

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection Flow Cytometry data was collected using BD DIVA software (version 8.0). TZMBL results were collected using AID ELISPOT reader. Microscopy images were visualised using Olympus-ASW (version 2.9).

Data analysis Flow cytometry data was analysed using FlowJo (V10). Microscopy analysis was done using OlyVia (version 2.9), FIJI (Madison version 2.0) and Huygens professional (20.04). All graphs/statistical analysis were generated using Prism- Graph pad (version 8.4.3). RNAseq data was analysed using TopHat2 software package (Version 2.1.1) and SAMMate (version 2.6.1)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

RNAseq data has been uploaded in the Gene Expression Omnibus (GEO) database under accession code GSE166639. Data availability statement is available in the manuscript Source data for all figures is available in the Source Data File.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences Behavioural & social sciences Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample sizes were not predetermined. For all key experiments at least three individual donors were analysed, while additional donors were used where ever possible. Due to low cell numbers in certain subsets (particularly langerin+ cDC2) it was not feasible to do some assays in larger numbers, however in each experiment we showed consistent trends in our results (which often reached statistical significance) and therefore believe sample size was sufficient.
Data exclusions	No data were excluded from the analysis
Replication	All findings presented were reproducible. For all functional assays findings were repeated at least three times with consistent results. For quantifying proportion of the mononuclear phagocyte subsets in human tissue there were a range of replications due to the varying commonality of receiving different tissues, with minimum replicates of 3 up to 52.
Randomization	There was no randomization in this study as we did not have different treatment/experimental groups and therefore was not relevant.
Blinding	There was no blinding in this study as we did not have different treatment/experimental groups and therefore was not relevant.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

Methods

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input type="checkbox"/>	<input checked="" type="checkbox"/> Human research participants
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used

Company: BD. Ab: CD103. Fluorophore: PE. Clone: Per-ACT8. Catalogue #: 340998 Lot #: 7250686
 Company: BD. Ab: CD11c. Fluorophore: PE-CF594. Clone: B-Ly6. Catalogue #: 562393 Lot #: 9336670
 Company: BD. Ab: CD14. Fluorophore: BUV737. Clone: M5E2. Catalogue #: 612763 Lot #: 140604
 Company: Miltenyi. Ab: CD14. Fluorophore: Vioblue. Clone: TUK4. Catalogue #: 130-113-152.
 Company: BD. Ab: CD14. Fluorophore: BV421. Clone: M5E2 Catalogue #: 565283 Lot #: 0.0064424
 Company: BD. Ab: CD141. Fluorophore: BV711. Clone: 1A4. Catalogue #: 563155 Lot #: 9213223
 Company: Miltenyi. Ab: CD141. Fluorophore: APC. Clone: AD5-14h12. Catalogue #: 130-113-314. Lot #: 5190605428
 Company: BD. Ab: CD161(CLEC5b). Fluorophore: APC. Clone: DX12. Catalogue #: 550968 Lot #: 3291608
 Company: Biolegend. Ab: CD169 (Siglec-1). Fluorophore: PE. Clone: 7-239. Catalogue #: 346004 Lot #: B223208
 Company: eBioscience. Ab: CD172a (SIPRa). Fluorophore: PerCP-eFluor710. Clone: 15-414. Catalogue #: 46-1729-42
 Company: Biolegend. Ab: CD172a (SIPRa). Fluorophore: APC/FIRE 750. Clone: SE5A5 Catalogue #: 323818 Lot #: B240662
 Company: BD. Ab: CD184 (CXCR4). Fluorophore: PE. Clone: 12G5. Catalogue #: 555974 Lot #: 5260751
 Company: Miltenyi. Ab: CD195 (CCR5). Fluorophore: PE. Clone: REA245. Catalogue #: 130-117-356. Lot #: 5200108299
 Company: BD. Ab: CD1a. Fluorophore: BV510. Clone: HI149. Catalogue #: 563481 Lot #: 9109913
 Company: BD. Ab: CD1c. Fluorophore: PE. Clone: F10/21A3 Catalogue #: 564900 Lot #: 8243815
 Company: Miltenyi. Ab: CD1c. Fluorophore: PE-Vio770. Clone: AD5-8E7. Catalogue #: 130-113-865. Lot #: 5190626261
 Company: Beckman Coulter. Ab: CD206 (MR). Fluorophore: PE. Clone: 3.29B1.10. Catalogue #: IM2741. Lot #: 15
 Company: BD. Ab: CD206 (MR). Fluorophore: BUV805. Clone: 19.2. Catalogue #: 742042 Lot #: 9154863

Company: Miltenyi. Ab: CD207 (Langerin). Fluorophore: Vioblue. Clone: MB22-9F2. Catalogue #: 130-106-096. Lot #: 5191016216
 Company: Beckman Coulter. Ab: CD207 (Langerin). Fluorophore: PE. Clone: DCGM4. Catalogue #: IM3577.
 Company: BD. Ab: CD209 (DC-SIGN). Fluorophore: APC. Clone: DCN46. Catalogue #: 551545
 Company: R&D. Ab: CD299 (L-SIGN). Fluorophore: PE. Clone: 120604 Catalogue #: FAB162P. Lot #: LDR0311061
 Company: R&D. Ab: CD301 (CLEC10a). Fluorophore: PE. Clone: 744812 Catalogue #: FAB48881P Lot #: ACTQ0112011
 Company: BD. Ab: CD33 (Siglec-3). Fluorophore: APC. Clone: WM53. Catalogue #: 551378 Lot #: 5247669
 Company: R&D. Ab: CD367 (CLEC4a). Fluorophore: PE. Clone: 216110 Catalogue #: FAB1748P. Lot #: LGQ0213041
 Company: R&D. Ab: CD368 (CLEC4d). Fluorophore: PE. Clone: 413512 Catalogue #: FAB2806P. Lot #: ABJQ0113031
 Company: BD. Ab: CD371 (CLEC12a). Fluorophore: AF674. Clone: 50C1. Catalogue #: 562568 Lot #: 7047639
 Company: BD. Ab: CD4. Fluorophore: APC. Clone: RPA-T4. Catalogue #: 555349 Lot #: 3303853
 Company: BD. Ab: CD45. Fluorophore: BV786. Clone: HI30. Catalogue #: 563716 Lot #: 813700
 Company: BD. Ab: CD45. Fluorophore: PE. Clone: HI30 Catalogue #: 555483 Lot #: 7096274
 Company: BD. Ab: CD80. Fluorophore: PE. Clone: L307.4. Catalogue #: 557227
 Company: BD. Ab: CD83. Fluorophore: APC. Clone: HB15e. Catalogue #: 551073 Lot #: 9009957
 Company: BD. Ab: CD86. Fluorophore: APC. Clone: 2331 Catalogue #: 555660 Lot #: 8235916
 Company: eBioscience. Ab: CD91. Fluorophore: eFluor660. Clone: A2MR-a2. Catalogue #: 50-0919-41 Lot #: E24664-101
 Company: R&D. Ab: CLEC14a. Fluorophore: APC. Clone: 743940 Catalogue #: FAB7436A Lot #: ACUQ0112021
 Company: R&D. Ab: CLEC5a. Fluorophore: APC. Clone: 283834 Catalogue #: FAB2384A Lot #: abju0115081
 Company: R&D. Ab: CLEC5C. Fluorophore: APC. Clone: 239127 Catalogue #: FAB1900A-100 Lot #: LSS0313121
 Company: R&D. Ab: CLEC6a (Dectin2). Fluorophore: APC. Clone: 545943 Catalogue #: FAB3114P Lot #: ABFE0213101
 Company: Miltenyi. Ab: CLEC7A (DECTIN1). Fluorophore: PE. Clone: REA515. Catalogue #: 130-122-009 Lot #: 5160406364
 Company: Miltenyi. Ab: CLEC9A (CD370). Fluorophore: PE. Clone: 8F9. Catalogue #: 130-099-615 Lot #: 5141210153
 Company: BD. Ab: DC SIGN. Fluorophore: PE. Clone: DCN46 Catalogue #: 551265 Lot #: 2097966
 Company: Biolegend. Ab: DEC205. Fluorophore: PE. Clone: HD30. Catalogue #: 342204 Lot #: B159801
 Company: Miltenyi. Ab: HLA DR. Fluorophore: PerCP. Clone: AC122. Catalogue #: 130-113-966 Lot #: 5200108204
 Company: BD. Ab: HLA-DR. Fluorophore: BVU395. Clone: G46-6. Catalogue #: 564040 Lot #: 9310
 Company: Miltenyi. Ab: Langerin. Fluorophore: PE-Vio770. Clone: MB22-9F2. Catalogue #: 130-100-586 Lot #: 5191023228
 Company: BD. Ab: Mouse IgG1. Fluorophore: APC. Clone: MOPC-21. Catalogue #: 555751 Lot #: 8081995
 Company: BD. Ab: Mouse IgG1. Fluorophore: PE. Clone: MOPC-21C. Catalogue #: 550617 Lot #: 2160609
 Company: Miltenyi. Ab: Mouse IgG1. Fluorophore: APC. Clone: IS5-21F5. Catalogue #: 130-113-200.
 Company: Beckman Coulter. Ab: Mouse IgG1. Fluorophore: PE. Clone: 679.1Mc7. Catalogue #: IM0670U
 Company: ebioscience. Ab: Mouse IgG1. Fluorophore: eFluor660. Clone: P3.6.2.8.1. Catalogue #: 50-4714-80. Lot #: E12137-1634.
 Company: R&D. Ab: Mouse IgG1. Fluorophore: PE. Clone: 11711 Catalogue #: IC002P. Lot #: LGZ2913031
 Company: BD. Ab: Mouse IgG2a. Fluorophore: PE. Clone: ZERO8894. Catalogue #: 555574 Lot #: 0.008894
 Company: Miltenyi. Ab: Mouse IgG2a. Fluorophore: PE. Clone: 5140124038 Catalogue #: 130-091-835. Lot #: 5140124038
 Company: R&D. Ab: Mouse IgG2a. Fluorophore: APC. Clone: 20102 Catalogue #: IC003A Lot #: LHA1013111
 Company: R&D. Ab: Mouse IgG2b. Fluorophore: APC. Clone: 133303 Catalogue #: IC0041A Lot #: I1011041
 Company: BD. Ab: Mouse IgG2b. Fluorophore: APC. Clone: 27-35 Catalogue #: 555745 Lot #: 36953
 Company: Beckman Coulter. Ab: P24. Fluorophore: RDI. Clone: KC57. Catalogue #: 6604667 Lot #: 7433056
 Company: MedimAb. Ab: P24. Fluorophore: APC. Clone: 28B7. Catalogue #: MM-0289-APC Lot #: 28902029113
 Company: Merck. Ab: SAMHD1. Fluorophore: Unconjugated Clone: I-19-18. Catalogue #: MABF933 Lot #: 3172409
 Company: eBioscience. Ab: SIGLEC1. Fluorophore: PE eFluor610. Clone: 7-239. Catalogue #: 61-1699-42 Lot #: 2105871
 Company: R&D. Ab: Siglec16. Fluorophore: APC. Clone: 706022 Catalogue #: FAB6819A Lot #: ACFR0111061
 Company: R&D. Ab: Siglec9. Fluorophore: APC. Clone: 191240 Catalogue #: FAB1139A-100 Lot #: AACT0214011
 Company: Biolegend. Ab: XCR1. Fluorophore: APC. Clone: S15046E. Catalogue #: 372606 Lot #: B300821.
 Company: R&D. Host species: Goat. Ab: Langerin. Clone: Polyclonal Catalogue #: AF2088. Lot #: KQJD115051.
 Company: abcam. Host species: Rabbit. Ab: CD14. Clone: ERP3652. Catalogue #: ab133503 Lot #: GR211954-4.
 Company: abcam. Host species: Rabbit. Ab: CD11c. Clone: EP1347Y. Catalogue #: ab216655
 Company: Invitrogen. Host species: Donkey. Ab: anti goat. Fluorophore: AF488. Catalogue #: A11055. Lot #: 49732A.
 Company: Invitrogen. Host species: Donkey. Ab: anti rabbit. Fluorophore: DyLight755. Catalogue #: SA5-10043. Lot #: TA2506986.

Validation

All antibodies used were commercially available and validated by corresponding manufacturers. The following companies have general validation/reproducibility statements. BD (<https://wwwbdbiosciences.com/us/go/reproducibility/overview>). Biolegend (<https://www.biolegend.com/en-us/reproducibility>). Beckman Coulter (<https://www.beckman.com.au/reagents/coulter-flow-cytometry/antibodies-and-kits/single-color-antibodies>).

CD103. PE. <https://wwwbdbiosciences.com/us/applications/research/t-cell-immunology/regulatory-t-cells/surface-markers/human/pe-mouse-anti-human-cd103-ber-act8/p/340998>

CD11c. PECF594. <https://wwwbdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/pe-cf594-mouse-anti-human-cd11c-b-ly6/p/562393>

CD14. BVU737. <https://wwwbdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/buv737-mouse-anti-human-cd14-m5e2/p/612763>

CD14. Vioblue. <https://www.miltenyibiotec.com/AU-en/products/cd14-antibody-anti-human-tuk4.html?countryRedirected=1#vioblue:100-tests-in-200-ul>

CD14. BV421. <https://wwwbdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-human->

antibodies/cell-surface-antigens/bv421-mouse-anti-human-cd14-m5e2/p/565283

CD141. BV711. <https://wwwbdbiosciences.com/us/applications/research/stem-cell-research/mesoderm-markers/human/bv711-mouse-anti-human-cd141-1a4/p/563155>

CD141. APC. <https://www.miltenyibiotec.com/AU-en/products/cd141-bdca-3-antibody-anti-human-ad5-14h12.html#gref>

CD161 (CLEC5b). APC. <https://wwwbdbiosciences.com/eu/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/apc-mouse-anti-human-cd161-dx12/p/550968>

CD169 (Siglec-1). PE. <https://www.biolegend.com/en-us/products/pe-anti-human-cd169-sialoadhesin-siglec-1-antibody-6290>

CD172a (SIPRa). PerCP-eFluor710. <https://www.thermofisher.com/antibody/product/CD172a-SIRP-alpha-Antibody-clone-15-414-Monoclonal/46-1729-42>

CD172a (SIPRa). APC/FIRE 750. <https://www.biolegend.com/en-us/products/apc-fire-750-anti-human-cd172ab-sirpalphabeta-antibody-14802>

CD184 (CXCR4). PE. <https://wwwbdbiosciences.com/eu/applications/research/t-cell-immunology/regulatory-t-cells/surface-markers/human/pe-mouse-anti-human-cd184-12g5/p/555974>

CD195 (CCR5). PE. <https://www.miltenyibiotec.com/AU-en/products/cd195-ccr5-antibody-anti-human-reafinity-rea245.html#gref>

CD1a. BV510. <https://wwwbdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/bv510-mouse-anti-human-cd1a-hi149/p/563481>

CD1c. PE. <https://wwwbdbiosciences.com/eu/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/pe-mouse-anti-human-cd1c-f1021a3/p/564900>

CD1c. PE-Vio770. <https://www.miltenyibiotec.com/AU-en/products/cd1c-bdca-1-antibody-anti-human-ad5-8e7.html#gref>

CD206 (MR). PE. <https://www.beckman.com.au/reagents/coulter-flow-cytometry/antibodies-and-kits/single-color-antibodies/cd206/im2741>

CD206 (MR). BUV805. <https://wwwbdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/buv805-mouse-anti-human-cd206-192/p/742042>

CD207 (Langerin). Vioblue. <https://www.miltenyibiotec.com/AU-en/products/cd207-langerin-antibody-anti-human-mb22-9f5.html?countryRedirected=1#gref>

CD207 (Langerin). PE. <https://www.beckman.com.au/reagents/coulter-flow-cytometry/antibodies-and-kits/single-color-antibodies/cd207/im3577>

CD209 (DC-SIGN). APC. <https://wwwbdbiosciences.com/us/applications/research/stem-cell-research/mesoderm-markers/human/apc-mouse-anti-human-cd209-dcn46/p/551545>

CD299 (L-SIGN). PE. https://www.rndsystems.com/products/human-dc-signr-cd299-pe-conjugated-antibody-120604_fab162p

CD301 (CLEC10a). PE. https://www.rndsystems.com/products/human-clec10a-cd301-pe-conjugated-antibody-744812_fab48881p

CD33 (Siglec-3). APC. <https://wwwbdbiosciences.com/eu/applications/research/stem-cell-research/hematopoietic-stem-cell-markers/human/negative-markers/apc-mouse-anti-human-cd33-wm53-also-known-as-wm-53/p/551378>

CD367 (CLEC4a). PE. https://www.rndsystems.com/products/human-dcir-clec4a-pe-conjugated-antibody-216110_fab1748p

CD368 (CLEC4d). PE. https://www.rndsystems.com/products/human-clec4d-clecf8-pe-conjugated-antibody-413512_fab2806p

CD371 (CLEC12a). AF674. <https://wwwbdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/alexa-fluor-647-mouse-anti-human-cd371-clec12a-50c1/p/562568>

CD4. APC. <https://wwwbdbiosciences.com/eu/applications/research/t-cell-immunology/th-1-cells/surface-markers/human/apc-mouse-anti-human-cd4-rpa-t4/p/555349>

CD45. BV786. <https://wwwbdbiosciences.com/us/applications/research/stem-cell-research/cancer-research/human/bv786-mouse-anti-human-cd45-hi30/p/563716>

CD45. PE. <https://wwwbdbiosciences.com/eu/applications/research/stem-cell-research/cancer-research/human/pe-mouse-anti-human-cd45-hi30/p/555483>

CD80. PE. <https://wwwbdbiosciences.com/eu/applications/research/b-cell-research/surface-markers/human/pe-mouse-anti-human->

cd80-l3074-also-known-as-l307/p/557227

CD83. APC. <https://wwwbdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/apc-mouse-anti-human-cd83-hb15e/p/551073>

CD86. APC. <https://wwwbdbiosciences.com/eu/applications/research/t-cell-immunology/regulatory-t-cells/surface-markers/human/apc-mouse-anti-human-cd86-2331-fun-1/p/555660>

CD91. eFluor660. https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&productssubtype=antibody_primary&productId=%2050-0919-41

CLEC14a. APC. https://www.rndsystems.com/products/human-clec14a-apc-conjugated-antibody-743940_fab7436a

CLEC5a. APC. https://www.rndsystems.com/products/human-mdl-1-clec5a-apc-conjugated-antibody-283834_fab2384a

CLEC5C. APC. https://www.rndsystems.com/products/human-nkp80-klrf1-apc-conjugated-antibody-239127_fab1900a

CLEC6a (Dectin2). APC. https://www.rndsystems.com/products/human-dectin-2-clec6a-pe-conjugated-antibody-545943_fab3114p

CLEC7A (DECTIN1). PE. <https://www.miltenyibiotec.com/AU-en/products/dectin-1-antibody-anti-human-reafinity-rea515.html?countryRedirected=1#pe:30-tests-in-60-ul>

CLEC9A (CD370). PE. <https://www.miltenyibiotec.com/AU-en/products/cd370-clec9a-antibody-anti-human-8f9.html#gref>

DC SIGN. PE. <https://wwwbdbiosciences.com/us/applications/research/stem-cell-research/mesoderm-markers/human/pe-mouse-anti-human-cd209-dcn46/p/551265>

DEC205. PE. <https://www.biolegend.com/en-us/products/pe-anti-human-cd205-dec-205-antibody-5901?GroupID=GROUP28>

HLA DR. PerCP. <https://www.miltenyibiotec.com/AU-en/products/hla-dr-antibody-anti-human-ac122.html?countryRedirected=1#gref>

HLA-DR. BUV395. <https://wwwbdbiosciences.com/us/applications/research/stem-cell-research/mesenchymal-stem-cell-markers-bone-marrow/human/negative-markers/buv395-mouse-anti-human-hla-dr-g46-6/p/564040>

Langerin. PE-Vio770. <https://www.miltenyibiotec.com/AU-en/products/cd207-langerin-antibody-anti-human-mb22-9f5.html?countryRedirected=1#pe-vio-770:100-tests-in-1-ml>

Mouse IgG1. APC. <https://wwwbdbiosciences.com/eu/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/apc-mouse-igg1-isotype-control-mopc-21/p/555751>

Mouse IgG1. PE. <https://wwwbdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-mouse-antibodies/cell-surface-antigens/pe-mouse-igg1-isotype-control-mopc-31c/p/550617>

Mouse IgG1. APC. <https://www.miltenyibiotec.com/AU-en/products/isotype-control-antibody-mouse-igg1-is5-21f5.html#apc:30-tests-in-60-ul>

Mouse IgG1. PE. <https://www.beckman.com.au/reagents/coulter-flow-cytometry/antibodies-and-kits/single-color-antibodies/isotype-control/im0670u>

Mouse IgG1. eFluor660. <https://www.fishersci.co.uk/shop/products/igg1-k-mouse-efluor-660-clone-p3-6-2-8-1-isotype-control-ebioscience-2/15321910>

Mouse IgG1. PE. https://www.rndsystems.com/products/mouse-igg-1-pe-conjugated-antibody_ic002p

Mouse IgG2a. PE. <https://wwwbdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/pe-mouse-igg2a-isotype-control-g155-178/p/555574>

Mouse IgG2a. PE. <https://www.citeab.com/antibodies/2090934-130-091-835-mouse-igg2a-pe-monoclonal>

Mouse IgG2a. APC. https://www.rndsystems.com/products/mouse-igg2a-apc-conjugated-antibody_ic003a

Mouse IgG2b. APC. https://www.rndsystems.com/products/mouse-igg2b-apc-conjugated-antibody_ic0041a

Mouse IgG2b. APC. <https://wwwbdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/apc-mouse-igg2b-isotype-control-27-35/p/555745>

P24. RDI. <https://www.beckman.com.au/reagents/coulter-flow-cytometry/antibodies-and-kits/single-color-antibodies/hiv-1-core-antigen/6604667>

P24. APC. <https://medimabs.com/product/hiv-1-p24-human-monoclonal-antibody-28b7-apc-labeled/>

SAMHD1. Unconjugated https://www.merckmillipore.com/AU/en/product/Anti-SAMHD1-Antibody-clone-I19-18,MM_NF-MABF933

SIGLEC1. PE eFluor610. <https://www.fishersci.co.uk/shop/products/cd169-siglec-1-mouse-anti-human-pe-eFluor-610-clone-7-239-ebioscience-2/15580467>

Siglec16. APC. https://www.rndsystems.com/products/human-siglec-16-apc-conjugated-antibody-706022_fab6819a

Siglec9. APC. https://www.rndsystems.com/products/human-siglec-9-apc-conjugated-antibody-191240_fab1139a

XCR1. APC. <https://www.biolegend.com/en-us/search-results/apc-anti-human-xcr1-antibody-14678>

Goat Langerin. https://www.rndsystems.com/products/human-langerin-cd207-antibody_af2088

Rabbit CD14. <https://www.abcam.com/cd14-antibody-epr3652-ab133503.html>

Rabbit CD11c. <https://www.abcam.com/cd11c-antibody-ep1347y-bsa-and-azide-free-ab216655.html>

Donkey anti goat AF488 <https://www.thermofisher.com/antibody/product/Donkey-anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11055>

Donkey anti rabbit DyLight755 <https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/SA5-10043>

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	JLTR - NIH AIDS repository (Cat #11586) TZMBL1 - NIH AIDS repository (Cat # 100)
Authentication	Cell lines are both reporter cell lines which will only report (Fluoresce green or turn blue) when infected with HIV. Furthermore STR profiling was conducted on both cell lines.
Mycoplasma contamination	Mycoplasma testing was regularly performed and cells lines were shown to be mycoplasma free
Commonly misidentified lines (See ICLAC register)	Neither cell line are listed in the most recent ICLAC database of commonly misidentified cell lines.

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics	Healthy human abdominal and genital tissues were obtained within 30 minutes of surgery from patients undergoing plastic surgery (Abdomen, labia and vagina), circumcision (foreskin), gender reassignment (glans penis, fossa navicularis, penile urethra), hysterectomy (cervix) or colorectal surgery (anal canal, perineum, colon, rectum). All patients were aged between 16-71 and gave written consent. All tissues were de-identified.
Recruitment	Human tissue was collected from a range of different surgeons across Sydney whom are named as authors in this study. There were no self-selection biases, any individual who was undergoing relevant surgery was asked at consult if they would like to donate their discarded tissue to the study and then consented to the study. There were no specific requirements for patients to meet for tissue to be included in this study, however macroscopically "diseased" tissue was sent to pathology and therefore omitted from this study.
Ethics oversight	The study was approved by the Western Sydney Local Health District (WSHLD) Human Research Ethics Committee (HREC); reference number (4192) AU RED HREC/15 WMEAD/11.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Cells were isolated from human anogenital and colorectal tissue as well as abdominal skin using enzymatic digestions. For abdominal skin, grafts were taken using a skin graft knife and passed through a skin graft mesher. For anogenital skin and type II mucosal tissue, underlying fat/connective tissue was removed with a scalpel before small scalpel cuts were made to the epithelial surface to mimick that of the skin graft mesher. Tissue were incubated overnight at 4°C with 0.14U/ml dispase in RPMI. Skin/type II mucosa was briefly washed in PBS and epidermis and dermis mechanically seperated using forceps and cut into 1-2mm pieces for digestion in collagenase type IV (200U/ml) with DNase1 (100U/ml) in RPMI at 37°C for two hours. Undigested tissue was removed using a tea strainer and supernatant than passed through a 100µm cell strainer and pelleted three times in PBS. Epidermal suspension underwent Ficoll-Paque enrichment while dermal suspensions were enriched using CD45 MACS bead selection. For type I mucosa no dispase treatment was required, tissue was digested in type IV collagenase over two consecutive 30 minute digestions.

Tissues included in this study were as follows -
 Skin: Abdomen, Labia, Glans, Inner and outer foreskin, Perineum.
 Type II mucosa: Vagina, Fossa navicularis, Anal Canal, Ectocervix
 Type I mucosa: Endocervix, Penile urethra, Rectum, Colon

Instrument

BD fortessa, BD Cantoll (transfer assays only), BD Symphony (Siglec-1 blocking experiment only) BD influx (FACS)

Software

Collected using BD DIVA (V 8.0) and analysed using FlowJo (V10).

Cell population abundance

All sorted populations were purity checked by running post-sorted samples back through the sorter and checking their surface expression. Purity checks were 99% accurate. Cell abundance varied with tissue source and size as well as cell subset from 5000-500000 sorted cells

Gating strategy

All antibodies were titrated on PBMCs and primary abdominal skin cells to determine optimal concentrations for separation of negative and positive cells.

FSC/SSC to gate broadly on immune cells. CD45+ HLADR+ cells were gated, followed by excluding dead cells by Live/Dead viability dye. Doublets were excluded using FSC-A/FSC-H. T cells were excluded as CD3+ cells and B cells excluded as CD19+.

From here populations were gated out as follows:

Macrophages: Autofluorescent+ CD14+
 cDC1: Autofluorescent- CD14- XCR1+
 langerin- cDC2: Autofluorescent- XCR1- CD14- CD1c+ (Mucosal tissue) CD1a+ (skin) Langerin-
 langerin+ cDC2: Autofluorescent- XCR1- CD14- CD1c+ (Mucosal tissue) CD1a+ (skin) Langerin+
 CD14+ MDDC: Autofluorescent- XCR1- CD14+ CD1a- (skin) CD1c+ CD11c+
 CD14+ MDM: Autofluorescent- XCR1- CD14+ CD1a- (skin) CD1c- CD11c-
 HIV+ cells were gated as p24 (KC57) RDI+ p24 (28B7) APC+.

All population markers were on bifurcated markers and clearly defined positive from negative. All CLR/markers of interest were gated using FMOs or isotype controls.

- Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.