

Understanding the mode of activation of plasmacytoid dendritic cells in different skin disorders

Neža Lesjak¹, Jeremy Di Domizio^{1*}¹ Department of Dermatology, CHUV University Hospital and University of Lausanne (UNIL), Lausanne, Switzerland

* Corresponding authors: Jeremy.Di-Domizio@chuv.ch

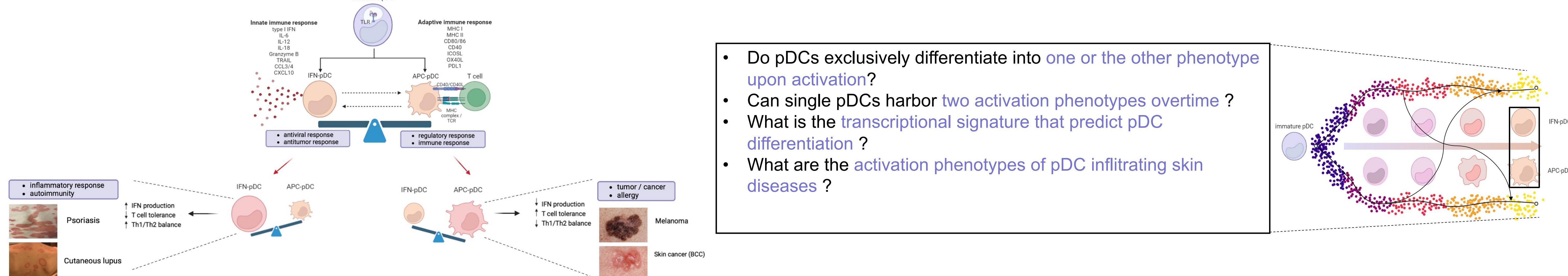
BACKGROUND and HYPOTHESIS

Plasmacytoid dendritic cells (pDCs) are a unique dendritic cell subset which represents from 0.2 - 0.8% of human peripheral blood mononuclear cells (PBMCs). These rare circulating cells express Toll-like receptors TLR7 and TLR9 and can produce large amounts of type I interferons (IFNs) upon viral stimulation, thereby playing a critical role in linking innate and adaptive immunity (Ref 1).

However, in inflammatory autoimmune diseases such as psoriasis and lupus, pDCs are abnormally activated. Conversely, in many cancers pDCs infiltrate tumors but produce less IFN-alpha, promoting immune suppression and tumor growth (Ref 2).

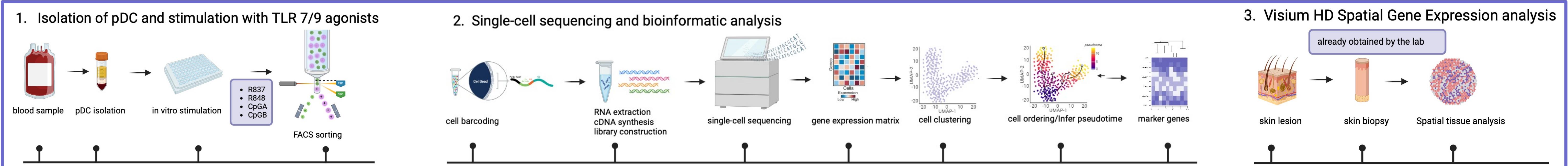
pDCs have shown phenotypic heterogeneity upon activation leading to two distinct populations: IFN production (IFN-pDCs) or antigen-presenting capacities (APC-pDCs). However, pDC differentiation into these two distinct types remains poorly understood.

We hypothesized that plasmacytoid dendritic cell diversification upon activation leads to two main phenotypes named IFN-pDC and APC-pDC, that can be predicted by the identification of specific transcriptional modules using scRNASeq.



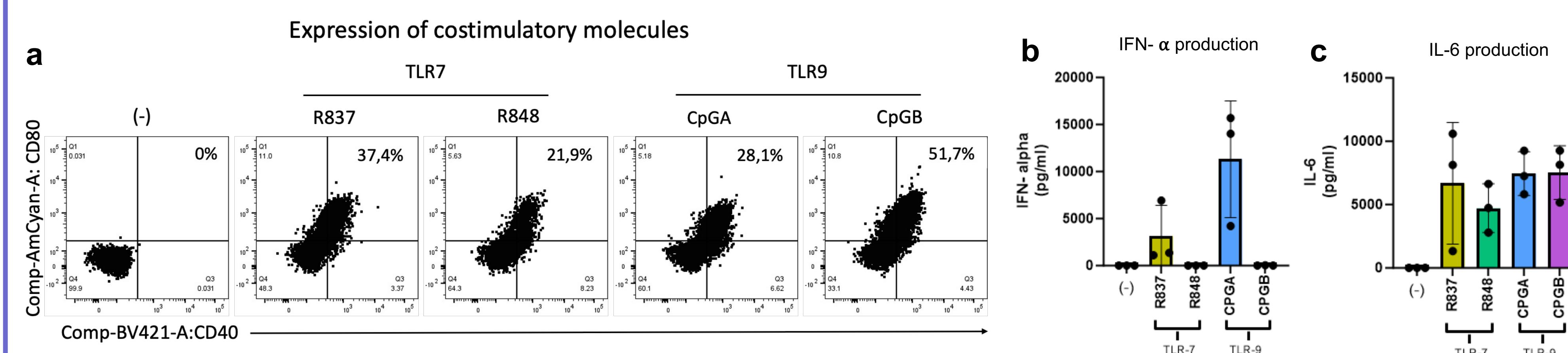
- Do pDCs exclusively differentiate into one or the other phenotype upon activation?
- Can single pDCs harbor two activation phenotypes overtime?
- What is the transcriptional signature that predict pDC differentiation?
- What are the activation phenotypes of pDC infiltrating skin diseases?

METHODS



PRELIMINARY RESULTS

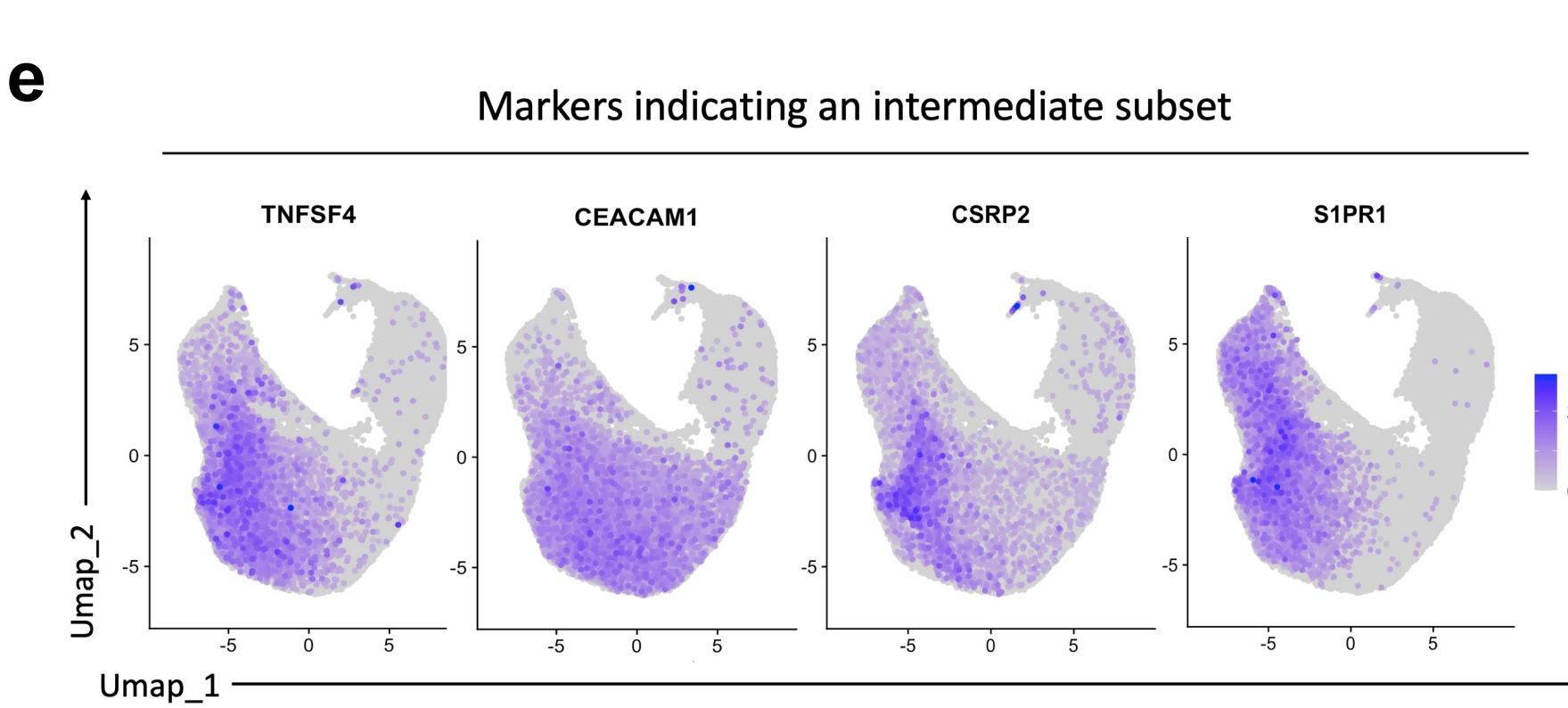
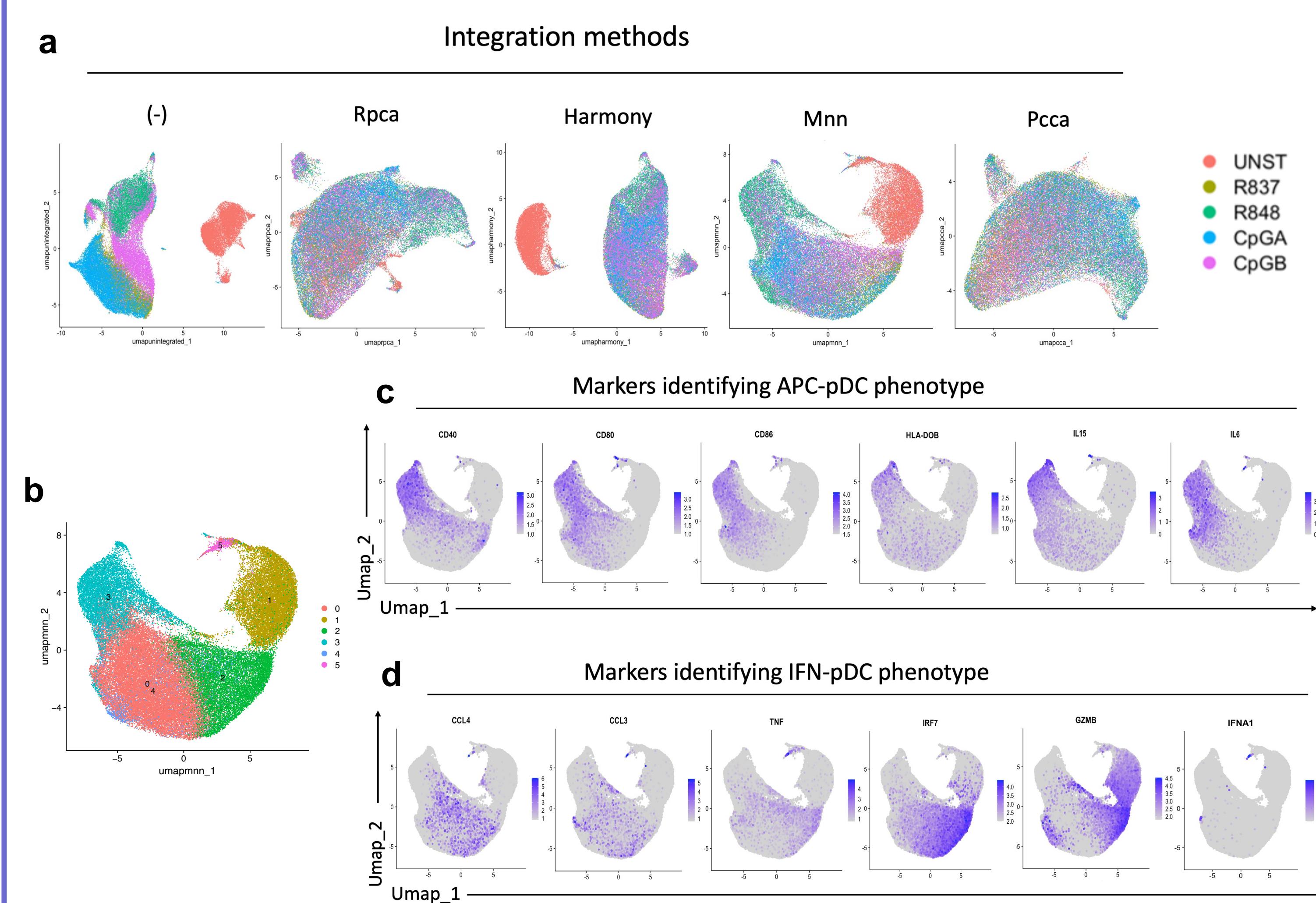
1. Verifying pDC phenotypes upon stimulation with four different agonists



To determine whether plasmacytoid dendritic cells (pDCs) exhibit distinct phenotypic profiles in response to different stimuli, we stimulated freshly isolated pDCs using Imiquimod (R837), Resiquimod (R848), CpGA, and CpGB. The cells were subsequently analyzed using surface markers including IFN- α , CD40, CD80, and CD83. Our results indicate the presence of an antigen-presenting cell (APC)-like phenotype following stimulation with CpGB (a TLR-9 agonist) and R837 (a TLR-7 agonist) (Fig. 1a). To further investigate cytokine production, a cytometric bead array (CBA) was performed to detect interferon signalling, utilizing antibodies against IFN- α and IL-6. Notably, we observed that stimulation with R837 not only promoted an APC signature but also led to the production of IFN- α . This suggests a potential role for scRNA-seq in further elucidating the distinction between TLR agonist responses (Fig. 1b). In addition to IFN type I production, pDCs demonstrated IL-6 expression, indicating cellular activation and maturation (Fig. 1c).

In summary, these data demonstrate that the two phenotypes (IFN and APC) can be obtained following stimulation with different TLR-7/9 agonists.

2. Identifying the subsets in single cell RNA sequencing

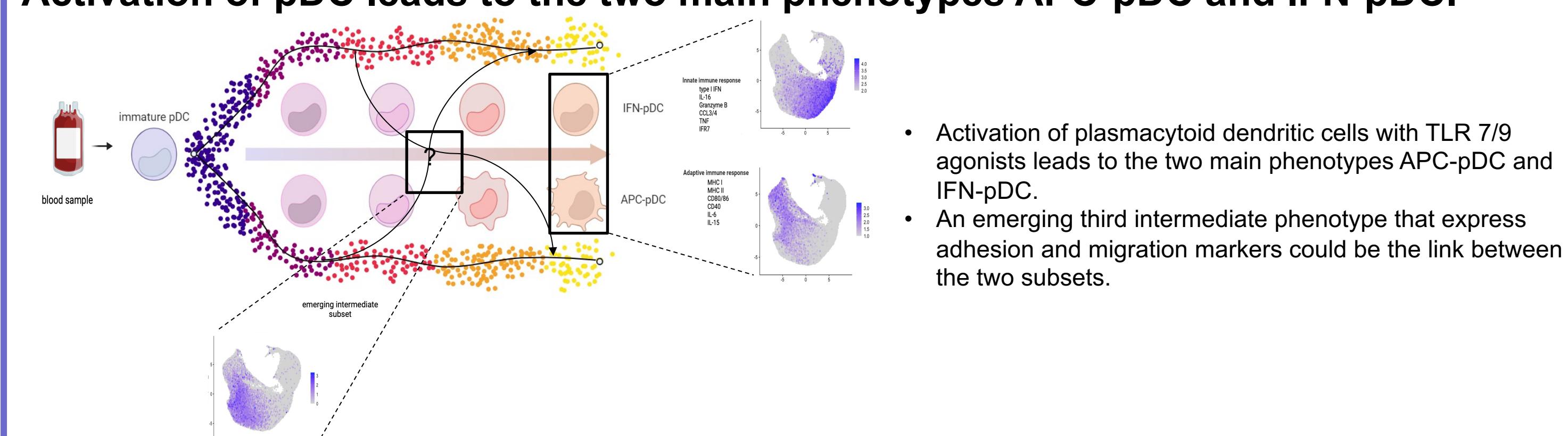


Following single-cell RNA sequencing of pDCs stimulated for 24 hours, we employed various bioinformatic tools to identify cellular subsets. After testing several integration methods, mutual nearest neighbor (MNN) integration was selected to produce a more suitable data transformation, particularly when visualized via UMAP, enabling clear identification of clusters corresponding to cells stimulated with different TLR agonists (Fig. 2a). We then wanted to characterize the different clusters (Fig. 2b). We found that cluster 3 expressed APC markers: HLA-DOB, CD80, CD40, CD86, IL-15, IL-6. (Fig. 2c). Cluster 2 expressed markers of the IFN-pDC phenotype: CCL4, CCL3, TNF, IRF7, GZMB and IFNA-1. Interestingly, IFNA-1 expression was nearly absent (Fig. 2d). Upon further analysis of the most representative markers within MNN-derived clusters, we identified an emerging pDC subset characterized by an intermediate expression profile, clustering between the two primary subsets: APC-pDCs and IFN-pDCs. This newly classified intermediate subset is defined by the expression of four prominent markers: TNFSF4, CEACAM1, CSRP2, and S1PR1 (Fig. 2e). These markers are well-known for their roles in mediating cellular adhesion, immune interactions, and functions related to binding, cell proliferation, and migratory capacity.

These findings demonstrate that the two subsets (APC-pDC and IFN-pDC) of pDCs can be distinguished at the single cell level, suggesting that they harbor different transcriptomes. Moreover, we observed an emerging third subset that might be an intermediate stage during pDC differentiation.

SUMMARY

Activation of pDC leads to the two main phenotypes APC-pDC and IFN-pDC.



- To be able to track pDC differentiation over time, we will add another timepoint at 6h.
- To confirm our results *in vivo* and to identify the pDC subsets that infiltrate different skin diseases, we will analyze pDC phenotypes in spatial transcriptomics datasets previously generated by the group.

ONGOING RESEARCH

