# Single-cell profiling reveals dendritic cell signatures associated with autoimmune inflammation

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**Figure S1. Protein expression profiles of DC sub-populations.** CD1C protein expression was determined as the fluorescence intensity recorded during index sorting for 285 CD1c+ cDCs isolated from eight healthy adults where index sorting was available (Data not collected for one adult in HCAdult cohort). Each dot represents a single cell. (A) Stratification of data based on the presence/absence of *CD1C* transcripts. The bars and lines indicate the mean and SEM, respectively. Significance was determined using the Mann–Whitney *U* test. (B) Correlation between protein expression (fluorescence intensity) and gene expression in cells with detectable *CD1C* gene expression. Spearman’s *r* and the two-tailed *P* value are shown. (C) CD123 (left) and CD141 (right) protein expression levels of CD1c+ cDCs and pDCs from eight healthy adults recorded during index sorting (Data not collected from one adult in HCAdult cohort). pDCs are segregated based on the presence/absence of *CD86* transcripts. Each dot represents the MFI of single cells analyzed from one individual. The bars and lines indicate the mean and SEM, respectively. \**P* < 0.05 (two-tailed paired *t* test).

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**Figure S2. Single-cell gene expression levels of DC subsets.** CD1c+ cDCs (A) and pDCs (B) were single-cell-sorted from freshly isolated PBMCs from healthy adults (HCAdult); patients with RA, SLE, or recent-onset T1D; and a second healthy cohort that was age-matched to the T1D cohort (HCChild). The cells were subjected to single-cell gene expression analysis. Each dot represents an individual cell. Only genes with a significant difference in expression between at least one disease cohort and the corresponding age-matched healthy cohort are shown based on Hurdle analysis with FDR correction and a significance threshold of 0.001 (indicated by an asterix).

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**Figure S3. Protein expression analysis of pDCs from patients with RA and SLE, and healthy adults.** The expression levels of IRF7, phosphorylated IRF7, IRF8, IFNAR1 and GZMB were determined by intracellular flow cytometric analysis. (A) pDCs were identified as CD123+CD303+HLADR+LIN−. (B) Representative histograms from a RA patient and a healthy adult. (C) Normalized MFI values for the panel of markers. Each dot represents an individual subject and the line represents the mean. \**P <* 0.05 (one-way ANOVA)

### Table S1. Genes and primers used for single-cell gene expression analyses

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| **Gene**  | **5’ primer** | **3’ primer** |
| *CCR3* | TGTCTCGTTCTCCCTCTGCT | AGCCACATTGTAGGGTGTCC |
| *CD1C* | TTCTTCTCCCAGGTGGTGAC | AGTTGCCCTTGGACCAGTTA |
| *CD40* | GCTTGTCCAAGGGTGACATT | GTGAGAGCTGTGTCCTGCAC |
| *CD86* | TTCCCTGATGTTACGAGCAA | GAGGCCGCTTCTTCTTCTTC |
| *CXCR4* | GGTGGTCTATGTTGGCGTCT | TGCAATAGCAGGACAGGATG |
| *FCGR1* | ACAGCCACTCAGACCTCGAC | TTATCCTTCCACGCATGACA |
| *FCGR3* | CGGTGCAGCTAGAAGTCCAT | CAGAAGTAGGAGCCGCTGTC |
| *GNAS* | CTGCTCGCTGAGAAAGTCCT | AGCAGTAGTGACGCCCATCT |
| *GZMB* | GCTGCAGTAGCATGATGTCG | GGTGGCTTCCTGATACGAGA |
| *GZMB* | GCTGCAGTAGCATGATGTCG\* | ACTGTTGGGGAAGCTCCATA\* |
| *IDO1* | TCACAGACCACAAGTCACAGC | TCCAGTTTGCCAAGACACAG |
| *IFNA1/13* | TAGACAAATTCTGCACCGAAC | GGGATCTCATGATTTCTGCTCT |
| *IFNAR1* | TGAGTCTGTCGGGAATGTGA | TGCGAAATGGTGTAAATGAGTC |
| *IRF3* | GTTCTGTGTGGGGGAGTCAT | CCTTGTACTGGTCGGAGGTG |
| *IRF4* | GAGCCAAGCATAAGGTCTGC | TCTGGGTAGGGGAACAGGA |
| *IRF7* | AGGCAGAGCCGTACCTGTC | CTACCTGCTGGGGGTCTGT |
| *IRF8* | ACGAGGTTACGCTGTGCTTT | TGATCAGCTCGTCGATTTCA |
| *LAMP3* | TCAGCCATCGTCAGTCAAGA | ATTTTCGGGTGCCACAGTT |
| *Ly75* | TGAATGTGAACATGGTTTTGG | TCCTTGTGCATATCGAACTGA |
| *NRP1* | ACCAACCCCACAGATGTTGT | AGTTTCTGTCCCCTTGGTTG |
| *PTPN6* | GAACTGCTCCGATCCCACTA | CATGACCTTGATGTGGGTGA |
| *STAT3* | GCTTTTGTCAGCGATGGAGT | GCTGCAACTCCTCCAGTTTC |
| *TAP1* | CGCCTCACTGACTGGATTCT | TGGAAAAACTCCGTCTCCTG |
| *TGFB* | CACAACTCCGGTGACATCAAA | TACCTGAACCCGTGTTGCT |
| *TGFB* | CAACTCCGGTGACATCAAAA\* | TACCTGAACCCGTGTTGCTT\* |
| *TLR3* | AGGCGGGTGTTTTTGAACTA | CGCAAACAGAGTGCATGGT |
| *TLR7* | AACCATGTGATCGTGGACTG | TTGACCCCAGTGGAATAGGT |
| *TLR9* | AACTGCAGCACCCTCAACTT | TCGTGGTAGAGGTCCAGCTT |
| *TNF*  | TGAGGTACAGGCCCTCTGAT | CCCCAGGGACCTCTCTCTAA |
| *TNF* | TGAGGTACAGGCCCTCTGAT\* | CCCGAGTGACAAGCCTGTAG\* |
| *TYROBP* | ATTGCAGTTGCTCTACGGTGA | CTGATAAGGCGACTCGGTCT |
| *XCR1* | CCATCTTCCACAAGGTGCTT | ATGGCGAAGATGAGCTTGAC |

\*Alternative primer pairs used for qPCR.