Human anogenital monocyte-derived dendritic cells and langerin+cDC2 are major HIV target cells

Tissue mononuclear phagocytes (MNP) are specialised in pathogen detection and antigen presentation. As such they deliver HIV to its primary target cells; CD4 T cells. Most MNP HIV transmission studies have focused on epithelial MNPs. However, as mucosal trauma and inflammation are now known to be strongly associated with HIV transmission, here we examine the role of sub-epithelial MNPs which are present in a diverse array of subsets. We show that HIV can penetrate the epithelial surface to interact with sub-epithelial resident MNPs in anogenital explants and define the full array of subsets that are present in the human anogenital and colorectal tissues that HIV may encounter during sexual transmission. In doing so we identify two subsets that preferentially take up HIV, become infected and transmit the virus to CD4 T cells; CD14+CD1c+ monocyte-derived dendritic cells and langerin-expressing conventional dendritic cells 2 (cDC2).
There is still no cure or vaccine for HIV/AIDS and 37 million people remain infected. Antiretroviral therapy (ART) is efficient at controlling infection but is a lifelong treatment which is costly to manage and associated with toxicities. Only 57% of HIV+ individuals receive ART and there are 1.8 million new infections each year. ART can be given to healthy ‘at risk’ individuals as pre-exposure prophylaxis (PrEP) which has shown to be effective in reducing transmission. However, this is not a universal solution because of poor access to PrEP in low income countries and variable uptake in Western countries. Furthermore, the effects of long-term administration of PrEP to healthy individuals are unknown and can be associated with decreased condom use, increased sexually transmitted infections and concomitant genital tract inflammation, enhancing HIV transmission, especially in sub-Saharan Africa. Furthermore, PrEP regimens have recently been shown to be ineffective in the context of an inflamed mucosa. Therefore, an effective vaccine and cure are still needed.

HIV is now transmitted sexually in almost all cases. In order to develop a vaccine (or more effective PrEP regimens) the precise definition of the initial HIV target cells in the anogenital mucosa is necessary, especially mononuclear phagocytes (MNP). These consist of Langerhans cells (LC), dendritic cells (DC) and macrophages which express the HIV entry receptors CD4 and CCR5 allowing them to be directly infected. Importantly, they also express a large repertoire of lectin receptors including C-Type Lectins (CLR) and Sialic acid-binding immunoglobulin-type lectins (Siglec), many of which can bind HIV and mediate endocytic uptake of the virus. As professional antigen presenting cells, LCs and DCs play a critical role in HIV transmission by transferring the virus to CD4+ T cells when they replicate resulting in CD4 T cell death and depletion and consequent immunosuppression. Epidermal LCs have been shown to take up HIV and transfer it to T cells in vagina, cervix and foreskin and we and others have recently shown that epidermal DC populations also participate in this process. We previously showed that HIV transfer to CD4+ T cells occurs in two successive phases from epidermal LCs, epidermal CD11c+ DCs and in vitro derived monocye-derived (MDDC) cells. The first phase of transfer occurs within 2 h and is dependent on lectin mediated uptake of the virus and declines rapidly with time. The second phase occurs from 72 h onwards and increases with time as newly formed virions bud off from the surface of cells that have become productively infected via CD4/CCR5 mediated entry into viral synapses.

Mucosal trauma which breaches anogenital and colorectal epithelium and associated inflammation are likely to enhance HIV acquisition as it also allows the virus direct access to deeper target cells in the underlying lamina propria. Despite this, the role of lamina propria MNP subsets in HIV transmission has been largely understudied. These MNPs are present in several distinct subsets which include CD1c+ and CD12+ cells. In addition, there are several subsets of CD14+ cells which include autofluorescent tissue resident macrophages and non-autofluorescent CD14+ cells which have been conventionally referred to as DCs and have an established role in transmitting HIV to CD4 T cells in both intestinal and cervical tissue. However, in healthy tissue CD14+ CD1c- cells which have recently been redefined as monocyte-derived macrophages (MDM) have a role in HIV transmission as macrophages are very weak antigen presenting cells for naive T cells and do not migrate to lymph nodes and thus are less likely to deliver the virus to CD4 T cells than DCs. In inflamed tissue CD14+ CD1c+ cells have been described which transcriptionally align with DCs and are well described in vivo MDDCs. Recently similar cells have also been described in healthy lung tissue.

In this study we have thoroughly defined the MNP subsets that are present in the sub-epithelium (lamina propria and dermis) of all human anogenital and colorectal tissues and found two cell subsets that are key players in HIV uptake and transmission: CD14+ CD1c+ ex vivo MDDCs and langerin- DC2. Compared to other MNPs both subsets took up HIV more efficiently and became more infected. We found that ex vivo CD14+ CD1c+ MDDCs were most efficient at transferring HIV to CD4 T cells at late time points which correlated with their high CCR5 expression and that langerin+ DC2 transferred the virus most efficiently at early time points.

Results
Defining human mucosal mononuclear phagocyte subsets by flow cytometry. We have previously optimised protocols for the efficient isolation and definition of MNPs from human skin with minimal surface receptor cleavage and in an immature state to most closely resemble their functional state when they encounter pathogens. We firstly modified our skin flow cytometry gating strategy for use in mucosal tissues (Fig. 1A). All known tissue MNP subsets were identified which were: epidermal CD11c+ DCS and LCs and dermal cDC1, cDC2, CD14+ autofluorescent macrophages and dermal non-autofluorescent CD14+ cells. We also designed the panel to differentiate between non-autofluorescent ex vivo CD14+CD1c- MDMs and ex vivo CD14+CD1c+ MDDCs. We used this gating strategy to define the relative proportions of each dermal MNP subset in the full range of human anogenital and colorectal tissues that are the actual sites where HIV transmission occurs, as well as abdominal skin for comparison. This included tissues comprised of skin (labia, outer foreskin, glans penis and anal verge), Type II mucosa (vagina, fossa navicularis, ectocervix and anal canal) and Type I mucosa (endocervix, penile urethra, rectum and colon) (Fig. 1B). In order to confirm these trends in situ we also used fluorescence microscopy to show similar trends in cell population density using inner foreskin (Fig. 2A). The relative proportions of cDC1 were relatively consistent across all tissues with the exception of outer foreskin which had slightly higher proportions. In abdominal skin we found that dermal cDC2 were the overwhelmingly predominant cell population in contrast anogenital and colorectal tissues where these cells were present in much smaller proportions. Notably, however, a greater proportion of the total cDC2 population expressed langerin in these tissues compared to abdomen (Fig. 2B). Conversely, CD14-expressing cells were present in higher proportions in anogenital and colorectal tissues compared to abdomen and the relative proportions were significantly higher in mucosal tissues compared to anogenital skin (Fig. 2C). Despite the fact that in vivo MDDCs have predominantly been described as an inflammatory cell we found that they were substantially present across all our uninfammed tissues.

Transcriptional profiling. As CD14+ cells have been shown to play an important role in HIV transmission we firstly focussed our study on these cells. As we found both ex vivo CD14+CD1c+ MDDC and ex vivo CD14–CD1c– MDM in all our uninfammed tissue samples we carried out RNAseq analysis to compare the transcription profile of these cells to other tissue MNPs; LCs, dermal langerin- cDC2 and their langerin-expressing counterparts (Fig. 3A). As expected, LCs were the most distinct population and, in agreement with the literature, cDC2 clustered together regardless of langerin expression and CD14+ CD1c- cells were transcriptionally very similar to autofluorescent macrophages whereas CD14+CD1c+ cells aligned more closely with DCs. Comparison of ex vivo MDM cells with ex vivo MDDC cells showed 501 differentially expressed genes. Ex vivo
MDDCs expressed lower levels of the lentiviral restriction factor APOBEC3G which is a known inhibitor of HIV-1 infectivity\(^42\). We also measured the protein expression levels of SAMHD1 given its known role as a myeloid HIV restriction factor. It was expressed at slightly higher levels in ex vivo MDDCs than in ex vivo MDMs although this was not statistically significant. However, it was expressed at significantly higher levels in cDC1 (Fig. 3B).

**Determination of surface receptor expression on skin mononuclear phagocytes.** Previously we have carried out gene expression studies to examine CLR expression by ex vivo-derived skin DC subsets derived by collagenase digestion and compared them to model in vitro derived cells\(^43\). However, we did not include cDC1 or autofluorescent macrophages nor did we divide the non-autofluorescent CD14-expressing cells according to CD1c expression or cDC2 by langerin expression. Importantly, we also did not examine the surface expression of these proteins. We therefore used our flow cytometry gating strategy (Fig. 1A) to determine the surface expression levels of a wide range of surface molecules including HIV entry receptors, costimulatory molecules and lectin receptors involved in pathogen recognition (CLRs and Siglecs) on each MNP subset obtained via enzymatic digestion (CD14, CD1a, CD1c, CD11c, Langerin) and Siglecs) on each MNP subset obtained via enzymatic digestion (CD14, CD1a, CD1c, CD11c, Langerin).

**Fig. 1 Definition of human dermal/lamina propria mononuclear phagocytes by flow cytometry.** A Following collagenase digestion six distinct CD3-CD19-CD45+HLA-DR+ mononuclear phagocyte subsets from human abdominal skin were defined, (i) tissue resident macrophages (green) were defined as autofluorescent CD14+, (ii) type 1 conventional dendritic cells (cDC1; purple) were defined as autofluorescent-, XCR1+, CD14-, type 2 conventional dendritic cells (cDC2; blue) were defined as autofluorescent-, XCR1-, CD14+, CD1c+, and could be split into two populations, (iii) a langerin+ population (light blue) and (iv) a langerin+ population (dark blue), (v) CD14+CD1c- cells (red) were defined as autofluorescent-, XCR1+, CD14+, CD1c-, (vi) CD14+CD1c+ cells (orange) were defined as autofluorescent-, XCR1-, CD14+, CD1c+. Representative plot of n = 52 abdominal donors is shown. B Relative proportions of each subset of mononuclear phagocyte as a percentage of CD45+HLA-DR+ gate across the human anogenital/colorectal tracts were determined and mean ± standard deviation plotted. Statistics for subsets in each tissue were generated using the Kruskal-Wallis test: two-tailed Dunn’s multiple comparisons, comparing against abdominal skin tissue. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. (abdominal tissue (Abdo) = 52, labia = 8, glans penis = 8, perineum = 3, outer foreskin = 7, inner foreskin = 5, vagina = 6, fossa navicularis (FN) = 8, anal canal = 5, ectocervix = 4, endocervix = 3, penile urethra = 8, rectum = 7, colon = 5). Underneath, a pie chart for each tissue shows the mean proportion of each dermal/lamina propria subset across the human anogenital/colorectal tracts.
CCR5. These two subsets also differed from other MNPs subsets in that they expressed far fewer lectin receptors. LCs only expressed langerin, DCIR, DEC205, SigleC-3 and Siglec-9 as well as very low levels of CLEC7A, CLEC4D and CLEC4E. cDC1 expressed slightly more lectin receptors than LCs but generally at lower levels than other MNPs and uniquely expressed CLEC9A consistent with the literature. Other than langerin expression, we did not identify any differences in surface expression molecules between langerin-expressing and non-expressing cDC2.

We next focused our attention on key differences in the CLR profiles between DC like cells and macrophage like cells. Inflammatory MDDCs have been reported to express CD141 and CD1a31,37 but we showed these were also not expressed on the surface our cells derived from uninflamed tissue (Fig. 3C). Interestingly, the HIV binding CLR DC-SIGN, previously thought to be a DC marker and shown to be involved in capture of HIV and transfer to T cells11,31–33,44–47, was not expressed by any ex vivo DC subsets or ex vivo CD14+CD1c+ MDDCs (although gene expression was detected). It was expressed highly by ex vivo autologous macrophages and in vitro derived MDDCs and at low levels by both ex vivo CD14+CD1c− and in vitro MDMs (Fig. 3C, D). Similarly Siglec-1, a lectin shown to be important in DC-mediated HIV uptake45,48,49 was expressed most highly by autologous macrophages and ex vivo MDMs but at lower levels on ex vivo MDDCs lower still on cDC1 and cDC2 and not at all by in vitro derived MDDCs (Fig. 3C, D). Finally, CLEC5A was expressed very highly by cDC2 and ex vivo MDDCs but at lower levels on other CD14+ cells. Other than this, all CD14+ ex vivo derived cells showed very similar surface expression profiles. Taken together the expression profiles of DC-SIGN, Siglec-1 and CLEC5A add further weight to the hypothesis that CD14+CD1c+ cells are DC-like and CD14+CD1c− cells are macrophage like.

In addition to what is described above, model in vitro derived MDDCs and MDM differed from bona fide ex vivo derived cells in several important ways; similar to LCs, they did not express L-SIGN, CLEC7A, CLEC10A and Siglec-6 but, unlike LCs did not express CLEC4D or langerin. They also expressed much higher levels of CD4 and DEC205 than any other cell type.

Finally, we investigated differences in surface receptors between immature and mature cells. Using the spontaneous migration method we were only able to reliably isolate langerin− cDC2 and ex vivo CD14+CD1c+ MDDCs as described previously39 (Fig. 3E). As expected, cells isolated by this method expressed much higher levels of CD80 and CD86 than cells isolated by enzymatic digestion confirming their mature state. Interestingly cDC2, but not ex vivo MDDCs expressed CD83. Cells isolated by spontaneous migration expressed much lower (or none) of the following molecules: CLEC4A, CLEC4K (langerin), CLEC4L (DC-SIGN), CLEC8A, CLEC10A, CLEC12A and Siglec-1, while...
CLEC4E was down regulated by ex vivo MDDCs but not cDC2. Conversely CLEC13B (DEC205) was upregulated. In terms of HIV restriction factor APOBEC3G gene expression by RNAseq for CD14+ cDC2 and right: CD14+ cDC2 cells were morphologically more similar to DCs with none or few vacuoles present.

CD14+ dermal cell morphology and tissue residency. As DCs and macrophages are morphologically different, we next compared the morphology of dermal ex vivo MDDCs, ex vivo MDMs and cDC2 using Giemsa staining (Fig. 4A). Consistent with the transcriptional profiling and surface expression profile, we found that ex vivo MDMs looked like macrophages with a ‘fried egg’ like appearance and containing many large intracellular vacuoles whereas ex vivo MDDCs cells were morphologically more similar to DCs with none or few vacuoles present.

DCs and macrophages also differ in tissue residency which has important implications for transmission of HIV to CD4 T cells; DCs migrate out of tissue to lymph nodes which are rich in CD4 T cells whereas macrophages remain tissue resident. Consistent with this migration differential, DCs can be isolated from tissues by the spontaneous migration method (albeit in an activated state) whereas macrophage isolation requires enzymatic digestion.

We therefore compared the ability of dermal ex vivo MDDCs and ex vivo MDMs to migrate out of tissue spontaneously (Fig. 4B). Consistent with their DC-like transcriptional and morphological phenotype ex vivo MDDCs migrated out of tissue whereas ex vivo MDMs did not, consistent with their macrophage like phenotype. To confirm that the ex vivo MDMs were still present within the tissue, we digested the tissue after spontaneous migration and found that they were still present. We
next sorted ex vivo MDM and ex vivo MDDCs after isolation by enzymatic digestion and confirmed that ex vivo MDMs did not upregulate CD1c with culture, whereas ex vivo MDDCs showed a slight downregulation of CD1c, suggesting the population of cells which migrated out of tissue were bona fide CD14⁺CD1c⁺ MDDCs (Fig. 4C). As CCR7 is a key chemokine receptor required for DC migration out of tissue and shown in lung to be expressed by ex vivo MDDC, we compared the CCR7 gene expression levels in both cells types and showed that CCR7 was expressed much more highly on ex vivo MDDCs (Fig. 4D). Finally, as it is postulated that HIV or other stimuli, such as bacterial components leaked through damaged epithelium due to local trauma, would lead to activation of DCs and migration of HIV-laden DCs, we stimulated abdominal skin tissue with the TLR7 agonist imiquimod and showed that this significantly increased the number of ex vivo MDDCs that migrated out of the tissue (Fig. 4E). Taken together this shows that ex vivo MDDCs migrate out of tissue whereas ex vivo MDMs do not.

CD14⁺ dermal cell MNPs take up HIV via Siglec-1. We next compared the ability of ex vivo derived CD14-expressing cell subsets to take up HIV after 2 h of exposure using both the lab-adapted Bal strain and the transmitted/founder Z3678M strain. All subsets efficiently took up the virus but ex vivo MDDCs took up significantly more (Fig. 5A). We were also able to observe both ex vivo MDDCs and ex vivo MDMs interacting with HIV in situ in penile urethra using our RNAscope HIV detection assay indicating that HIV can penetrate into the sub-epithelium and interact with these cells in situ (Fig. 5B). CD14-expressing cells have previously been shown to play a role in HIV transmission and we and others have shown that DC-SIGN is a key HIV binding receptor expressed on these cells and is necessary for uptake and transfer to CD4 T cells. Furthermore, in all specimens Siglec-1 was expressed significantly higher levels of Siglec-1 than cells that did not take up HIV, indicating that Siglec-1 expression correlates with HIV uptake. However, we observed here that another key HIV binding lectin receptor, Siglec-1, was expressed by all CD14⁺ sub-epithelial cells and at much higher levels than CD14⁻ MNP subsets. We therefore examined Siglec-1 expression on HIV infected and by stander cells derived from labia, inner foreskin and colon. In all CD14-expressing cell types we found that cells that had taken up HIV expressed significantly higher levels of Siglec-1 than cells that did not take up HIV, indicating that Siglec-1 expression correlates with HIV uptake. Furthermore, in all specimens Siglec-1 was expressed most highly in autofluorescent tissue resident macrophages followed by ex vivo MDM then ex vivo MDDCs. This finding was confirmed in colonic tissue (Fig. 5D). Using cells derived from labia, foreskin and colon we showed that a Siglec-1 antibody was able to partially block HIV uptake further implying a role of...
Fig. 5 HIV uptake of dermal CD14-expressing mononuclear phagocytes. A MNP s were liberated from human abdominal skin using and CD45^+HLA-DR^+ live cells FACS isolated. Mixed dermal populations were incubated for 2 h with HIV_{Bal} or HIV_{Z3678M} or mock treated, thoroughly washed and stained for surface markers and two antibody clones to HIV p24 (KC57 and 28B7) for flow cytometry analysis to determine percentage dual p24^+ cells. Left: HIV_{Bal} results graphed with each donor represented by individual dots, donor matched by connected lines (n = 7). Statistics were generated using RM one-way ANOVA with Holm-Sidak’s multiple comparisons with adjusted P values shown. Right: representative contour plots for HIV_{Z3678M} shown, gating on dual p24^+ cells for each subset. B Human penile urethra explants were treated with HIV for 2 h before being fixed and paraffin embedded. After sectioning, HIV RNA was visualised using RNAscope probe. C Representative images of Siglec-1 in HIV uptake by CD14-expressing cells (Fig. 5E). We measured the expression of these molecules on ex vivo cells from abdominal skin and found that supernatants derived from infected abdominal ex vivo MDDCs cultures were able to infect greater numbers of CD4 TZMBL cells than other CD14-expressing populations (Fig. 7A). We then confirmed that the combined CD14-expressing cells derived from abdominal tissue and ex vivo MDDCs derived from colonic tissue could transfer HIV to activated primary CD4 T cells derived from PBMCs (Fig. 6B). We found no statistically significant difference between the three subsets’ ability to transfer the virus, although autofluorescent tissue resident macrophages (which expressed the highest levels of Siglec-1) were able to transfer the virus much more efficiently in 2 of the 4 donors. Therefore, Siglec-1 expression does not explain why ex vivo MDDCs cells take up HIV more efficiently than other CD14-expressing cells.

Ex vivo MDDCs are most susceptible to HIV infection. We next investigated the ability of dermal CD14-expressing cells to become productively infected with HIV and to transfer the virus to CD4 T cells at later time points. As the expression of the HIV entry receptors CD4 and CCR5 are essential for productive HIV infection, we firstly measured the surface expression of these molecules on ex vivo cells from abdominal skin and found that ex vivo MDDCs expressed higher levels of CCR5 than other CD14-expressing populations (Fig. 7A). We then confirmed this observation in cells derived from colon tissue which is more relevant to HIV transmission (Fig. 7B). Corresponding with their higher CCR5 surface expression, we also found that supernatants derived from infected abdominal ex vivo MDDCs cultures were able to infect greater numbers of CD4 TZMBL cells than other CD14-expressing subsets (Fig. 7C). To confirm that cells were productively infected at these late time points (as opposed to virus being held on the surface) we infected CD4 T cells with supernatants derived from combined CD14^+ cells that had been treated with HIV in the presence of AZT and showed that a reduction in the number of CD4 T cells that became infected (Fig. 7D). Despite using our optimised ex vivo derived skin MNP culture methods, we were unable to measure direct infection of
CD14-expressing cell subsets by flow cytometry (as we have done previously with other ex vivo derived skin MNPs13,21) as only small cell yields could be derived for each specific cell subsets (particularly ex vivo MDDCs), even from very large pieces of abdominal skin, and too few live cells could be detected after 96 h of culture. Corresponding with their higher levels of HIV infection, we finally showed that ex vivo MDDCs were the most efficient CD14-expressing cells at transferring HIV to CD4 T cells (Fig. 7E).

Langerin+ cDC2 are enriched in anogenital tissues and the most efficient cells at HIV uptake and infection. We next turned our attention to sub-epithelial cDC2 which have been understudied in HIV transmission. We have recently shown that these cells exist in the epidermis of human anogenital cutaneous tissues where they preferentially interact with HIV and transmit it to T cells21. Interestingly, we found that, similar to epidermis, langerin+ sub-epithelial cDC2 were significantly enriched in anogenital tissues compared to abdominal skin (Fig. 2B). We also found that within 2 h these cells took up much more HIV than their langerin− counterparts and cDC1 using both a lab-adapted and transmitted founder HIV strain (Fig. 8A) and could be visualised interacting with the virus in situ using RNAscope following topical infection of foreskin explants (Fig. 8B). Correspondingly, they also transferred HIV to CD4 cells more efficiently at 2 h than langerin− cDC2 (Fig. 8C). Interestingly, although langerin+ cDC2 did not express higher levels of CCR5 (Fig. 9A) or CD4 (Fig. 9B) than their langerin− counterparts, they nevertheless produced higher levels of both HIV lab-adapted BaL strain and a transmitted founder strain after 96 h indicating they supported higher levels of infection (Fig. 9C) and we were able to block this effect with AZT (Fig. 9D). We attempted to block HIV uptake and transfer to CD4 cells using a langerin antibody, as we have done previously with LCs13, and also to carry out a 96 h MNP-T cell transfer assay but unfortunately these experiments proved impossible due to very small numbers of langerin-expressing sub-epithelial cells that are able to be extracted from tissue.

Fig. 6 1st phase HIV transfer from CD14-expressing mononuclear phagocytes to CD4+ T cells. A Sorted CD14-expressing dermal cells were incubated with HIVBal for 2 h and then thoroughly washed off. JLTR cells were added to MNPs at a ratio of 4:1 and cultured for a further 96 h in human fibroblast conditioned media. Transfer of HIV to T cells was assessed using flow cytometry to plot the square root percent of GFP+ T cells, with each dot representing an individual donor (n = 5). B Left: CD14-expressing MNPs were sorted and 2-h transfer ability to JLTR cells compared to primary activated CD4 T cells derived from PBMCs was assessed (n = 3). Primary CD4 T cells were cultured at a ratio of 2T cells:1 MNP. The number of infected T cells was assessed by flow cytometry using Live/dead NIR and intracellular P24 staining. Three individual MNP donors were plotted with squares representing transfer to primary CD4 T cells and triangles representing transfer to JLTR cells. Right: CD14+ CD1c+ MDDC from four human colonic tissue donors were sorted and 2-h transfer assays to CD4 T cells from PBMCs were setup as above, plotted as box and whisker plots, box representing the upper and lower quartile, central line representing the median, the whiskers the minimum and maximum and each donor represented by an individual dot (n = 4). Representative contour plots from one individual donor are shown. Macrophages (green), CD14+ CD1c− MDM (red) and CD14+ CD1c+ (orange).
Fig. 7 HIV infectability and transfer capacity of CD14-expressing dermal mononuclear phagocytes. A CCR5 expression was determined on CD14-expressing MNPs liberated from collagenase type IV digested tissue. Left: histogram representing one abdominal skin donor with black, unfilled histogram representing FMO control. Right: geometric mean fluorescent intensity (gMFI) minus FMO control plotted from abdominal skin (n = 4), plotted as box and whisker plots, box representing the upper and lower quartile, central line representing the median, the whiskers the minimum and maximum of each cell subset and each donor represented by an individual dot. Statistics were generated using a Friedman test. Macrophages (green), CD14+ MNP (red) and CD14+ CD1c+ (orange).

B CCR5 expression was investigated in colon (n = 4) plotted as box and whisker plots, box representing the upper and lower quartile, central line representing the median, the whiskers the minimum and maximum of each cell subset and each donor represented by an individual dot. Statistics were generated as in A. C CD14-expressing MNP subsets were FACS isolated and incubated with HIVBal for 2 h before being thoroughly washed off. Cells were then cultured for an additional 96 h in human skin fibroblast conditioned media. Cell supernatants were taken and assessed for secreted HIV using a TZMBL infection assay, with the number of infected cells per 10,000 MNPs calculated and graphed. Individual dots represent three donors matched by connecting lines. Statistics were generated as in A. D Combined CD14-expressing MNPs were sorted and pre-treated for 1 h with 50 µM azidothymidine (AZT) before 2 h culture with HIVBal. Cells were washed three times and incubated for a further 48 h with AZT and then washed three more times. Cells were cultured for another 48 h before cell supernatants were collected and secreted HIV was assessed using a TZMBL infection assay. Each donor represented by individual point matched with joining lines, circles represent untreated cells and squares represent AZT treated cells, column representing the mean. E JLTRs were added to cell cultures from C after supernatants removed, at a ratio of 4:1 and cultured for a further 96 h. Transfer of HIV to T cells was determined using flow cytometry to assess the percent of GFP+ JLTRs. Raw data was square root normalised and plotted, with each dot representing four individual donors with connecting lines matching donors. Statistics were generated using a Friedman test. Macrophages (green), CD14+ CD1c- MDM (red) and CD14+ CD1c+ (orange).

Discussion
In this study we have investigated the role that sub-epithelial MNPs play in HIV transmission. We began by thoroughly defining the relative proportions MNPs that are present in all human anogenital and colorectal tissues which are the sites of HIV transmission. In doing so we revealed that CD14-expressing cells predominate in these tissues in contrast to abdominal skin. CD14-expressing cells were found in greater proportions in cells predominate in these tissues in contrast to abdominal skin. We identified two MNPs subsets that may play a dominant role in HIV transmission; ex vivo CD14+CD1c+ MDDCs and langerin+ cDC2, both of which were present in higher proportions in human anogenital and colorectal tissues compared to abdominal skin.

Tissue macrophages express CD14 and have been classically defined in tissue by their high levels of autofluorescence26,52. Non-autofluorescent tissue CD14+ cells have traditionally been referred to as CD14 DCs which have been well characterised in their ability to bind to and capture HIV and transmit it to CD4 T cells in model systems11,17,53–56 as well as in intestinal tissue32–34 and more recently in cervical tissue15. The ability of these cells to transmit the virus to CD4 T cells has been assumed to be associated with the potent antigen presenting function of DCs. However, transcriptional profiling has recently led to non-autofluorescent CD14+ CD1c– cells in skin to be redefined as in vivo monocyte-derived macrophages40. This is puzzling as macrophages are thought to be weak antigen presenting cells to naïve T cells, especially when compared to DCs and they do not migrate out of tissue to lymph nodes52. As ex vivo CD14+CD1c– MDDCs have been described in inflamed tissue30,31,37 we therefore carried out transcriptional profiling and found that, in agreement with the literature, CD14+CD1c– cells transcriptionally aligned very closely with macrophages40 and that CD14+CD1c+ cells aligned more closely with DCs37. Adding weight to the hypothesis that these cells were DC-like, we showed that CD14+CD1c+ cells morphologically resembled DCs and,
expressed CCR7 and spontaneously migrated out of tissue as DCs are known to do, unlike CD14+CD1c− cells which morphologically resembled macrophages, did not express CCR7 and remained tissue resident. This migration was enhanced in the presence of the TLR7 agonist imiquimod. These results support the literature which shows that CD1c is an essential marker to define cDC2 and inflammatory MDDCs have been shown to express CD14 and CD1c in patients with rheumatoid arthritis and cancer31,37. Indeed, it has been recently showed that all human CD1c+ inflammatory DCs derived from ascites are monocyte-derived cells and therefore not cDC1 or cDC2 which are derived from specific bone marrow precursors38. However, these cells have not been previously defined in healthy human anogenital tissue which form the portals of HIV entry and we show that they did differ from inflammatory MDDCs in that they did not express CD1a, CD141 or DC-SIGN.

We next investigated the way CD14-expressing cells interacted with HIV-1_{Bal} and a transmitted/founder clinical HIV-1 strain and found that ex vivo MDDCs cells took up significantly more HIV within 2 h than ex vivo MDMs and autofluorescent macrophages. They also expressed higher levels of the HIV entry receptor CCR5 and correspondingly supported higher levels of productive infection resulting in higher levels of infectious virion secretion and higher levels of transfer of the virus to CD4 T cells. They also expressed lower levels of the HIV restriction factor APOBEC3G which could further account for the higher levels of secreted virions by these cells although the HIV Vif protein is known to inhibit the function of this protein. We also investigated

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**Fig. 8 HIV uptake and 1st phase transfer capacity of dermal dendritic cells.** **A** Enzymatically liberated cells from abdominal skin were FACS isolated to obtain a CD45+HLA-DR+ population and cultured with HIV_{Bal} or HIV_{Z3678M} or Mock treated for 2 h before being washed off three times in PBS. Cells were stained for surface markers and two p24 clones, before analysis by flow cytometry. Left: HIV_{Bal} treated cells percentage of dual p24+ cells plotted as box and whisker plots, box representing the upper and lower quartile, central line representing the median, the whiskers the minimum and maximum of each cell subset and each donor represented by an individual dot (n = 7, n = 3 for cDC1 due to low cell numbers). Statistics were generated using a mixed effects analysis with two-tailed Tukey’s multiple comparisons with adjusted P values shown. Right: representative contour plots for HIV_{Z3678M} gating on dual p24+ cells. **B** Human inner foreskin explants were treated with HIV for 2–3 h, before being fixed, paraffin embedded and sectioned. HIV was visualised using RNAscope alongside immunofluorescence for CD11c, CD14 and langerin as well as DAPI to identify Langerin− cDC2 (top) and langerin+ cDC2 (bottom). Representative image from n = 3 donors. **C** FACS isolated cDC2 divided by langerin expression were cultured with HIV_{Bal} for 2 h before being washed off three times. JLTR cells (CD4 T cells with GFP under the HIV promoter) were then co-cultured for 96 h in fibroblast conditioned media. Flow cytometry was used to analyse the percent of GFP+ T cells. Data was square root normalised and graphed with each donor represented by an individual dot and lines connecting matched donors (n = 4). Statistics were generated using a two-tailed paired T test. cDC1 (purple), langerin− cDC2 (light blue) and langerin+ cDC2 (dark blue).
the expression of the key HIV restriction factor SAMHD1 and found that it was expressed at roughly equal levels across MNP subsets except cDC1 which expressed higher levels of this protein. It is of note that the SAMHD1 antibody clone we used did not distinguish whether the SAMHD1 was phosphorylated (no restriction) or not (antiviral). Uptake of HIV by MNPs at early time points is mediated by lectin receptors and previously DC-SIGN has shown to be key a lectin receptor involved in HIV uptake by CD14-expressing cells and has also been implicated in transfer to CD4 T cells. Similarly Siglec-1 has also been implicated. However, ex vivo MDDCs did not express DC-SIGN so this receptor cannot be responsible for the efficient early uptake observed at 2 h by these cells. This is an important observation as soluble DC-SIGN designed to block HIV interacting with MNPs has been proven ineffective in blocking HIV transmission. Ex vivo MDDCs did however express Siglec-1, but at lower levels than ex vivo MDMs and autofluorescent macrophages which expressed the highest Siglec-1 levels. We therefore investigated the role of Siglec-1 in early uptake and found that in all CD14-expressing cell types, those cells that contained HIV expressed higher levels of Siglec-1 than uninfected cells. Furthermore, a Siglec-1 blocking antibody was able to partially block HIV uptake which corresponded with the levels of Siglec-1 surface expression. Therefore, the mechanism which allows ex vivo MDDCs to take up HIV more efficiently than other CD14-expressing cells remains to be elucidated and will be the subject of a future study. However, other than DC-SIGN we did not detect any difference in the expression profiles of known HIV binding CLRs between ex vivo MDDCs and other CD14+ cells. Ex vivo MDDCs did however express higher levels of CLEC5A which opens an avenue of investigation. It is of note that ex vivo CD14+ tissue resident macrophages and MDMs were able to take up HIV (albeit significantly less so than MDDCs). The possibility therefore exists that these cells act as virus reservoirs that transmit viral particles to DCs which then migrate and transmit the virus to T cells.

Despite their importance in antigen presentation, cDC2 have been understudied in HIV transmission with almost all studies focussing on langerin-expressing LCs or DC-SIGN expressing CD14+ cells. Recently we reported that cDC2-like cells were present in the epidermis of anogenital tissues where they predominately comprised LCs and preferentially become infected with HIV.
and transmitted the virus to CD4 T cells. Pena-Cruz and colleagues made similar observations in vaginal epithelium. Importantly, we showed that in anogenital tissues the majority of these cells expressed langerin. We therefore investigated the role of lamina propria cDC2 in HIV transmission. We noticed that a greater proportion of sub-epithelial cDC2 in anogenital and colorectal tissues expressed langerin than in abdominal dermis. This was especially the case in the inner foreskin, penile urethra, vagina and rectum. Importantly, we found that langerin-expressing cDC2 were much more efficient at HIV uptake after 2 h than their non-langerin-expressing counterparts and were correspondingly much more efficient at transferring the virus to CD4 T cells at the same early time point. In fact, these cells were the most efficient of all MNPs at transfer of HIV to CD4 T cells, meaning despite their relatively lower frequency compared to other MNP cell types, they are nevertheless likely to be key players in HIV transmission. Furthermore, despite the fact that no differences in surface CCR5 expression were detected between the two cell types, langerin-expressing cDC2 also secreted higher levels of infectious HIV virions after 96 h using both the lab-adapted Bal strain and a clinical transmitted/founder strain. Other than langerin expression these two cell types expressed an identical array of surface lectin receptors so we hypothesise that langerin must be mediating these effects. In LCs langerin is well known to bind HIV and mediate efficient HIV uptake so we hypothesise here that these cells efficiently bind HIV via langerin which concentrates HIV on the cells surface allowing for greater HIV-CCR5 binding and infection as well as endocytic uptake. We made multiple attempts to test this hypothesis using a langerin blocking antibody as we did previously with LCs but unfortunately these experiments proved impossible using the very small numbers of this cell type we could extract from abdominal or genital skin or mucosal tissue.

It is of note that under the condition used in this study, virus spread from T cells to T cells may occur. It is possible that the efficiency of T cell-to-T cell spread may be differentially altered in the presence of different MNPs (e.g. via cytokine release). We believe this is unlikely to be substantial especially in the context of early phase (2 h) transfer which is from MNP intracellular caves which is unlikely to be a powerful inducer of cytokine release. However, if this mechanism does contribute it clearly varies amongst MNP subsets in targeting spread to CD4 T cells and therefore it is of no less relevant to transmission.

A clear strength of this study is that it has been exclusively conducted using human tissues including the anogenital and colorectal tissues that HIV may encounter during sexual transmission. However, these kinds of experiments also come with limitations. They are very laborious and time consuming and only a very small number of cells can be isolated for each specific subset, especially ex vivo MDDCs and even more so for langerin + cDC2 which were both a key focus of this study. This severely limits the parameters that can be included in our assays and many experiments proved impossible such as blocking assays using langerin + cDC2. Furthermore, these cells are very difficult to culture once isolated from tissue and despite our optimised cell extraction and ex vivo culture protocols functional assays requiring 96 h of culture were extremely difficult to perform. We did manage to perform HIV infection assays using infected MNP culture supernatants but there were too few live cells to gate on to measure direct infection of any MNP cell type by flow cytometry as we have done previously for epidermal cells and we were unable to perform transfer assays at 96 h for langerin + cDC2. These constraints also meant that we could only repeat a few key observations with a clinical transmitted/founder strain. All uptake assays were confirmed at least once using this strain and also the cDC2 infectivity assays. In all experiments similar trends were observed with both virus strains. Furthermore, we have previously observed similar trends using the same two strains of HIV in epidermal LCs and CD11c+ DCs.

In conclusion, we have identified two sub-epithelial MNPs that may play a role in transmission of HIV; ex vivo CD14 + CD1c + MDDCs and langerin + cDC2. Both were able to preferentially take up the virus within 2 h and support higher levels of HIV infection than other MNP subsets. Previously, many studies have focussed on the role of LCs in sexual transmission of HIV as these were considered most likely to interact with HIV as they are closest to the epithelial surface. We also demonstrated the importance of a second epidermal DC subset, resembling activated cDC2. However, as genital trauma and inflammation are clearly associated with HIV transmission, especially in sub-Saharan Africa, and current PrEP regimens, if available, are not efficient at blocking transmission across an inflamed mucosa, it is important that the role of sub-epithelial MNPs are also examined as we have done here. This is relevant to vaccine design as, to protect against initial infection, the route of transmission and which local immune defence mechanisms can be harnessed or impaired needs to be understood. The current targets of systemic vaccines as broadly neutralising antibodies, antibody dependent cytoxicity and systemic CD8 T cells may not be enough. Local immunity in the anogenital mucosa such as resident memory T cells could be induced by mucosal vaccines or maintained by local tissue DCs after initial stimulation by systemic vaccine adjuvants in lymph nodes draining the site of application. Therefore, it is important to determine which specific subsets of MNPs pick up the virus and deliver it to specific subsets of CD4 T cells. Here we have identified two MNP subsets of interest and together with our recently discovered epidermal CD11c+ DCs and LCs, the next step is to determine with which T cells subsets these cells interact. Finally, defining the interactions between HIV and its mucosal target cells should aid in the development of blocking agents that can be used in modified PrEP regimens to block MNP infection via the anogenital and colorectal mucosa.

**Methods**

**Sources of tissues and ethical approval.** This study was approved by the West Sydney Local Area Health District (WSLHD) Human Research Ethics Committee (HREC); reference number HREC/2013/8/44/3777 AU RED HREC/13/ WMEAD/232. Healthy human tissue was obtained from a range of plastic surgeons and written consent was obtained from all donors.

**Tissue processing.** MNP were isolated from abdominal tissue using our optimised collagenase-based digestion process. Skin was collected immediately after surgery, stretched out and sectioned using a skin graft knife (Swann-Morton, Sheffield, UK) and the resulting skin grafts passed through a skin graft mesher (Zimmer Biomet, Warsaw, IN, USA). The meshed skin was placed in RPMI1640 (Lonza, Switzerland) with 0.14 U/ml dispase (neutral protease, Worthington Industries, Columbus, OH, USA) and 50 μg/ml Gentamicin (Sigma-Aldrich, St Louis, MO, USA) and rotated at 4 °C overnight. The skin was then washed in PBS and dermis and epidermis were mechanically separated using forceps. Dermal collagenase type II (1 mg/mL, Worthington Industries) and 200 U/ml collagenase Type IV (Worthington) at 37 °C for 120 min in a rotator. The cells were then separated from undigested dermal and epithelial tissue using a tea strainer. The supernatants were then passed through a 100-μm cell strainer (Greiner Bio-One, Monroe, NC, USA) and pelleted. The cell pellet was then passed again through a 100-μm cell strainer and washed twice more in PBS. The epidermal suspension was spun on a Ficoll-Paque PLUS (GE Healthcare Life Sciences, Little Chalfont, United Kingdom) gradient and the immune cells harvested from the Ficoll-PBS interface. Dermal cells were enriched for CD14 + CD1c + cDC2 expressing cells using CD145 magnetic bead separation (Miltenyi Biotec, San Diego, CA, USA). Cell suspensions were then counted and/or labelled for flow cytometric phenotyping of surface expression markers or for flow sorting. This protocol was modified for anogenital tissues as follows: for skin and type II mucosa (labia, foreskin, glans penis, fossa navicularis, vagina, ectocervix and anal canal), small shallow scalpels were made to the epithelial surface before overnight dispase II treatment; for type I mucosa (endocervix, penile urethra, rectum and colon) no dispase treatment was required and tissue was digested using two
successive 30 min digestions with collagenase type IV. For spontaneous migration assays, following mechanical separation of dermis and epidermis tissue was cultured for 24 h in RPMI 10% FCS, 50 U/ml DNase I and 25 µg/ml gentamicin before cells were collected from culture media. For TLR stimulated experiments culture media were added to culture plates, treated as above. 

Preparation of in vitro monocyte-derived dendritic cells and macrophages. CD14+ monocytes were derived from human blood and cultured for 6 days in RPMI with 500 U/mL interleukin (IL)-4 and 300 U/mL granulocyte-macrophage colony-stimulating factor (GM-CSF) to produce in vitro derived MDSC or in human serum to generate in vitro derived MDM as described previously. Cells were then treated as above. 

Flow cytometry and sorting. Cells were labelled in aliquots of 1 x 10⁶ per 100 µl of buffer, according to standard protocols. Nonviable cells were excluded by staining with Live/Dead Near-IR dead cell stain kit (Life Technologies, Carlsbad, CA, USA). Flow cytometry was performed on Becton Dickson (Franklin Lakes, NJ, USA) LSRSortessa, CantoII and Symphony flow cytometers with BD DIVA software. For cellular proliferation assays, cells were cultured for 24 h in RPMI 10% FCS, 250 µM 2′-mercaptoethanol, 50 U/ml DNase I and 25 µg/ml gentamicin before being washed with two changes of PBS. Supernatants were cultured with TZMBL cells (HeLa cell derivatives expressing high levels of CD4 and CCR5 and containing the β-galactosidase reporter gene under the HIV promoter) for a further 72 h on 96-well flat-bottom plates. Supernatants were removed and T2MBL development solution added (0.5 M potassium ferricyanide, 0.5 M potassium cyanide and 50 mg/ml X-gal) and incubated for 1 h to detect β-galactosidase reporter gene expression. Development solution was removed, and cells fixed with 4% (v/v) PFA (diluted in PBS) for 15 min. Infection was quantified using an EliSpot plate reader (AID, Strasbourg, Germany). For AZT blocking assays, cells were pre-treated with 25 µg/mL AZT (National Institutes of Health AIDS reagent programme) for 1 h at 37 °C before addition of HIV. At 48 h AZT was thoroughly washed off and the assay continued as described above.

HIV uptake assay. Ex vivo dervial monopule subsets were tested for their ability to take up HIV virions. Cells were isolated from human skin by enzymatic digestion and live HLA-DR+ cells were isolated (Supplementary Fig. 1C). Sorted cells were cultured for 2 h with HIV or HIV2淫m in the presence of 10% FCS and the HIV infection rate determined using the dual p24 assay. For Siglec-1 blocking assays, cells were pre-treated with 20 µg/mL Siglec-1 mAb (Invitrogen, H5N 7D2) for 30 min before HIV was added.

HIV transfer assay. Ex vivo dervial monopule subsets were tested for their ability to transfer HIV to activated T cells as described previously for Epidermal MNPs. Cells were liberated from abdominal skin as described above and individual subsets were FACS isolated (Supplementary Fig. 1A). A minimum of 1 x 10⁴ and 3 x 10⁴ cells were cultured with HIVinf for 1st and 2nd phase transfer assays respectively, at an MOI of 1 for 2 h before being washed off three times with PBS. For 1st phase transfer assays, ILTR cells (CD4 T cells which express GFP under the HIV promoter) were co-cultured at a ratio of 4:1 (T cells:MNPs) or activated CD4+ PBMCs at a ratio of 4:1 (T cells:MNPs) or activated CD4+ PBMCs at a ratio of 4:1 (T cells:MNPs) before cloning cylinders (8 x 8 mm, Sigma-Aldrich) were adhered to the tissue using surgical glue (B Braun, Germany). Explants were cut around the cloning cylinder and placed onto gel foam sponge (Pfizer, New York, NY, USA) soaking in DC culture media in a 24-well plate. HIV was diluted in 100 µl of PBS at a TCD5₀ of 3500 and then added to cloning cylinders. In all, 100 µl of PBS was added to mock samples to prevent tissue drying out. Explants were cultured for 2–3 h before virus/PBS was removed and cylinders washed out 3x with PBS. After removal of cloning cylinders, tissue explants were placed in 4% PFA (Electron Microscopy Sciences, Hatfield, PA, USA) for 24 h before paraffin embedding.

RNAseq and immunofluorescent staining of tissue. All microscopy staining was carried out on 4 µm paraffin sections. Sections were blocked at 60 °C for 45 min, dehydrated in xylene followed by 100% ethanol and dried. Detection of HIV RNA was achieved using the RNAseq 2.5HD Reagent Kit-RED (Cat: 323620, ACD Bio, Newcastle, UK) as previously described. Sections underwent antigen retrieval in RNAseq target Retrieval buffer (RNAseq Kit) at 95 °C for 20 min in a decloaking chamber (Biocare, Pacheco, CA, USA) for 20 min in a decloaking chamber (Biocare, Pacheco, CA, USA) and then incubated with protease pre-treatment-3 (RNAseq kit) for 20 min at 40 °C. Sections were washed in Milli-Q water and incubated with custom-made HIV-1 labelling antibodies (Invitrogen, H5N 7D2) for 2 h at 37 °C followed by the biotinylated secondary antibody (Invitrogen). Slides were washed 2x for a total of 5 min. Slides were air dried and treated with Biotin-X (Vector Laboratories, SP-6000, Burlingame, CA, USA) for 10 min, washed in Milli-Q water and then incubated with protease pre-treatment-3 (RNAseq kit) for 20 min at 40 °C. Slides were washed in Milli-Q water and incubated with custom-made HIV-1 labelling antibodies (Invitrogen, H5N 7D2) for 2 h at 37 °C followed by the biotinylated secondary antibody (Invitrogen). Slides were washed 2x for a total of 5 min. Slides were air dried and treated with Biotin-X (Vector Laboratories, SP-6000, Burlingame, CA, USA) for 10 min, washed in Milli-Q water and then incubated with protease pre-treatment-3 (RNAseq kit) for 20 min at 40 °C. Slides were washed in Milli-Q water and incubated with custom-made HIV-1 labelling antibodies (Invitrogen, H5N 7D2) for 2 h at 37 °C followed by the biotinylated secondary antibody (Invitrogen). Slides were washed 2x for a total of 5 min. Slides were air dried and treated with Biotin-X (Vector Laboratories, SP-6000, Burlingame, CA, USA) for 10 min, washed in Milli-Q water and then incubated with protease pre-treatment-3 (RNAseq kit) for 20 min at 40 °C. Slides were washed in Milli-Q water and incubated with custom-made HIV-1 labelling antibodies (Invitrogen, H5N 7D2) for 2 h at 37 °C followed by the biotinylated secondary antibody (Invitrogen). Slides were washed 2x for a total of 5 min. Slides were air dried and treated with Biotin-X (Vector Laboratories, SP-6000, Burlingame, CA, USA) for 10 min, washed in Milli-Q water and then incubated with protease pre-treatment-3 (RNAseq kit) for 20 min at 40 °C. Slides were washed in Milli-Q water and incubated with custom-made HIV-1 labelling antibodies (Invitrogen, H5N 7D2) for 2 h at 37 °C followed by the biotinylated secondary antibody (Invitrogen). Slides were washed 2x for a total of 5 min. Slides were air dried and treated with Biotin-X (Vector Laboratories, SP-6000, Burlingame, CA, USA) for 10 min, washed in Milli-Q water and then incubated with protease pre-treatment-3 (RNAseq kit) for 20 min at 40 °C. Slides were washed in Milli-Q water and incubated with custom-made HIV-1 labelling antibodies (Invitrogen, H5N 7D2) for 2 h at 37 °C followed by the biotinylated secondary antibody (Invitrogen). Slides were washed 2x for a total of 5 min. Slides were air dried and treated with Biotin-X (Vector Laboratories, SP-6000, Burlingame, CA, USA) for 10 min, washed in Milli-Q water and then incubated with protease pre-treatment-3 (RNAseq kit) for 20 min at 40 °C.
Statistical analysis. For comparisons on unmatched donor data Kru skal–Walls with Dunn’s multiple comparison tests were performed to correct for the multiple contrasts made. Repeated measures one-way ANOVA with a Holm–Sidak’s multiple comparisons tests were used for experiments comparing more than two groups with donor matched experiments with equal number of data points. For experiments with unequal numbers of donor matched data points mixed effects analysis with Tukey’s multiple comparisons were performed. All multiple comparison–calculated adjusted P values. Normality was assessed using an Anderson–Darling test. Spearman’s tests were used to assess heteroscedasticity. All statistics were performed using GraphPad Prism Version 8.4.3.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data presented in this study are available from the corresponding author upon reasonable request. Source data used to generate figures are provided in the source data file. The source data underlying the Gene Expression Omnibus (GEO) database under accession code: GSE166639. Source data are provided with this paper.

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Author contributions
J.W.R. guided all the experiments in the manuscript. R.A.B. helped guide key experiments to begin the project and provided extensive intellectual input throughout. K.M.B. assisted and gave intellectual input to all experiments. E.E.V. assisted with colonic tissue processing and analysis. H.R. assisted with tissue processing and HIV functional assays. H.R. gave extensive intellectual input for microscopy experiments. T.R.O. helped with tissue processing and MNF quantification by microscopy. A.A. performed the TLR stimulated cell migration experiments. J.D.G. guided the RNAseq, P.V. and G.P.P. conducted the RNAseq analysis. J.F. conducted the Giema staining. N.N. provided intellectual input into the infection and transfer assay experiments. J.K.L., L.B., P.H., M.P.G., A.D.R., F.R., G.C., G.J.I. and A.J.B. provided human tissue specimens and intellectual input. E.P. assisted with statistical analysis. E.H. provided the clinical transmitted founder isolate. S.N.B., M.A.H. and A.L.C. provided significant intellectual input. A.N.H. conceived of and guided the study.

Competing interests
The authors declare no competing interests.

Additional information
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