Supplemental Information

Unsupervised High-Dimensional Analysis Aligns Dendritic Cells across Tissues and Species

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Figure S1 (related to Figure 1). Mapping the potential macrophage contamination across tissues and identification of mouse pDC

(A) Representative FACS plots showing the expression of CD11c, CD26, XCR1 and CD172a on CD64$^{hi}$F4/80$^{hi}$ macrophages (orange gate in Figure 1) in the spleen, liver, lung, small intestine and large intestine of wild-type mice (flow panel see Table S1). Cells were pre-gated as single live CD45$^{+}$ cells. The grey gates correspond to the gates used to identify cDC in Figure 1. Data are representative of 3 independent experiments. (B) Representative FACS plots showing the identification of pDC as CD11b$^{lo}$120G8$^{hi}$CD172a$^{int}$B220$^{int}$Ly6C$^{int}$CD11c$^{int}$ cells in the lung and the spleen of wild-type mice. (C) Intracellular staining for IRF4 and IRF8. The profiles of cDC1, cDC2, macrophages and pDC are shown. Data are representative of 2 independent experiments.
Figure S2 (related to Figure 3). Comparison of the classical and proposed cDC analysis strategies using spleens from CD11c\textsuperscript{cre} x XBP1\textsuperscript{fl/fl} mice

Cells from spleens of 5 Xbp1\textsuperscript{fl/fl} (cre\textsuperscript{-}) and 4 Itgax\textsuperscript{cre} x Xbp1\textsuperscript{fl/fl} (cre\textsuperscript{+}) were stained with the extracellular mouse panel (Figure 1 - Table S1). (A,B) Representative flow cytometry plots showing identification of cDC1 and cDC2 in cre\textsuperscript{-} and cre\textsuperscript{+} mice via the (A) classical and (B) proposed manual gating strategies. Numbers represent proportion of each cDC populations as a % of total cDC. (C) Histograms showing CD11c expression by cre\textsuperscript{-} (shaded grey) and cre\textsuperscript{+} (black line) cDC1 and cDC2. (D) Single live CD45\textsuperscript{+} cells for each sample were exported and concatenated. FlowSOM was then used for the automated identification of the cDC1 and cDC2 nodes in all samples (see Figure 3). Proportion of cDC1 and cDC2 in cre\textsuperscript{-} and cre\textsuperscript{+} as identified using the classical manual gating strategy, the proposed manual gating strategy or FlowSOM, *p<0.05, **p<0.01, ***p<0.001. One Way ANOVA with Bonferroni post-test. The FlowSOM algorithm was run 5 times to ensure reproducibility of the results. (E) Representative concatenated minimal spanning tree showing identification of cDC1 (blue) and cDC2 (green) by FlowSOM. Histograms show CD11c expression by cDC1 and cDC2 in CRE\textsuperscript{-} (shaded grey) and cre\textsuperscript{+} (black line) mice as identified by FlowsSOM. (F) Identification of cDC1 and cDC2 amongst concatenated cre\textsuperscript{-} and cre\textsuperscript{+} or cre\textsuperscript{-} or cre\textsuperscript{+} live CD45\textsuperscript{hi}Lin\textsuperscript{lo}F4/80\textsuperscript{lo} cells by tSNE analysis. Expression level heat maps of selected markers in concatenated tSNE plots, allowing the identification of clusters of cells (circled) displaying the phenotype of cDC1 (blue) and cDC2 (green).
A Mouse Spleen

B Human Spleen

C Macaque Spleen

D Blood Lung

E Human Macaque

Counts CD64 CD64
**Figure S3 (related to Figure 4). Gating strategy to define cDC1 and cDC2 in mouse, human and macaque spleen**

(A) Gating strategy for the identification of mouse CADM1^{hi}XCR1^{hi}CD172a^{lo} cDC1 (blue gate) and CADM1^{lo}XCR1^{lo}CD172a^{hi} cDC2 (green gate). (B) Gating strategy for the identification of human CADM1^{hi}XCR1^{hi}CD172a^{lo} cDC1 (blue gate), CADM1^{lo}XCR1^{lo}CD1c^{hi}CD172a^{hi} cDC2 (green gate) and CD123^{hi} pDC (pink gate). XCR1 staining was performed using mcherry-conjugated XCL1-vaccibodies. Histograms showing the expression of CADM1, CD26, CD11c and IRF8 by XCR1^{hi}CD172a^{lo} cDC1 and XCR1^{lo}CD1c^{hi}CD172a^{hi} cDC2 are shown. (C) Gating strategy for the identification of macaque CADM1^{hi}XCR1^{hi}CD172a^{lo} cDC1 (blue gate), CADM1^{lo}XCR1^{lo}CD1c^{hi}CD172a^{hi} cDC2 (green gate) and CD123^{hi} pDC (pink gate). XCR1 staining was performed using mcherry-conjugated XCL1-vaccibodies. Histograms showing the expression of CADM1, CD26, CD11c and IRF8 by XCR1^{hi}CD172a^{lo} cDC1 and XCR1^{lo}CD1c^{hi}CD172a^{hi} cDC2 are shown. (D) Overlaid contour plots showing IRF4 and IRF8 expression by pDC (pink), cDC1 (blue) and cDC2 (green) in human (upper plots) and macaque (lower plots) blood (left plots) and lung (right plots). (E) Histograms displaying the expression of CD64 by human (left plot) or macaque (right plot) cDC1 (blue), cDC2 (green) and monocyte/macrophages (orange) in the spleen. Data are representative of at least three independent experiments using cells from different individuals.
A. Human Lung from Figure 5
Live CD45+Lin−CD123−

B. Human Lung from Figure 7

C. Human Lung from Figure 5
Live CD45+Lin−HLA-DR−

D. Human Skin

E. Macaque Dermis

F. Macaque Epidermis
Figure S4 (related to Figure 4). Lung CD172a<sup>hi</sup>CD1c<sup>lo</sup> cells are not cDC2 and identification of cDC1 and cDC2 in human and macaque skin

(A) Gating strategy for the identification of human CD14<sup>hi</sup>CD16<sup>lo</sup> monocytes/macrophages (CD14<sup>+</sup>, orange gate), CD14<sup>int</sup>CD16<sup>hi</sup> monocytes/macrophages (CD16<sup>*</sup>, brown gate), CADM1<sup>hi</sup>CD172a<sup>lo</sup> cDC1 (blue gate), CD172a<sup>hi</sup>CD11c<sup>hi</sup>CD1c<sup>hi</sup> cDC2 (green gate) and CD172a<sup>hi</sup>CD11c<sup>hi</sup>CD1c<sup>lo</sup> cells (red gate). (B) IRF4 and IRF8 expression of the cell subsets defined in panel A. (C) Manual gating of human lung cells starting from single live CD45<sup>+</sup>Lineage<sup>-</sup>HLADR<sup+</sup> cells. In the CD14/CD16 plot, CD14<sup>hi</sup>CD16<sup>lo</sup> monocyte/macrophages (gate 1), CD14<sup>hi</sup>CD16<sup>hi</sup> monocyte/macrophages (gate 2), CD14<sup>lo</sup>CD16<sup>hi</sup> monocyte/macrophages (gate 3) are defined. The remaining CD14<sup>lo</sup>CD16<sup>lo</sup> cells (gate 4) are next analyzed for HLADR/CD123 expression to define Lineage<sup>-</sup>HLADR<sup+</sup>CD123<sup+</sup> pDC (gate 6). Remaining HLADR<sup<hi>hi</sup>CD123<sup<lo>lo</sup> cells (gate 5) are next analyzed for CD172a/CADM1 expression to define CADM1<sup>hi</sup>CD172a<sup>lo</sup> cDC1 (gate 8) and remaining CADM1<sup>lo</sup>CD172a<sup>hi</sup> cells are analyzed for CD11c/CD1c to define CD172a<sup>hi</sup>CD11c<sup>hi</sup>CD1c<sup>lo</sup> cells (gate 9) and CD172a<sup>hi</sup>CD11c<sup>hi</sup>CD1c<sup>hi</sup> cDC2 (gate 10). (D) tSNE dot plot of all the individual gates (gate 1 to gate 10) defined in panel A. Regions in the tSNE plots corresponding to cDC1 (blue region), cDC2 (green region), pDC (pink region) and CD14<sup>lo</sup>CD16<sup+</sup> monocyte/macrophages (orange region) are shown in each tSNE plot. (E) Gating strategy for the identification of CD26<sup>hi</sup>CADM1<sup>hi</sup>CD172a<sup>lo</sup> cDC1 (blue gate) and CADM1<sup>lo</sup>CD172a<sup>hi</sup>CD1c<sup>hi</sup> cDC2 (green gate) among migrating cells collected from human skin. (F) Gating strategy for the identification of CADM1<sup>hi</sup>CD172a<sup>lo</sup> cDC1 (blue gate) and CADM1<sup>lo</sup>CD172a<sup>hi</sup>CD1c<sup>hi</sup> cDC2 (green gate) among cells obtained from digested macaque dermis (upper panels) and epidermis (lower panels). In both human and macaque skin, Langerhans cells (LC, purple gate) were excluded based on their CD1a<sup>hi</sup>CD11c<sup>lo</sup> profile and cDC1 and cDC2 were confirmed as being IRF8<sup>hi</sup>IRF4<sup>lo</sup> and IRF8<sup>lo</sup>IRF4<sup>hi</sup>, respectively. Data are representative of at least three independent experiments using cells from different individuals.
Figure S5 (related to Figure 6). Classical contour plots and histograms of mouse and human CyTOF data

(A,B) Classical 2D contour plots of selected markers for (A) the mouse and (B) the human concatenated (all different organs depicted in Figure 6) CyTOF data of overlaid pDC (pink), cDC1 (blue) and cDC2 (green) are shown. (C-H) Classical histograms of markers selected for their differential expression in (C-E) mouse spleen (green histograms), lung (red histograms) and gut (blue histograms), or (F-H) human blood (PBMC, orange histograms), spleen (green histograms), lung (red histograms) and gut (blue histograms) are shown for (C, F) cDC1, (D, G) cDC2 and (E, H) pDC. (I) Classical 2D contour plots showing the expression of CD5 and CD2 by human pDC (One-SENSE clusters 5 and 6 from Figure 6D pooled) detected in the blood (PBMC, orange), spleen (green), lung (red) and gut (blue) allowed to define a subset of CD5+CD2+ pDC (upper panels). In the lower panels, the expression of CD11c and CD39 by CD5+CD2+ pDC defined in the upper CD5/CD2 contour plots (brown in the blood, dark green in the spleen, purple in the lung and dark blue in the gut) is shown as compared to the remaining pDC.
Figure S6 (related to Figure 7). CD11c/MHCII expression profile determined by CyTOF of Lymph node and Lung Lin-CD45+ cells at various timepoints following intranasal LPS administration

(A) The gate used to export cells analyzed by One-SENS in Figure 9 is shown in each contour plot. (B) Classical dot plots showing the expression of the lineage markers for cDC1 (CD26 & CADM1), cDC2 (CD172a & CD11b) and monocyte-derived cells (F4/80 & CD64) by cells from the different One-SENSE clusters (defined in Figure 8) in the lung (left panels) and in the draining lymph node (right panels).
Table S1. Antibodies and secondary reagents used for the extracellular mouse flow cytometry panel. Related to Figure 1.

| Laser | Antibody | Clone | Fluorochrome | Company | Catalogue # | Dilution | Step | Time (mins) |
|-------|----------|-------|--------------|---------|-------------|----------|------|-------------|
| n/a   | CD16/32 (Fc Block) | 2.4g2 | - | Bioceros BV | - | 1/400 | 1 | 45 |
| F4/80 | BM8     | Biotin | FITC | eBioscience | 13-4801-85 | 1/200 | 1 | 45 |
| 488nm | CD26    | M194-112 | FITC | BD | 559652 | 1/100 | 1 | 45 |
|       | P84     | PerCP-eFluor710 | eBioscience | 46-1721-82 | 1/200 | 2 | 30 |
| 405nm | Fixable Live/Dead | - | eFluor560 | eBioscience | 65-0866-18 | 1/500 | 1 | 45 |
|       | M1/69   | eFluor450 | eBioscience | 48-0242-82 | 1/500 | 2 | 30 |
|       | Streptavidin | - | QD605 | Invitrogen | Q10101MP | 1/400 | 2 | 30 |

561nm | XCR1 | ZET | PE | Biologend | 148204 | 1/400 | 2 | 30 |
|       | CD3   | 145-2C11 | PE-Cy5 | Tonbo Biosciences | TON855-0031-U100 | 1/300 | 2 | 30 |
|       | CD19  | 1D3 | PE-Cy5 | eBioscience | 15-0193-83 | 1/300 | 2 | 30 |
|       | CD45R (B220) | RA3-6B2 | PE-Cy5 | BD | 553091 | 1/300 | 2 | 30 |
|       | CD161 (NK1.1) | PK136 | PE-Cy5 | Biologend | 108716 | 1/300 | 2 | 30 |
|       | CD11b | M1/70 | PE-Cy7 | BD | 552850 | 1/500 | 2 | 30 |
|       | CD11c | N418 | PE-eFluor610 | eBioscience | 61-0114-82 | 1/400 | 2 | 30 |
| 633nm | CD44 | X54-5/7.1 | AF647 | eBioscience | 559529 | 1/200 | 4 | 30 |
|       | CD45 | 30-F11 | AF700 | eBioscience | 56-0451-82 | 1/200 | 2 | 30 |
|       | MHCIi | M5/114.15.2 | APC-eFluor780 | eBioscience | 47-5321-82 | 1/500 | 2 | 30 |

Table S2. Antibodies and secondary reagents used for the intracellular mouse flow cytometry panel. Related to Figure 1.

| Laser | Antibody | Clone | Fluorochrome | Company | Catalogue # | Dilution | Step | Time (mins) |
|-------|----------|-------|--------------|---------|-------------|----------|------|-------------|
| n/a   | CD16/32 (Fc Block) | 2.4g2 | Unconjugated | Bioceros BV | - | 1/400 | 1 | 45 |
| F4/80 | BM8     | Biotin | FITC | eBioscience | 13-4801-85 | 1/200 | 1 | 45 |
| 488nm | IRF4    | M17   | Unconjugated | Santa Cruz Biotech | sc-6059 | 1/400 | 3 | 45 |
| 405nm | Fixable Live/Dead | - | eFluor560 | eBioscience | 65-0866-18 | 1/500 | 1 | 45 |
|       | CD24    | M1/69 | eFluor450 | eBioscience | 48-0242-82 | 1/500 | 2 | 30 |
|       | Streptavidin | - | QD605 | Invitrogen | Q10101MP | 1/400 | 2 | 30 |
|       | CD64    | X54-5/7.1 | AF647 | eBioscience | 559529 | 1/200 | 4 | 30 |
| 561nm | XCR1 | ZET | PE | Biologend | 148204 | 1/400 | 2 | 30 |
|       | CD3    | 145-2C11 | PE-Cy5 | Tonbo Biosciences | TON855-0031-U100 | 1/300 | 2 | 30 |
|       | CD19   | 1D3 | PE-Cy5 | eBioscience | 15-0193-83 | 1/300 | 2 | 30 |
|       | CD45R (B220) | RA3-6B2 | PE-Cy5 | BD | 553091 | 1/300 | 2 | 30 |
|       | CD161 (NK1.1) | PK136 | PE-Cy5 | Biologend | 108716 | 1/300 | 2 | 30 |
|       | CD11b | M1/70 | PE-Cy7 | BD | 552850 | 1/500 | 2 | 30 |
|       | CD11c | N418 | PE-eFluor610 | eBioscience | 61-0114-82 | 1/400 | 2 | 30 |
| 633nm | Anti-Goat IgG | A2047 | AF647 | Invitrogen | A-21447 | 1/1000 | 4 | 30 |
|       | CD45 | 30-F11 | AF700 | eBioscience | 56-0451-82 | 1/200 | 2 | 30 |
|       | MHCIi | M5/114.15.2 | APC-eFluor780 | eBioscience | 47-5321-82 | 1/500 | 2 | 30 |
Table S3. Antibodies and secondary reagents used in human and macaque flow cytometry experiments. Related to Figure 4.

| Target                        | Conjugate         | Isotype          | Clone   | Dilution | Provider               |
|-------------------------------|-------------------|------------------|---------|----------|------------------------|
| **primary antibodies**        |                   |                  |         |          |                        |
| CADM1                         | Purified          | chicken IgY      | 3E1     | 1/400    | MBL                    |
| CD1a                          | Biotin            | mouse IgG2a      | NA1/34-HLK | 1/12.5   | AbD Serotec            |
| CD1a                          | AF700             | mouse IgG2a      | NA1/34-HLK | 1/20     | Novus Bio              |
| CD1c                          | PE/Cy7            | mouse IgG1       | L161    | 1/200    | Biolegend              |
| CD2                           | BV421             | mouse IgG1       | L161    | 1/20     | Biolegend              |
| CD11b                         | BV711             | rat IgG2b        | M1/70   | 1/80     | BD Biosciences         |
| CD11c                         | BV605             | mouse IgG1       | 3.9     | 1/20     | Biolegend              |
| CD14                          | BV711             | mouse IgG2a      | M5E2    | 1/20     | Biolegend              |
| CD14                          | ECD               | mouse IgG2a      | RMO52   | 1/10     | Beckman Coulter        |
| CD16                          | APC/Cy7           | mouse IgG1       | 3G8     | 1/40     | Biolegend              |
| CD19                          | BV650             | mouse IgG1       | SJ25C1  | 1/20     | BD Biosciences         |
| CD20                          | BV650             | mouse IgG2b      | 2H7     | 1/20     | BD Biosciences         |
| CD26                          | PE/Cy7            | mouse IgG2a      | BA5b    | 1/20     | Biolegend              |
| CD26                          | PE                | mouse IgG2a      | BA5b    | 1/20     | Biolegend              |
| CD45                          | V500              | mouse IgG1       | HI30    | 1/20     | BD Biosciences         |
| CD45 (non-human primate)      | V500              | mouse IgG1       | D058-1283 | 1/25    | BD Biosciences         |
| CD64                          | AF700             | mouse IgG1       | 10.1    | 1/40     | BD Biosciences         |
| CD123                         | BUV395            | mouse IgG2a      | 7G3     | 1/40     | BD Biosciences         |
| CD172a                        | Purified          | mouse IgG1       | DH59B   | 1/400    | KingFischer Biotech Inc |
| IRF4                          | PE                | rat IgG1         | 3E4     | 1/1000   | eBioscience            |
| IRF8                          | PercP/eFluor710   | mouse IgG1       | V3GYWCH | 1/1000   | eBioscience            |
| MHCII (HLADR)                 | BV785             | mouse IgG2a      | L243    | 1/20     | Biolegend              |
| **secondary reagents**        |                   |                  |         |          |                        |
| Live/Dead Fixable             | Blue dye          | N/A              | N/A     | 1/1000   | Life Technologies      |
| Streptavidin                  | BUV737            | N/A              | N/A     | 1/100    | BD Biosciences         |
| anti-Chicken IgY              | AF647             | Donkey Fab'2     | N/A     | 1/200    | Jackson Immunoresearch |
| Mouse IgG1 Zenon labelling kit| AF488             | Fab              | N/A     | 5µL/µg mAb | Life Technologies     |
Table S4. Human and macaque flow cytometry panels. Related to Figure 4.

| Laser | 355nm | 355nm | 355nm | 405nm | 405nm | 405nm | 405nm | 488nm | 488nm | 561nm | 561nm | 561nm | 640nm | 640nm | 640nm |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Detector | A | C | C | A | B | C | D | E | F | A | B | A | B | C | A | B | C | A | B | C |
| BP Filter | 740/35 | 375/2 | 8 | 45050 | 780/60 | 710/50 | 670/30 | 610/20 | 525 | 50 | 450 | 50 | 685/35 | 525 | 50 | 780/60 | 670/30 | 610 | 20 | 585/15 | 780/60 | 730/45 | 670/14 |
| Fluorochrome | BU737 | BU737 | BU737 | Blue Dye | BV711 | BV650 | BV605 | V500 | BV421 | PE/C | PE/C | PE/C | PE/C | PE/C | PE/C | AF488 | AF488 | AF488 | AF488 |
| Panel used in Figures 5, S2 and S4 | XCL1-VB1a | mCherry-biotin | streptavidin | BU737 | CD123 | Live/Dead | HLA DR | CD1 | 4 | CD3 | CD19 | CD19 | CD19 | CD19 | CD19 | CD19 | CD19 | CD19 | CD19 |
| Panel used for tSNE analyses in Figure 7(E-L) | XCL1-VB1a | mCherry-biotin | streptavidin | BU737 | CD123 | Live/Dead | HLA DR | CD1 | 4 | CD3 | CD19 | CD19 | CD19 | CD19 | CD19 | CD19 | CD19 | CD19 | CD19 |

Table S5. Antibodies used in mouse CyTOF experiments. Related to Figure 6.

| Antibody | Clone | Provider |
|----------|-------|----------|
| CD19 | 6D5 | Life Technologies |
| CD45 | 30-F11 | Biolegend |
| MCHII | Y3P | Biolegend |
| B220 | RA3.341/61 | Biolegend |
| CD11A | FD441.8 | Biolegend |
| LY6A/E (SCA1) | D7 | Biolegend |
| CD8A | S3-6.7 | Biolegend |
| LY6G | IA8 | BD Biosciences |
| LY6C | HK1.4 | BD Biosciences |
| CD226 | 10E5 | eBioscience |
| SIGLEC F | E50-2440 | BD Biosciences |
| CD62L | MEL14 | Biolegend |
| CD49B | DX5 | Biolegend |
| CD11B | M170 | Biolegend |
| CD3 | 145.C211 | Biolegend |
| CD26 | DPP4 | Biolegend |
| BST2 | 120G8 | AbD Serotec |
| CADM1 (biotin) | 3E1 | MBL |
| TER119 | TER119 | Biolegend |
| F4/80 | C1:A3-1 | SEROTEC |
| FcER1 | 1-MAR | eBioscience |
| CD43 | S11 | Biolegend |
| CD326 (EPCAM) | G8.8 | eBioscience |
| CD11C | N418 | Biolegend |
| CD103 | 2E7 | Biolegend |
| CD64 | X54-5/7.1 | Biolegend |
| CD48 | HM48-1 | Biolegend |
| CD34 | RAM34 | BD Biosciences |
| BTLA (CD272) | 6G3 | eBioscience |
| CD115 | AF598 | Biolegend |
| CD40 | 3/23 | Biolegend |
| SIGLECH | 551 | Biolegend |
| CLEC9A (PE) | PE001 | Biolegend |
| CD172 | PB4 | BD Biosciences |
| CD24 | M169 | Biolegend |
| ESAM | 1G8 | eBioscience |
| CD90 | T24/31 | Bixocell |
Table S6. Antibodies used in human CyTOF experiments. Related to Figure 6.

| Target     | Clone  | Company          |
|------------|--------|------------------|
| CD14       | TUK4   | Life Technologies|
| CD45       | HI30   | FLUIDIGM         |
| CD15       | HI98   | Biolegend        |
| CD57       | HCD57  | Biolegend        |
| CD7        | 6B7    | Biolegend        |
| CD5        | UCHT2  | Biolegend        |
| CD62L      | DREG-56| BD Biosciences   |
| CD66ACE    | ASL-32 | Biolegend        |
| CX3CR1     | K0124E1| Biolegend        |
| CD20       | 2H7    | Biolegend        |
| CD86       | IT2.2  | BD Biosciences   |
| CLA        | HECA-452| Biolegend       |
| HLADR      | L3243  | Biolegend        |
| CD80       | L307.4 | BD Biosciences   |
| CADM1 (biotin) | 30   | MBL              |
| CD1c       | L161   | Biolegend        |
| FcεR1      | AER-37 (CRA1) | eBioscience |
| CD3        | UCHT1  | Biolegend        |
| CD123      | 6H6    | BD Biosciences   |
| CD19       | HIB19  | Biolegend        |
| CXCR3      | 49801  | R&D              |
| CD56       | NCAM16.2| BD Biosciences  |
| CD226      | 11A8   | Biolegend        |
| CLEC9A     | 8F9    | Biolegend        |
| CD172a     | SESA5  | Biolegend        |
| CD49d      | 9F10   | Biolegend        |
| CD1a       | HI49   | BD Biosciences   |
| CD141      | 1A4    | BD Biosciences   |
| CD303      | 201A   | Biolegend        |
| CD71       | CY1G4  | Biolegend        |
| CD103      | B-LY7  | eBioscience      |
| CCR7       | 150503 | R&D              |
| CD25       | M-A251 | Biolegend        |
| BLTA (PE)  | MIH26  | Biolegend        |
| CD34       | 581    | Biolegend        |
| CD2        | RPA-2.1| Biolegend        |
| CD39       | A1     | Biolegend        |
| CD26       | BA5B   | Biolegend        |
| CD11c      | B-LY6  | Biolegend        |
| CD11b      | ICRF44 | Biolegend        |
| CD16       | 3G8    | Fluidigm         |
Table S7. Overview of the markers used for the identification of cDC1 and cDC2 across tissues and species. Related to Figure 5.

|       | cDC1 Mouse | cDC1 Human | cDC2 Mouse | cDC2 Human | cDC2 Macaque |
|-------|------------|------------|------------|------------|--------------|
| XCR1  | hi         | hi         | —          | —          | —            |
| CADM1 | hi         | hi         | hi         | lo         | lo           | —/lo         |
| CD24  | hi         | /          | /          | lo/int/hi# | /            | /            |
| CLEC9A| hi         | hi         | hi         | —          | —            | —            |
| CD172a| —          | —          | —          | hi         | hi           | hi           |
| CD1c  | /          | —/lo       | —/lo       | /          | hi           | hi           |
| CD11b | lo         | —          | —          | hi*        | lo/int       | lo/int       |
| CD26  | hi         | hi         | 4****      | hi         | int          | —****        |
| CD11c | hi         | int        | lo/int     | hi*        | hi           | int/hi       |
| CD64  | lo         | int        | int        | lo**       | int          | int          |
| F4/80 | lo         | /          | /          | lo/int/hi**| /            | /            |
| CD14  | /          | —          | —          | —/lo       | —/lo         | /            |
| CD16  | /          | —          | —          | —          | —            | —            |
| MHCII/HLA-DR | hi | int/hi | int/hi | hi | hi | hi |
| IRF8  | hi         | hi         | hi         | lo         | lo           | lo           |
| IRF4  | lo         | lo         | lo         | hi         | hi           | hi           |

— = not expressed; lo, int, hi = low, intermediate and high expression
/ = Not determined
* = Part of the Dermal cDC2 express low levels of CD11c and CD11b (Figure 2)
** = Part of the Kidney cDC2 express high levels of CD64 (Figure 2)
*** = Dermal cDC2 express high levels of F4/80 levels (Figure 2)
*** = Lung and gut cDC2 express intermediate levels of F4/80 (Figure 3E)
**** = at the mRNA level
# = Dermal cDC2 are CD24-low (Figure 2), most but not all lung and gut cDC2 are CD24-high
Supplemental Experimental Procedures

Mice

All animals were housed under specific pathogen free conditions in individually ventilated cages in a controlled day-night cycle and given food and water ad libitum.

Generation of BM chimeras

7-8 week-old B6 CD45.1 x CD45.2 mice were lethally irradiated with two doses of 550 rads, 5 h apart, and then injected i.v. with $2 \times 10^6$ BM cells. BM cells were obtained from femurs and tibias of wild-type B6 CD45.1 or of $\text{Irf}4^{+/−}$ CD45.2 mice or $\text{Batf}3^{+/−}$ CD45.2 mice and mixed at a 50:50 ratio between CD45.1 and CD45.2 BM cells.

Intranasal LPS treatment

C57BL/6 mice were treated or not intranasally with 5µg of LPS (Invivogen, in 20µl PBS) and were euthanized at day 1 (D1), day 2 (D2) or day 3 (D3) after LPS administration (2 mice per group). 8 hours before euthanasia, mice were injected intraperitoneally with 0.25mg of BrefeldinA (sigma) in 200µl PBS.

Mouse tissues

Unless indicated differently, mice were perfused with PBS prior to dissection and organs were isolated, minced, digested at 37°C, and passed through a 100-µm cell strainer followed by lysis of erythrocytes. Lungs, spleens, kidneys and lymph nodes were cut into small pieces and digested for 30 min in RPMI-1640 (Gibco) containing 20 µg/ml Liberase TM (Roche) and 10 U/ml DNAse I (Roche). Following digestion kidney cell suspensions were subjected to a 100:40 percoll gradient, to isolate leukocytes. Livers were perfused with PBS prior to dissection. Livers were cut roughly into pieces and subsequently subjected to gentleMACS disaggregation according to the manufacturers’ protocol. After 20 min digestion in RPMI containing 1 mg/ml collagenase A (Roche) and 10 U/ml DNAse I, a second gentleMACS dissociation was performed. Intestines were soaked in PBS, opened
longitudinally and cut into 0.5 cm sections. Lamina propria cells were obtained by enzymatic
digestion as previously described (Bain et al., 2012; Scott et al., 2015). In short, after
removal of the epithelial layer, the remaining colonic tissue was digested in RPMI containing
0.425 mg/ml Collagenase V (Sigma), 0.625 mg Collagenase D (Roche), 1 mg/ml Dispase
(Gibco) and 10 U/ml DNAse I, while the remaining SI tissue was digested in RPMI
containing 0.6mg/ml Collagenase VIII and 10U/ml DNAse I. Mouse skin was processed as
described before (Tamoutounour et al., 2013). Ears were split in two parts (dorsal and
ventral). The skin was incubated overnight in PBS containing 2.5 mg/ml Dispass II (Roche)
and then cut into small pieces and incubated for 1 h at 37°C with a solution of RPMI
containing 1 mg/ml DNase and 1 mg/ml Collagenase type IV (Worthington Biochemical
Corporation) to obtain homogeneous cell suspension. Organs used for the CyTOF anlaysis
were digested for 30 min in Hank's balanced salt solution HBSS containing 10% FBS and
Collagenase type IV (0.2 mg/ml; working activity of 770 U/mg) (Sigma Aldrich). Before
staining, cell suspensions underwent pre-enrichment for CD45+ cells with anti-CD45
microbeads (Miltenyi) and were separated on an AutoMacs following manufacturer's
instructions (Miltenyi).

**Flow Cytometry stainings**

For the mouse tissues, Intracellular staining of IRF4 and IRF8 was done after fixing and
permeabilizing of the cells with the FOXP3 Transcription Factor Staining Buffer Set
eBioscience). Cells (5.10^6 cells/tube) were thawed, washed and incubated with Live/Dead
blue dye (30 min, 4°C; Invitrogen/Life Technologies) in PBS. Then, 5% heat-inactivated fetal
calf serum (FCS) was added (15 min, 4°C; Sigma Aldrich). Cells were labeled with
antibodies and mcherry-conjugated XCL1 vaccibodies (PBS-2% FCS and 2 mM EDTA, 30
min, 4°C), then washed and stained with secondary reagents. Cells were then stained
intracellularly for IRF8 and/or IRF4 using the eBioscience FOXP3 / Transcription Factor
Staining Buffer Set (eBioscience/Affimetrrix) following manufacturer's instructions. The
CD172a purified mAb was conjugated to Zenon-AlexaFluo488 immediately prior to use
following manufacturer's instructions (Invitrogen/Life Technologies). We also used bivalent
human XCL1-mCherry vaccibodies that bind human XCR1 (50 mg/ml; produced and
Mass cytometry staining, barcoding, acquisition and data pre-processing

For some markers, fluorophore or biotin conjugated antibodies were used as primary antibodies followed by secondary staining with anti-fluorophore metal-conjugated antibodies (anti-PE, Clone PE001, Biolegend, 5µg/mL) or metal conjugated streptavidin produced as previous described. Cells were plated and stained in a U-bottom 96 well plate (BD Falcon, Cat# 3077). First, cells were washed once with 200µL FACS buffer (4% FBS, 2mM EDTA, 0.05% Azide in 1X PBS) and then stained with 100µL of 200µM cisplatin (Sigma-Aldrich) for 5 min on ice to exclude dead cells. Cells were then stained with anti-CADM1 and anti-BTLA-APC primary antibodies in 50µL reaction volume for 30 min on ice. Cells were washed twice with FACS buffer and stained with 50µL heavy-metal isotope labeled secondary mAb cocktail for 30 min on ice. Cells were washed twice with FACS then once with PBS before fixing with 200µL 2% PFA (Electron Microscopy Sciences) in PBS overnight or longer. Following fix, cells were pelleted and resuspended in 200µL 1X perm buffer (Biolegend) and allowed to stand for 5 mins at room temperature. Cells were washed once with perm buffer and then PBS before barcoding. Bromoacetamidobenzyl-EDTA (BABE)-linked metal barcodes was prepared by dissolving BABE (Dojindo) in 100mM HEPES buffer (Gibco) to a final concentration of 2mM. Then isotopically purified PdCl$_2$ (Trace Sciences Inc.) was added to BABE solution to 0.5mM. Similarly, DOTA-maleimide (DM)-linked metal barcodes was prepared by dissolving DM (Macrocyclics) in L buffer (MAXPAR) to a final concentration of 1mM. Then, 50mM of RhCl$_3$ (Sigma) was added to DM solution to 0.5mM. Six metal barcodes were used: DM-Rh-103, BABE-Pd-104, BABE-Pd-105, BABE-Pd-106, BABE-Pd-108 and BABE-Pd-110. All BABE and DM-metal solution mixtures were immediately snap-frozen in liquid nitrogen and stored at -80°C. A unique dual combination of barcodes was chosen to stain each tissue sample. Barcodes Rh-103 was used at 1:300 dilution, Pd-104 at 1:2000, Pd-105 at 1:200, Pd-106 and Pd-108 at 1:1000, Pd-110 at 1:500. Cells were stained in 100µL barcodes in PBS for 30 min on ice. Cells were then washed in...
perm buffer and incubated in FACS buffer for 10 min on ice. Cells were then pelleted and
resuspended in 100µL of nucleic acid Ir-Intercalator (MAXPAR) in 2% PFA/PBS (1:2000), at
room temperature. After 20 min, cells were washed twice with FACS and twice with water
before final resuspension in water. In each set, cells were pooled from all tissue types,
enumerated, and diluted to a final concentration of 0.5x10^6 cells/mL for acquisition. One-
dimensional Soli-Expression by Nonlinear Stochastic Embedding (One-SENSE) analysis
was performed as recently described (Cheng et al., 2015). Briefly, after applying the Logicle
transform to mass cytometry data extracted from fcs files generated using CyTOF-TM
software (Becher et al., 2014), groups of parameters were selected (see figure legends) and
each run separately using t-distributed Stochastic Neighbor Imbedding (tSNE) (Amir el et
al., 2013; van der Maaten and Hinton, 2008). The Barnes-Hut implementation of tSNE
provided as part of the “Rtsne” R package was used and output into a single dimension was
selected. In this manner, each category of parameters was mapped into a single dimension
that was saved as additional parameter in fcs file format for subsequent analysis using
FlowJo software. FlowJo was used to construct two-dimensional dot or contour plots
showing the relationships between the newly created one-dimensional tSNE parameters.
Heat maps summarizing the meanings of each of these axes were created using R by
segregating each axis into 250 bins. Within each bin, the frequencies of cells with marker
expression above a manually determined threshold were plotted using a black to red color
scale as shown. These heat maps were aligned and plotted in parallel to each axis of the
two-dimensional One-SENSE dot plots.

**FlowSOM based unsupervised analysis and automated cell type detection**
The automated analysis is done by the FlowSOM algorithm (Van Gassen et al., 2015). The
data is manually gated on single live CD45^+ cells. For the IRF8 experiment, a maximum of 1
million cells per group is selected, in the other datasets all gated cells were used. The cells
are subsequently assigned to a Self-Organizing Map with a 7x7 grid. This groups similar
cells, resulting in 49 clusters or "nodes". Once the data is mapped onto the grid, a Minimal
Spanning Tree is build to visualize similar nodes in branches. To automate the identification
of specific cell types in the FlowSOM tree, as opposed to the unlabeled clusters, a cell
profile is established where selected markers get the label "high" or "low". In the current form of the algorithm, it is important to only include markers for which the expression is defined as high or low and to avoid any markers with a noisy or intermediate expression. Each node in the FlowSOM tree gets a score indicating its correspondence with this requested cell profile. This score is computed as the mean of its scores for each individual marker, which are each normalized between zero and one. The score for a marker is the difference between the median value of the node for that marker and the minimum or maximum (for resp. the low or high label) median node value that is present in the tree. All nodes with a final score of at least 0.95 times the highest score are selected as fitting the requested profile, where we work under the assumption that the cell type is indeed present in the analyzed sample. Once the nodes containing the cell types of interest are identified, we compute the percentage of selected cells with respect to the live cells and use this number to estimate the absolute cell count. Because of the random initialization of the FlowSOM grid, each run of the algorithm will have slightly different results. Therefore we confirmed that the cell counts computed by the automated analysis vary only minimally in 5 runs of the algorithm.

tSNE based unsupervised analysis

FCS files compensated for spillover between channels were exported using FlowJo. FCS files were then imported into the R environment via the read. FCS function in the flowCore (Hahne et al., 2009) package and intensity values of marker expression were extracted. The intensity values of marker expression were then logicle-transformed via the logicleTransform function in the flowCore package using parameters w=0.1, t=4000, m=4.5 and a=0. Subsequently up to 20000 cell events were randomly sampled from individual FCS files and combined. The dimensionality of the combined data was reduced to two using bh_tsne, an efficient implementation of t-Distributed Stochastic Neighbor Embedding (tSNE) via Barnes-Hut approximations (van der Maaten and Hinton, 2008). Lastly the 2D tSNE coordinates were inverse-logicle transformed and added to the original FCS files as additional channels. The analyses were performed using cytof-kit bioconductor package (Becher et al., 2014; Wong et al., 2015).
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