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Peer Review File

CCR7+ Dendritic Cells Expressing Both IL-23A and IL-12B Potentially Contribute to Psoriasis Relapse

Corresponding Author: Professor Honglin Wang

Parts of this Peer Review File have been redacted as indicated to remove third-party material.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This is an important study that is closely focused on expression and respionse toi UK-23 signaling in an IL4il, using both human disease tissue and relevant experimental models. It also shows the signal "getting through" to KC for their expression of S100A8/A9 and other markers of IL-17 response.
There are a number of recent papers on this topic, each with their own experimental conditions making comparison challenging. To keep this lively discussion going, I recommend thiat this paper be accepted as is. Best, JT Elder

Reviewer #2

(Remarks to the Author)
The manuscript by Sun et al. investigates the cellular source of IL-23, a key cytokine in psoriasis pathogenesis. The authors identify CCR7+ dendritic cells (DCs) as the dominant IL-23 producers in both human psoriatic skin and a mouse model of psoriasis. They demonstrate that these CCR7+ DCs express both IL-23A and IL-12B subunits. The study further reveals that ablation of CCR7 from DCs eliminates IL-23 signaling in a mouse model, while overexpression of IL-23a in these cells triggers psoriasis-like skin inflammation and psoriatic arthritis model. Additionally, the authors uses spatial analysis of psoriatic epidermis and identify CCR7+ DCs co-localize with CD161+ IL-17-producing T cells and KRT17+ keratinocytes. The author have carried out a thorough work using both mouse model of psoriasis and human tissue samples however, as the importance of IL23 is already known in psoriasis a solid mechanistic study of at least part of the processes described is missing and could have contributed to the novelty of the paper.

Specific issues are listed below:
A major concern with this manuscript is that the manuscript's conclusions about CCR7+ DCs being the primary source of IL-23 production conflict with previously published literature from their own group Nakamizo et al. (2021) identify CD14+ type 3 dendritic cells (DC3s) as the main producers of IL23A in psoriasis (Fig S4). The clearly show in their Figure 1 b, d,e,f.
also how are these data coincide with other reports showing that the DC-LAMP (LAMP3+ DCs) are not the ones producing the IL-23 (Lee et al. doi: 10.1084/jem.20030451 and Zaba et al. doi: 10.1084/jem.20071094.)

2. the novelty of the message is compromised by previous reported studies. in Ma et al.
Nat Commun. 2023 Jun 12;14(1):3455. doi: 10.1038/s41467-023-39020-4. related to the proximity and functional interaction of LAMP3+CCR7+ DCs with keratinocytes and IL-17 producing T cells.

3. the novelty With the role of LAMP3+CCR7+CD5+ DCs in driving Th17 was reported initially by Korenfeld et al. JClinsihght 2017 10.1172/jci.insight.96101 although they didn't indicate the production of IL-23.

4. Figure S6B , it is difficult to assess the expression of IL-23 from that staining in the specific subset that the author describes. it is showing all the CD11c+ cells. A better approach is to sort the subsets and stained the purified DC subsets on a slide.

5. Figure S4, in the Nakamizo, paper showing that DC3 makes the most IL23. So what is it??

6. CCR7 signaling was shown to mediate IL-23 production in a different setting. Kuwabara et al. 2009 doi: 10.4049/jimmunol.0800729. although not novel is that a better message for the current study?

7. The author don't provide any indication about what CD161 function on these T cells, and besides making IL17 and IL26 , what are their other characteristics? are they CD4, CD8? and if CD8, what is their similarity to the TC17 described in Ma et al.

Reviewer #3

(Remarks to the Author)
This research manuscript aims to identify the cellular source of IL-23 that drives psoriasis. It uses both human samples and mouse models for this purpose. The authors first use scRNAseq and spatial sequencing on human psoriasis vs healthy skin to identify localization of KRT17 keratinocytes and IL-17 producing T cells in diseased epidermis then identify CCR7+ DC as the main transcribers of IL-23. They then turn to the imiquimod (IMQ) psoriatic mouse model for functional studies. They use a diphtheria toxin approach to eliminate a comparable population of CCR7+ DC, then take an overexpression approach and observe not only skin lesions but also joint changes consistent with psoriatic arthritis. Moreover they observe ab T cells (the main mediators in human disease) in the model which are normally not particularly prominent in the IMQ mouse model.

The paper is well written. The figures are clear, some quite beautiful. The question of how to tackle disease resistance and recurrence in psoriasis is an important one clinically, and trying to look upstream at the pathogenic DC is a reasonable idea. As a whole, their data are suggestive of the finding that CCR7+DC are potentially the key source of IL-23 that drives IL17 production by T cells and in turn activates KRT17 keratinocytes, and the idea of a tripartite module between these three localized in the epidermis is intriguing. The development of a new model of psoriasis that better recapitulates human disease is a major strength of the paper, as the psoriasis field is plagued by seemingly innumerable models that are in various ways flawed.
Limitations of the manuscript are the heavy reliance on transcriptional profiling for much of the work, and gaps in presented data that limit the interpretation of the functional relevance of the proposed involved cells.
Please find below recommendations that may strengthen the paper

1. It would be ideal to have protein data in human skin to confirm the transcriptomic data (IHC or IF staining for example) showing the coexpression of the IL23 subunits with the CCR7+ DC.
2. Similarly, it would be helpful to show IL17 production by labeled T cells adjacent to the KRT17 keratinocytes. Fig 1H does not include any T cell markers, and non T cells can produce IL-17, and are present in psoriatic skin (Line 26 page 8 says they are T cells but I'm not seeing that in the figure). Alternatively, could the authors show IL17 protein (or maybe transcription factor) production by the KLRB1 / CD161 T cells, for example by flow. Essentially, use another approach that shows that it is indeed the CD161 ab T cells that are the cells producing IL-17 in human skin (rather than just the scRNAseq analysis, and the correlative spatial transcriptomic data).
3. Fig 2D –beautiful imaging, I'm struck by the fact there's just one cell in the field that is producing IL-23 (both subunits) yet that is enough to be a major mediator of disease? The text notes it's a 'small fraction' but there's no quantification provided and that implies the cells are indeed quite rare in patient samples.
4. Similarly, in the spatial transcriptomic colocalization figures, it seems surprising that in an entire psoriatic plaque that there are only a handful of regions/cells colocalizing IL17 and KRT17, etc, yet the manuscript argues this is a major player in disease pathogenesis. There also appear to be several areas of KRT17 positivity yet no or few IL17 positive cells. My assumption would have been greater colocalization, or greater number of 'modules' if a major axis in disease. Is there a way to identify these modules more easily (and cheaply!) that you could do on a larger number of sections per patient, and number of patients, to determine how common they really are?
5. I recognize this may be difficult so if it can't be done, I get it, but could you obtain pretreated skin and then after treatment skin in an area that commonly recurs like elbows, knees, or lower back, etc and stain for the CCR7 DC to show that indeed even after treatment for example with topical steroids, they remain in skin in the same number as before treatment? That would really cement the theory that they are persistent in skin and mediate disease recurrence.
6. Can you confirm that IL-23 protein expression is lost in the IL4i1cre mice (its shown in UMAPs but I still see red in both umaps and its unclear how much red on a umap, reflecting transcription in digested skin samples status post flow sorting etc really equates to in vivo cytokine production). Protein confirmation by IHC or other in situ analysis would be helpful…
7. …and then did knocking down IL-23 production by the IL4i1cre DC abrogate disease in the IMQ mice!!!? I was surprised this wasn't mentioned / shown, making me wonder if the mice still developed disease. If they did develop disease, is it because the DC of interest are still producing low level IL-23 or does it mean that IL-23 production by the CCR7 DC is not necessary for disease?
8. It would be nice to show that the Il4i1-IL23OE DC are indeed inducing IL17 production by T cells and that production is leading to disease ie to confirm the functionality of the pathway being proposed, as opposed to off target/bystander effects of overexpression that is generating disease. Only Suppl. Figure 8B shows IL-17 production by TCRb T cells and it's a bar graph. While comparatively the TCRb staining is shown by flow plots. Why? Can the authors show the flow plots for the IL-17? Or else could they show it in sections/microscopy?
a. If you neutralize IL-17 and/or use mice lacking ab T cells, do the OE mice still get the skin and joint disease?

Minor comments:
9. Please clarify in the manuscript results section text or title that the figure 6 data are in human – it is unclear in the main body of the text as the preceding figures were first human then mice.

10. I might be mistaken but the two normal samples shown for spatial transcriptomics look like scalp (both with numerous hair follicles/adnexal structures) though the table says 1 trunk / 1 scalp – I noticed it b/c both have a large burden of KRT17 cells despite being healthy/non psoriasis, which at first is eye-catching. Then on deeper examination, the KRT17 cells are all down in the adnexae rather than epidermis in both samples while the psoriasis KRT17 cells are in the epidermis. This makes sense biologically I suppose, but readers not familiar with skin histopath may be confused / question why KRT17 is so prevalent in healthy controls.

Version 1:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
The manuscript claims that CCR7^{+} (LAMP3^{+}) dendritic cells are the primary producers of IL-23 in psoriatic skin. reanalysis of the dataset cited from Nakamizo et al. (2021) reveals no detectable IL12B expression in either LAMP3^{+} or LAMP3^{-} DC subsets, which undermines the core conclusion regarding IL-12/IL-23 co-expression. PS074 express IL-23A and IL12B in their LAMP3+ and LAMP3- DCs, which PS-76 there is no expression of IL-12B at all. Not even in the LAMP3+ DCs, ie their conclusion is not supported.

Furthermore, the group has previously reported that DC3 are the main source of IL-23A in skin (the reviewer understands that its not both IL23A and IL12B), and this inconsistency is not addressed or reconciled, which could create confusion in the field.the relevance of the co expression of these subunits is not clear, obviously, treatments that were directed towards IL-12B compared to IL-23A were not as effective in psoriasis treatment and are not used (p40 inhibitor (ustekinumab) → blocks both IL-12 and IL-23, broader but less effective), p19 inhibitors (guselkumab, risankizumab, tildrakizumab, mirikizumab) → specific to IL-23, better psoriasis targeting.

if the authors argue that CCR7+ DCs and not DC3 are the main IL-23 producers in psoriasis, leading to Th17 (which also make IL-22) your finding aligning with findings from Ma et al. 2023,Korenfeld et al. 2017, Zaba et al. 2007, Lee et al. 2004 and others and should be discussed.

Reviewer #3

(Remarks to the Author)
the authors addressed all of my comments and have significantly strengthened the data in the paper/support of their interpretation.

Point-by-Point Response to the Reviewers' Comments

Reviewer #1:

This is an important study that is closely focused on expression and respionse toi UK-23 signaling in an IL4il, using both human disease tissue and relevant experimental models. It also shows the signal "getting through" to KC for their expression of S100A8/A9 and other markers of IL-17 response.

There are a number of recent papers on this topic, each with their own experimental conditions making comparison challenging. To keep this lively discussion going, I recommend thiat this paper be accepted as is. Best, JT Elder

Re: We are deeply grateful for your thoughtful evaluation of our manuscript and your enthusiastic endorsement of its publication. Our integration of spatial transcriptomics, functional assays in murine models, and human disease profiling underscores the goal to bridge clinical observations with mechanistic insights.

We fully agree that the dynamic nature of IL-23/IL-17 research, with its diverse experimental systems, poses challenges for direct comparisons. By employing complementary human and murine approaches, we aimed to resolve conflicting observations in the field and provide a unified framework for understanding DC- T17-keratinocyte crosstalk in psoriasis pathogenesis.

Your recommendation to accept the manuscript as-is is immensely motivating. We are eager to contribute this work to the ongoing discourse and look forward to collaborating with the scientific community to advance these findings toward improved clinical outcomes. Particularly, targeting $CCR7^{+}$ DC to prolong remission—a strategy distinct from current IL-23/IL-17 inhibitors—is on our research schedule stepping from the *Il4i1-Il23a*^{OE} mouse model which faithfully recapitulates the immunopathogenesis of human psoriasis.

Reviewer #2:

The manuscript by Sun et al. investigates the cellular source of IL-23, a key cytokine in psoriasis pathogenesis. The authors identify CCR7+ dendritic cells (DCs) as the dominant IL-23 producers in both human psoriatic skin and a mouse model of psoriasis. They demonstrate that these CCR7+ DCs express both IL-23A and IL-12B subunits. The study further reveals that ablation of CCR7 from DCs eliminates IL-23 signaling

in a mouse model, while overexpression of IL-23a in these cells triggers psoriasis-like skin inflammation and psoriatic arthritis model. Additionally, the authors uses spatial analysis of psoriatic epidermis and identify CCR7+ DCs co-localize with CD161+ IL-17-producing T cells and KRT17+ keratinocytes. The author have carried out a thorough work using both mouse model of psoriasis and human tissue samples however, as the importance of IL23 is already known in psoriasis a solid mechanistic study of at least part of the processes described is missing and could have contributed to the novelty of the paper.

Re: We thank you for the thoughtful assessment of our work and for highlighting the importance of mechanistic insights. We agree that the role of IL-23 in psoriasis is well-established, but the IL-23-producing cell identity has been complicated by conflicting reports. We aimed to advance this understanding by pinpointing the cellular source and spatial dynamics of IL-23 production (the bioactive IL-23 heterodimer, not the mere expression of IL-23A without forming a complex with IL-12B), which are critical for developing targeted therapies. Below, we clarify the mechanistic insights already embedded in our study and acknowledge opportunities for future work.

Mechanistic validation of CCR7$^+$ DC as IL-23 producers

(1) We demonstrated necessity: CCR7$^+$ DC ablation via *Il4i1*^{cre}DTR mice abolished IL-23 in psoriasis-like skin (**Fig. 3F-J**).

(2) We established sufficiency: Overexpression of IL-23 in CCR7$^+$ DC (via *Il4i1-Il23a*^{OE}) directly triggered αβT cell-driven pathology (**Fig. 4**) and PsA-like arthritis (**Fig. 5**), bypassing exogenous triggers like IMQ.

Spatial and functional crosstalk

Spatial transcriptomics (**Fig. 6A, B**) and ligand-receptor analysis (**Fig. 6C, D**) revealed CCR7$^+$ DC recruit CD161$^+$ T cells via CXCL16 and orchestrate IL-17 responses in KRT17$^+$ keratinocytes, forming a type 17 module.

Lineage and regulation

CCR7$^+$ DC likely derive from cDC2 (**Fig. 2F, 3C**), suggesting differentiation pathways that could be therapeutically modulated.

Future directions

While our study focuses on cellular and spatial mechanisms, we agree that deeper molecular insights (e.g., transcriptional regulation of IL-23 in CCR7$^+$ DC) would enhance novelty. We have expanded the Discussion to highlight this limitation and propose future work (revised Manuscript Text **Page 15, Lines 28-30**).

Thank you for the opportunity to strengthen our manuscript. We believe our findings provide a foundational framework for understanding IL-23's cellular dynamics, which is essential for resolving relapse in psoriasis.

Specific issues are listed below:

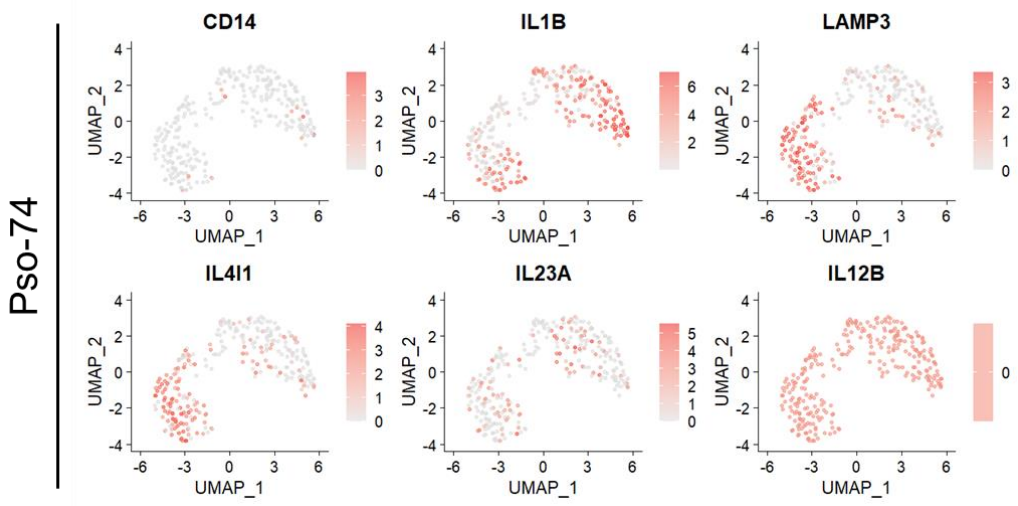
1. A major concern with this manuscript is that the manuscript's conclusions about CCR7+ DCs being the primary source of IL-23 production conflict with previously published literature from their own group Nakamizo et al. (2021) identify CD14+ type 3 dendritic cells (DC3s) as the main producers of IL23A in psoriasis (Fig S4). The clearly show in their Figure 1 b, d,e,f. also how are these data coincide with other reports showing that the DC-LAMP (LAMP3+ DCs) are not the ones producing the IL-23 (Lee et al. doi: 10.1084/jem.20030451 and Zaba et al. doi: 10.1084/jem.20071094.)

Re: We appreciate your critical evaluation and opportunity to clarify how our findings reconcile with prior literature. Below, we address the apparent contradictions and highlight the novel mechanistic insights from our study.

Nakamizo et al. (2021): DC3 and IL-23A vs. our study: CCR7$^+$ DC and IL-12B/IL-23A co-expression.

Key distinction: Nakamizo et al. identified DC3 (CD14$^+$IL1B$^+$) as IL-23A producers but did not analyze IL-12B (p40), which is indispensable for functional IL-23 heterodimer formation and cytokine secretion[1] (**Fig. 2C-E,** revised **Fig. S6B**).

Re-analysis of Nakamizo's data: Using their scRNA-seq dataset (GSE176509), we could not find cells co-expressing *IL23A* and *IL12B*, as *IL12B* was barely detected (**Fig. R1**).



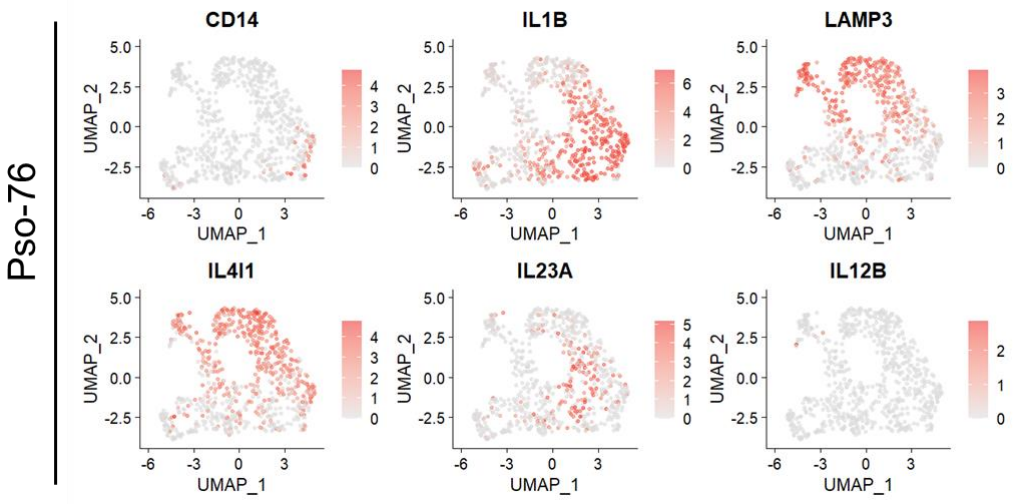


Fig. R1 Feature-plots of indicated genes in psoriatic samples from Nakamizo et al. 2021

Antibody specificity: Nakamizo's IHC for "IL-23" (ab45420) targets IL-23A alone, not the intact IL-23 heterodimer. Our flow cytometry (**Fig. 2E**) and scRNA-seq data (**Fig. 2C**) demonstrate that only CCR7$^+$ DC co-express both subunits.

Lee et al. (2004): IL-23p19 and p40 mRNA were strongly up-regulated in peripheral blood monocyte-derived human DCs after encountering a maturation stimulus, and that p19 mRNA was strongly expressed in cells bearing both immature and mature DC surface markers in psoriatic lesions (CD11c$^+$CD14$^-$CD83$^-$ and CD83$^+$).

Resolution of the detection means: Although Lee et al. did not use experimental approaches at single-cell resolution or for *in situ* detection of psoriatic skin, their findings did not rule out the fact that CCR7$^+$ DC are the primary source of bioactive IL-23 which requires co-expression of IL-23p19 and IL-12p40 in an individual cell.

Novel epidermis focus in our study: In contrast to the findings of Lee et al. 2004 showing that IL-12p40 was localized to the dermal compartment of psoriatic skin (Fig. 2 in Lee et al. 2004), our RNAscope (**Fig. 2D**) and immunofluorescence (**Fig. S6B**) data consistently demonstrated that CCR7$^+$ DC in psoriatic epidermis express both IL23A and IL12B. This indicates that the epidermal CCR7$^+$ DC serve as the primary source of functional IL-23 heterodimer, which is key to the maintenance and (re)activation of local T17 cells and keratinocytes. Our study emphasizes that the spatial distribution of immune cells and their intimate crosstalk within the skin microenvironment are crucial for disease development.

Zaba et al. (2007): Anti-TNF treatment decreased DC-LAMP$^+$ cells in psoriasis

Functional context: Zaba et al. showed DC-LAMP$^+$ (LAMP3$^+$) DCs responded to anti-TNF treatment (Fig. 4A in Zaba et al. 2007) but did not link them to IL-23 production. **Mechanistic advance:** We demonstrate that epidermal CCR7$^+$ DC (LAMP3$^+$) co-expressing IL-23p19 and IL-12p40 localize in adjacent to CD161$^+$ T cells and KRT17$^+$ keratinocytes (**Fig. 6**) and defines a spatially restricted "type 17 module" that is critical for psoriasis development.

Summary:

Our work resolves prior ambiguities by:

(1) Distinguishing IL-23A expression (DC3) from functional IL-23 production (CCR7$^+$ DC).

(2) Identifying CCR7$^+$ DC as the dominant cells co-expressing IL-23A and IL-12B *in situ* in psoriatic lesions.

(3) Defining the spatial interactions (CCR7$^+$ DC → CD161$^+$ T cells → KRT17$^+$ keratinocytes) in psoriatic epidermis that explain the role of IL-23 in disease maintenance and relapse.

We thank you for prompting this clarification and have amended the Discussion to explicitly address these points (revised Manuscript Text **Page 15, Lines 17-28**).

2. the novelty of the message is compromised by previous reported studies. in Ma et al. Nat Commun. 2023 Jun 12;14(1):3455. doi: 10.1038/s41467-023-39020-4. related to the proximity and functional interaction of LAMP3+CCR7+ DCs with keratinocytes and IL-17 producing T cells.

Re: We appreciate your attention to contextualizing our findings within the existing literature. While Ma et al. (2023) identified LAMP3$^+$CCR7$^+$ DC and IL-17$^+$ T cells as distinct populations in psoriasis, their work did not address the functional interplay or spatial coordination between these cell types, which is the central mechanistic novelty of our study. We here clarify our unique contributions.

(1) The cellular source of IL-23

Ma et al. did not pay attention to the source of IL-23. We re-analyzed their data (GSE173706) and found that CCR7$^+$ DC (*CD200*$^+$*CD1B*$^+$*LAMP3*$^+$*IL4I1*$^+$) but not DC3 (*CD14*$^+$*IL1B*$^+$) expressed the transcripts of *IL23A* and *IL12B* (**Fig. R2**), which is first reported in our manuscript.

[FIGURE REDACTED]

Fig. R2 Feature-plots of indicated genes in APC subsets from Ma et al. 2023

(2) Beyond coexistence: defining a functional module

Ma et al. catalogued CCR7$^+$ DC and IL-17$^+$ T cells but did not explore how these cells interact. Our spatial transcriptomics (**Fig. 6A, B**) and ligand-receptor analysis (**Fig. 6C, D**) reveal that CCR7$^+$ DC actively recruit CD161$^+$ T cells via CXCL16 and sustain IL-17 production through IL-23 secretion, forming a self-reinforcing "type 17 module" in the epidermis. Moreover, with our new data (revised **Fig. 6E**) showing CCR7$^+$ DC but not other types of myeloid cells reactivated memory T cells to produce IL-17 in the absence of exogenous antigens, we demonstrate that CCR7$^+$ DC sufficiently provide first (endogenous antigens), second (co-stimulatory molecules), and third (IL-23 and other cytokines) signals for memory T17 cell reactivation and psoriasis recurrence. We subsequently identified KRT17$^+$ keratinocytes as direct IL-17 responders (revised **Fig. 1E-H**) and showed their spatial coupling with CCR7$^+$ DC and T17 cells (**Fig. 6B**), linking immune activation to epidermal pathology.

(3) Mechanistic causality

We demonstrated functional necessity: CCR7$^+$ DC ablation abolished IL-23 production (**Fig. 3J**) and downstream IL-17 responses.

We established sufficiency: CCR7$^+$ DC-specific IL-23a overexpression drove αβT cell-mediated pathology (**Fig. 4**) and PsA-like arthritis (**Fig. 5**), which Ma et al. did not

investigate.

Overall, while Ma et al. mapped cellular diversity, our work defines the hierarchical organization and mechanistic drivers of the IL-23–IL-17 axis in psoriasis. This spatial-functional framework provides actionable insights for targeting disease recurrence. Thank you for the opportunity to emphasize the conceptual advance of our study.

3. the novelty With the role of LAMP3+CCR7+CD5+ DCs in driving Th17 was reported initially by Korenfeld et al. JCIinsihght 2017 10.1172/jci.insight.96101 although they didn't indicate the production of IL-23.

Re: We appreciate your attention to prior literature and clarify below how our findings extend beyond Korenfeld et al. (2017).

(1) Distinct cell populations

Korenfeld et al. identified $CD5^+$ DCs ($CD11c^+CD1c^+CD5^+$) as a heterogeneous population constituted of epidermal Langerhans cells and dermal $CD1a^{dim}$ DCs, distinct from the $CCR7^+$ DC ($CD200^+CD1B^+LAMP3^+IL4I1^+$) investigated in our study.

(2) Mechanistic novelty

Korenfeld et al. focused on T cell polarization to non-Th17 lineages. Their findings demonstrated that $CD5^+$ DCs drive inflammatory cytotoxic T lymphocyte (CTL), Th1, and Th22 cell responses; however, this mechanism does not represent a critical pathogenic pathway in psoriasis. Notably, their work did not establish the association between $CD5^+$ DCs and IL-23 production or T17 cell activation. In contrast, our study is the first to identify the IL-23-producing $CCR7^+$ DC subset as the key driver of the pathogenic T17 response in psoriasis. We demonstrated that this specialized DC population is the primary source of IL-23 and is responsible for driving αβT cell-dependent IL-17 production.

In summary, Korenfeld et al. described a broad role for $CD5^+$ DCs in adaptive immunity. In contrast, our work defines a unique $CCR7^+$ DC subset that specifically orchestrates the IL-23/IL-17 axis in psoriasis. This represents a conceptual and mechanistic advance in understanding disease pathogenesis.

4. Figure S6B, it is difficult to assess the expression of IL-23 from that staining in the specific subset that the author describes. it is showing all the CD11c+ cells. A better

approach is to sort the subsets and stained the purified DC subsets on a slide.

Re: We appreciate the critical assessment and suggestion for additional validation. We here clarify the rationale behind our approach and the strength of existing evidence.

Technical limitations of psoriatic skin samples

Our human psoriatic biopsies are limited to < 0.5 cm^2, yielding insufficient cells for subset-specific sorting and slide-based staining. This constraint is common in clinical dermatology studies.

Robust co-expression validation

(1) scRNA-seq analysis (**Fig. 2C**) showed *IL12B* and *IL23A* co-expression was restricted to CCR7$^+$ DC (*CD200*$^+$*CD1B*$^+$*LAMP3*$^+$*IL4I1*$^+$).

(2) RNAscope (**Fig. 2D**) validated in *situ* co-localization of *IL12B* and *IL23A* transcripts within *CD200*$^+$ cells, excluding macrophages or DC3.

(3) Flow cytometry (**Fig. 2E** and revised **Fig. S6C**) demonstrated that 90% of IL-12p40$^+$IL-23p19$^+$ cells within CD11c$^+$ DCs were CD1B$^+$CD200$^+$ (defining CCR7$^+$ DC), strongly supporting their identity as the dominant IL-23 producers.

(4) Immunofluorescence staining of CD200, IL-12p40 and IL-23p19 confirmed that CCR7$^+$ DC co-express both subunits of IL-23, making them the dominant producer of the IL-23 functional heterodimer (revised **Fig. S6B**).

5. Figure S4, in the Nakamizo, paper showing that DC3 makes the most IL23. So what is it??

Re: We thank you for raising this critical point and clarify below why our findings reconcile with—and extend beyond—Nakamizo et al.'s observations.

(1) IL-23 requires both subunits for bioactivity

Nakamizo et al. focused on *IL23A* (p19) expression in DC3 (Fig. S4 in Nakamizo et al. 2021), but IL-23 bioactivity depends on co-expression of IL-12B (p40) to form the functional heterodimer[1].

Our re-analysis of Nakamizo's scRNA-seq data (GSE176509) showed that *IL12B* was barely detected and thus cannot lead to the conclusion that DC3 are the source of IL-23 the cytokine (**Fig. R1**). In fact, Nakamizo et al. rigorously stated "Single-cell analysis of human skin identifies CD14$^+$ type 3 dendritic cells co-producing IL1B and IL23A in psoriasis".

(2) Contextualizing Nakamizo's findings

Nakamizo's IL-23 IHC (ab45420) detects IL-23A alone, not the intact IL-23

heterodimer. While DC3 may produce IL-23A, they cannot secrete bioactive IL-23 without IL-12B. Our data confirm that DC3 highly express *IL23A* but do not express *IL12B* (**Fig. 2C**), while CCR7$^+$ DC are the source of functional IL-23 heterodimer driving T17 responses.

The apparent discrepancy arises from differing definitions of "IL-23 production". By emphasizing subunit co-expression and spatial functionality, our work clarifies that CCR7$^+$ DC—not DC3—are the dominant IL-23 producers in psoriasis.

6. CCR7 signaling was shown to mediate IL-23 production in a different setting. Kuwabara et al. 2009 doi: 10.4049/jimmunol.0800729. although not novel is that a better message for the current study?

Re: We appreciate your insightful reference to Kuwabara et al. (2009) and clarify how our work builds upon and diverges from prior studies to advance mechanistic understanding.

(1) Beyond CCR7 signaling: defining a unique DC subset

Kuwabara et al. demonstrated that CCR7 ligands (CCL19/CCL21) drive IL-23 production in experimental autoimmune encephalomyelitis (EAE) **yet did not resolve** the specific cellular origin of IL-23 or define the responsible DC subset(s). Critically, CCR7 is ubiquitously expressed across CD45$^+$ leukocytes as we observed CCR7 expression on both T cell and APC populations (**Fig. R3A, B**). Thus, targeting CCR7 suppresses systemic IL-23 levels without addressing its cellular specificity.

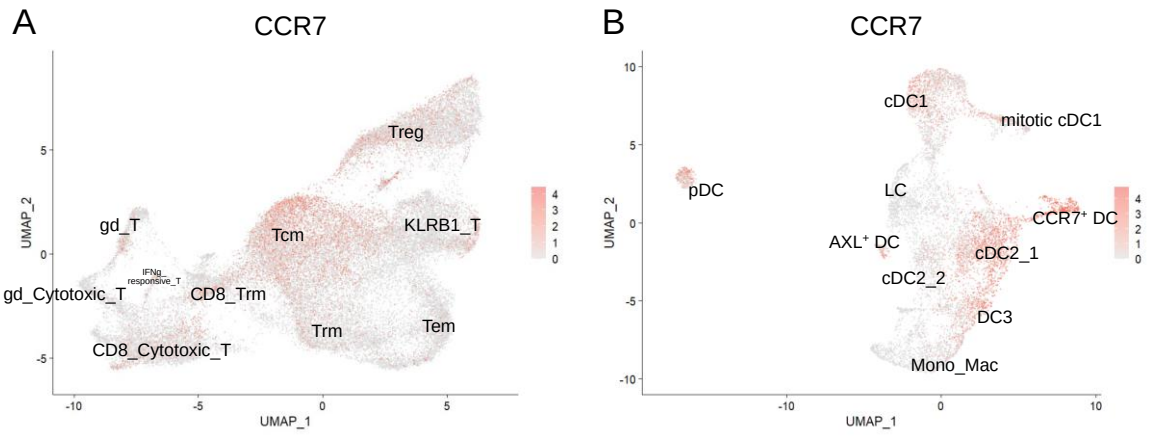


Fig. R3 *CCR7* expression in T cells and APCs

We addressed the cellular specificity via defining a novel DC subpopulation—CCR7$^+$ DC (*LAMP3*$^+$*CD200*$^+$*IL4I1*$^+$*CD1B*$^+$)—as the dominant source of IL-23. This discovery establishes a framework for precision therapeutic strategies aimed at targeting IL-23-

producing cells and resolving relapse for psoriasis and other chronic inflammatory diseases.

(2) Spatial and functional mechanism

We reveal how CCR7$^+$ DC orchestrate IL-23-driven pathology through recruitment of CD161$^+$ T cells via CXCL16 (**Fig. 6C, D**) and formation of a spatially restricted type 17 module with IL-17-producing T cells and KRT17$^+$ keratinocytes (**Fig. 6A, B**) in psoriatic epidermis. This mechanism is specific to psoriasis.

While Kuwabara et al. highlighted the importance of CCR7 in IL-23-mediated autoimmune disease model, our work identifies a novel DC subset and its spatial-functional role in psoriasis pathogenesis, offering actionable therapeutic targets. We have revised the Discussion to explicitly contrast these findings (revised Manuscript Text **Page 15, Lines 17-28**).

7. The author don't provide any indication about what CD161 function on these T cells, and besides making IL17 and IL26, what are their other characteristics? are they CD4, CD8? and if CD8, what is their similarity to the TC17 described in Ma et al.

Re: We thank you for raising these important questions about CD161$^+$ T cell heterogeneity and function. Below, we clarify the phenotypic and functional characteristics of these cells based on our data and contextualize them relative to prior studies.

(1) CD161$^+$ T cell subsets in psoriatic skin

Subset composition (Fig. S3B)

60% of CD161$^+$ (*KLRB1*$^+$) T cells were CD4$^-$CD8$^-$ (double-negative, DN), 20% were CD8$^+$, and 20% were CD4$^+$. These percentages were not different between normal and psoriatic skin.

IL-17 production (Fig. 1D)

We found that *IL17A* expression in psoriatic skin was largely confined to CD161$^+$ T cells (**Fig. 1B**) and averagely 10% of CD161$^+$ T cells produced *IL17A* (**Fig. 1C**). Among the CD161$^+$ T cells (composed of CD4$^+$, CD8$^+$ and DN T cells), there were equivalent proportions of CD8$^+$ T cells and DN T cells to predominantly produce *IL17A*, and CD4$^+$*IL17A*$^+$ cells were of a lower proportion compared to either CD8$^+$ *IL17A*$^+$ T cells or DN *IL17A*$^+$ T cells.

These findings aligned with our *Il4i1-Il23a*^{OE} mouse model, in which IL-17-producing

T cells were predominantly $CD8^+$ and DN T cells (revised **Fig. S8C**).

(2) Comparison to TC17 cells in Ma et al. (2023)

Ma et al. described that $CD8^+$ T cells predominantly produced IL-17A in psoriatic skin (GSE173706, **Fig. R4A**), but they overlooked DN T cells which also produced considerable amount of *IL17A*.

We extracted all $IL17A^+$ T cells (*IL17A* > 0) from Ma et al.'s data and analyzed *CD4* and *CD8A* expression of these cells to quantify T cell subsets. In our re-analysis, $CD4^+$ T cells meet the criteria: *CD4* > 0 and *CD8A* = 0; $CD8^+$ T cells meet the criteria: *CD4* = 0 and *CD8A* > 0; DN T cells meet the criteria: *CD4* = 0 and *CD8A* = 0; and DP T cells meet the criteria: *CD4* > 0 and *CD8A* > 0. We found that $CD8^+$ T cells and DN T cells equivalently constituted over 90% of total $IL17A^+$ T cells (**Fig. R4B**). This is highly consistent with our data.

We also checked if $IL17A^+$ T cells (composed of $CD4^+$, $CD8^+$ and DN T cells) from Ma et al.'s data were $CD161^+$ ($KLRB1^+$) T cells. The results showed that > 80% of the total $IL17A^+$ T cells were $CD161^+$ T cells (**Fig. R4C**), which is also consistent with our data showing that *IL17A* was largely confined to $CD161^+$ T cells.

[FIGURE REDACTED]

Fig. R4 Re-analysis of T17 cell characteristics in Ma et al. 2023

A, Feature-plots of indicated genes in T cells from Ma et al. 2023. **B**, Percentages of $CD8^+$, $CD4^+$,

DN and DP cells in *IL17A*$^+$ T cells. **C**, Percentages of *KLRB1*$^+$ cells in total, CD8$^+$, CD4$^+$, DN or DP T17 cells.

(3) CD161 functional implications

While our study focuses on CD161 as a marker of IL-17-producing T cells in psoriatic skin, the function of CD161 is largely unknown. This warrants future studies involving mice expressing human CD161 because mice do not have an endogenous homolog of this gene. We have added this limitation to Discussion in the revised manuscript (revised Manuscript Text **Page 16, Lines 27-30**).

(4) Beyond IL-17/IL-26: additional features

CD161$^+$ T cells additionally expressed *CCL20*, *CXCR6* and *CCR6* as hallmarks (revised **Fig. S3A**).

Reviewer #3:

This research manuscript aims to identify the cellular source of IL-23 that drives psoriasis. It uses both human samples and mouse models for this purpose. The authors first use scRNAseq and spatial sequencing on human psoriasis vs healthy skin to identify localization of KRT17 keratinocytes and IL-17 producing T cells in diseased epidermis then identify CCR7+ DC as the main transcribers of IL-23. They then turn to the imiquimod (IMQ) psoriatic mouse model for functional studies. They use a diphtheria toxin approach to eliminate a comparable population of CCR7+ DC, then take an overexpression approach and observe not only skin lesions but also joint changes consistent with psoriatic arthritis. Moreover they observe ab T cells (the main mediators in human disease) in the model which are normally not particularly prominent in the IMQ mouse model.

The paper is well written. The figures are clear, some quite beautiful. The question of how to tackle disease resistance and recurrence in psoriasis is an important one clinically, and trying to look upstream at the pathogenic DC is a reasonable idea. As a whole, their data are suggestive of the finding that CCR7+DC are potentially the key source of IL-23 that drives IL17 production by T cells and in turn activates KRT17 keratinocytes, and the idea of a tripartite module between these three localized in the epidermis is intriguing. The development of a new model of psoriasis that better recapitulates human disease is a major strength of the paper, as the psoriasis field is plagued by seemingly innumerable models that are in various ways flawed.

Limitations of the manuscript are the heavy reliance on transcriptional profiling for

much of the work, and gaps in presented data that limit the interpretation of the functional relevance of the proposed involved cells.

Re: We sincerely thank you for the thoughtful engagement with our work and appreciate the questions raised, which helped to strengthen the clarity and rigor of our manuscript.

Please find below recommendations that may strengthen the paper

1. It would be ideal to have protein data in human skin to confirm the transcriptomic data (IHC or IF staining for example) showing the coexpression of the IL23 subunits with the CCR7+ DC.

Re: We thank you for emphasizing the importance of protein-level validation. As detailed in revised **Fig. S6B**, we performed IF co-staining for IL-23p19 (IL-23A), IL-12p40 (IL-12B), and CD200 (a $CCR7^+$ DC marker) in human psoriatic skin. The results confirmed that $CCR7^+$ DC co-expressed both IL-23A, IL-12B proteins, which solidified $CCR7^+$ DC as the dominant IL-23 producers.

2. Similarly, it would be helpful to show IL17 production by labeled T cells adjacent to the KRT17 keratinocytes. Fig 1H does not include any T cell markers, and non T cells can produce IL-17, and are present in psoriatic skin (Line 26 page 8 says they are T cells but I'm not seeing that in the figure). Alternatively, could the authors show IL17 protein (or maybe transcription factor) production by the KLRB1/CD161 T cells, for example by flow. Essentially, use another approach that shows that it is indeed the CD161 ab T cells that are the cells producing IL-17 in human skin (rather than just the scRNAseq analysis, and the correlative spatial transcriptomic data).

Re: We appreciate your emphasis on validating the identity of IL-17–producing cells and performed IF co-staining in human psoriatic skin for CD161, IL-17A, and KRT17. The results showed that $IL\text{-}17A^+$ cells co-localize with $CD161^+$ T cells, which are spatially adjacent to $KRT17^+$ keratinocytes (revised **Fig. 1H**). This protein-level validation, combined with spatial transcriptomics, reinforced the conclusion that $CD161^+$ T cells mediate IL-17–KRT17 crosstalk.

3. Fig 2D –beautiful imaging, I'm struck by the fact there's just one cell in the field that is producing IL-23 (both subunits) yet that is enough to be a major mediator of disease? The text notes it's a 'small fraction' but there's no quantification provided and that implies the cells are indeed quite rare in patient samples.

Re: There are about 20 cells co-expressing *CD200*, *IL23A* and *IL12B* in the epidermis compartment presented in **Fig. 2D**. We quantified IL-23A$^+$IL-12B$^+$ cells to be < 15% among CD11C$^+$ cells of psoriatic skin in revised **Fig. S6D**. We then quantified the percentages of IL-23A$^+$IL-12B$^+$ DCs among total live cells in psoriatic skin and found that IL-23A$^+$IL-12B$^+$ DCs constituted < 0.25% of total skin cells of psoriatic samples (**Fig. R5**). These data suggest that DCs expressing both IL-23A and IL-12B are indeed rare in psoriatic skin.

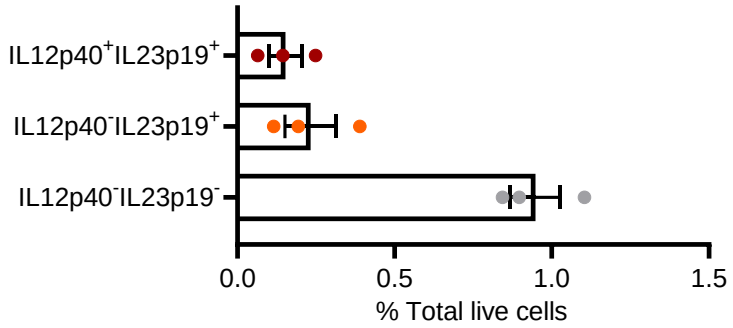


Fig. R5 IL-23A$^+$IL-12B$^+$ DC percentages in psoriatic skin

4. Similarly, in the spatial transcriptomic colocalization figures, it seems surprising that in an entire psoriatic plaque that there are only a handful of regions/cells colocalizing IL17 and KRT17, etc, yet the manuscript argues this is a major player in disease pathogenesis. There also appear to be several areas of KRT17 positivity yet no or few IL17 positive cells. My assumption would have been greater colocalization, or greater number of 'modules' if a major axis in disease. Is there a way to identify these modules more easily (and cheaply!) that you could do on a larger number of sections per patient, and number of patients, to determine how common they really are?

Re: We used 10x Visium v1 chip which has a resolution of ~55 μm and aggregates signals from multiple cells. While this allows broad tissue mapping, it may obscure rare but functionally critical cellular interactions. Our identification of even 1–2 modules per patient (**Fig. 1G** and **S5A**) likely reflects focal "hotspots" of active crosstalk rather than diffuse colocalization.

Also, there are indeed areas of *KRT17* positivity yet no or few *IL17A*/*IL17F* positive cells. Because T cells transiently express cytokine mRNAs no longer than 72~96 h and cytokine proteins no longer than 96 h after TCR engagement[2, 3], and keratinocytes sustain the upregulation of *KRT17* mRNA for longer than 48 h after IL-17 protein stimulation[4], it is possible that keratinocytes still express *KRT17* ("legacy" KRT17$^+$

zones) after the adjacent CD161$^+$ T cells stop to produce the mRNAs of *IL17A*/*IL17F*.

As IL-17 proteins are secreted by CD161$^+$ T cells (expressing IL17 mRNAs), it is quite reasonable that several spots within the KRT17$^+$ regions co-localized with IL17 mRNAs because cytokines amplify the function of CD161$^+$ T cells in a paracrine manner which affects dozens of cells surrounding the cytokine-producing T cell[5]. We calculated *KRT17*$^+$ spots (defined as the epidermal type 17 module) in comparison to *KRT14*$^+$ spots (covering all keratinocytes) in our spatial transcriptomic data and found that KRT17$^+$ regions varied from 10% to 35% in different samples (**Fig. R6A, B**). We also managed to use immunofluorescence staining of KRT17 on psoriatic skin sections for the quantification of KRT17$^+$ regions in the epidermis and found that KRT17$^+$ regions varied from 13% to 34% in different samples (**Fig. R6C, D**). Thus, immunofluorescence staining of KRT17 can be an easy and accurate way to quantify the epidermal type 17 modules in psoriatic skin.

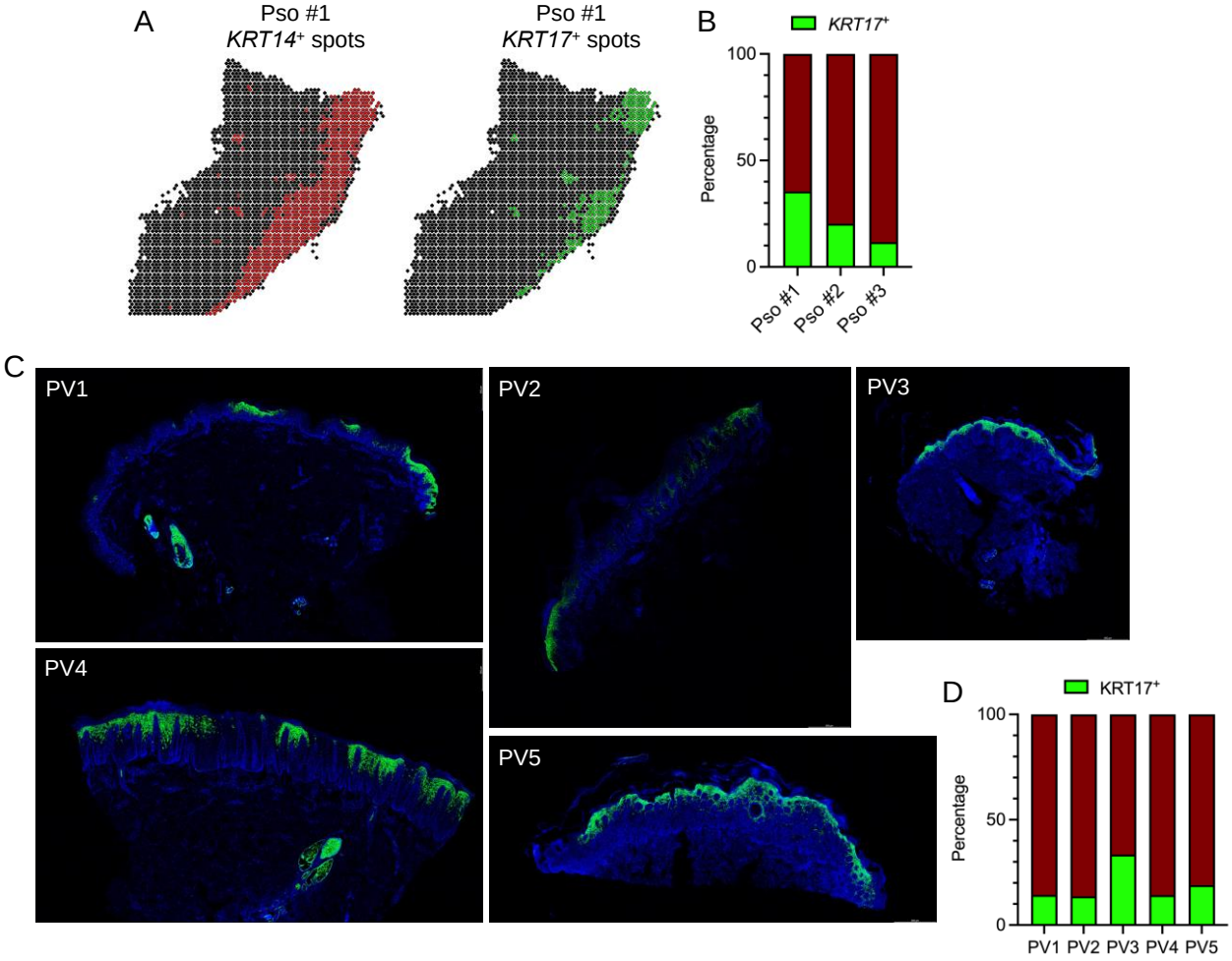


Fig. R6 Quantification of KRT17$^+$ regions in psoriatic epidermis

A, Spatial feature-plots of *KRT14*$^+$ (*KRT14* expression > 40) spots and *KRT17*$^+$ (*KRT17* expression >

30) spots in psoriatic skin section from a patient. **B**, Percentages of *KRT17*$^+$ spots relative to *KRT14*$^+$ spots. **C**, Immunofluorescence staining of KRT17 on psoriatic skin sections. **D**, Percentages of the areas stained positive for KRT17 within the epidermis compartment of psoriatic skin sections.

5. I recognize this may be difficult so if it can't be done, I get it, but could you obtain pretreated skin and then after treatment skin in an area that commonly recurs like elbows, knees, or lower back, etc and stain for the CCR7 DC to show that indeed even after treatment for example with topical steroids, they remain in skin in the same number as before treatment? That would really cement the theory that they are persistent in skin and mediate disease recurrence.

Re: We sincerely appreciate this thoughtful suggestion to investigate CCR7$^+$ DC persistence during treatment and recurrence. While we faced challenges in obtaining longitudinal biopsies from identical anatomic sites, we analyzed two independent public datasets to address this question.

1. **GSE114729** (topical steroid treatment): Bulk RNA-seq of epidermis from psoriasis patients (7 responders, 4 non-responders), pre- and post-treatment.

We performed deconvolution analysis using BayesPrism[6] and revealed that in the responsive patients, CCR7$^+$ DC proportions decreased post-treatment but rebounded upon relapse; while in non-responsive patients, CCR7$^+$ DC proportions remained unchanged correlating with therapeutic resistance (revised **Fig. S10B**).

2. **GSE228421** (Anti-IL-23 therapy): scRNA-seq of non-lesional (NL) and lesional skin from 5 psoriasis patients pre-treatment (D0), day 3 (D3), and day 14 (D14) post-IL-23 blockade.

We performed analyses on myeloid cell subsets and found that CCR7$^+$ DC, but not other DC subsets, were enriched in myeloid cells of lesional skin compared to that in non-lesional skin and the frequency of CCR7$^+$ DC, but not other DC subsets, declined post IL-23 mAb treatment (**Fig. R7**).

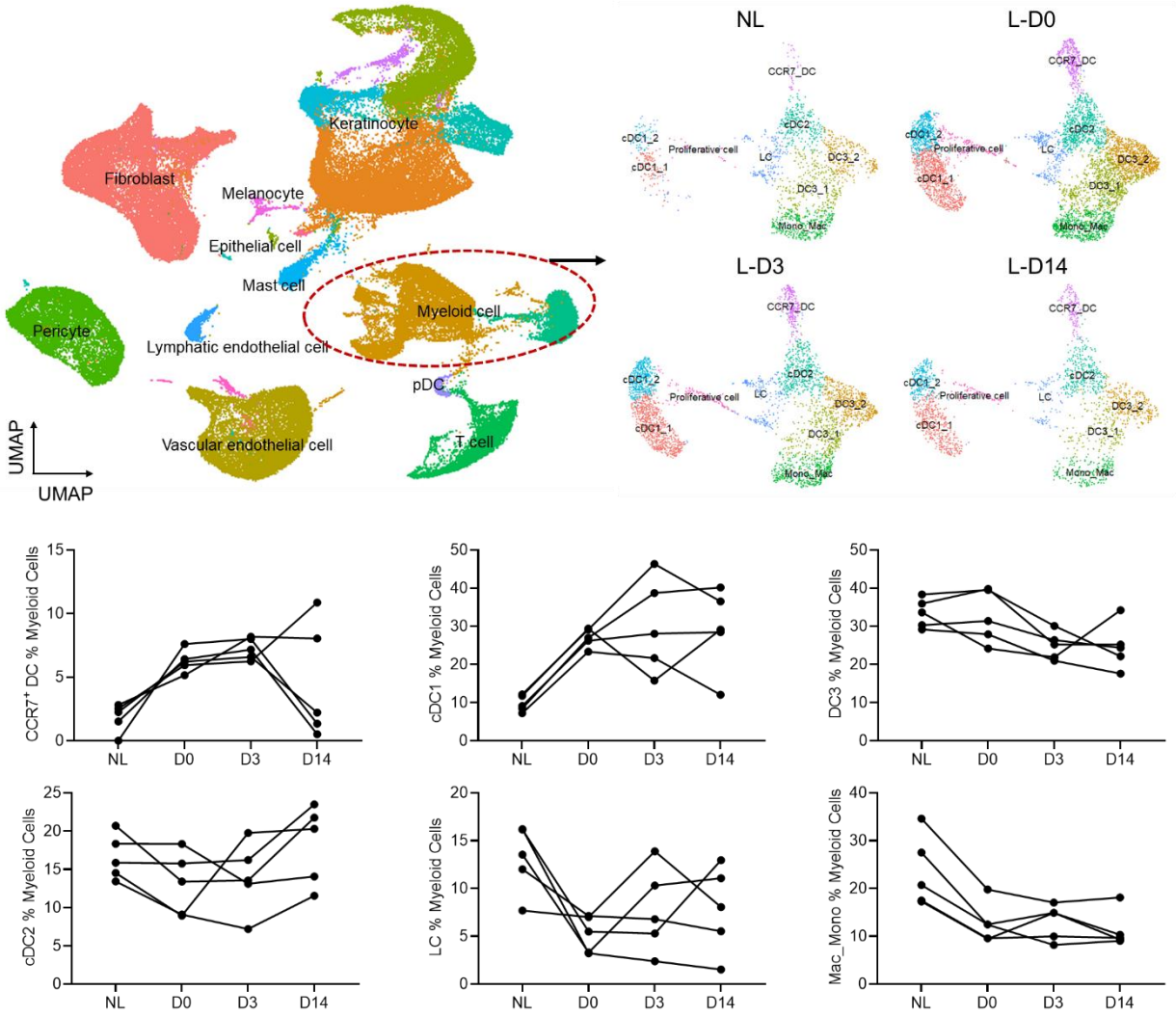


Fig. R7 Myeloid cell subset alterations after anti-IL-23 treatment in patients with psoriasis

These orthogonal datasets strongly support our conclusion that CCR7$^+$ DC are resilient pathogenic hubs that persist in treated skin and drive recurrence. Their depletion or functional silencing may be critical for sustained remission. Thank you for prompting this deeper exploration of CCR7$^+$ DC biology, which has significantly strengthened our mechanistic narrative. We have added this to revised **Fig. S10B** and expanded the Discussion to contextualize the clinical implications (revised Manuscript Text **Page 17, Lines 1-10**).

6. Can you confirm that IL-23 protein expression is lost in the IL4i1cre mice (its shown in UMAPs but I still see red in both umaps and its unclear how much red on a umap, reflecting transcription in digested skin samples status post flow sorting etc really equates to in vivo cytokine production). Protein confirmation by IHC or other in situ

analysis would be helpful…

Re: We performed immunofluorescence staining on skin samples from IMQ-treated *Il4i1*^{cre}DTR mice injected with DT or vehicle. The data confirmed the presence of cells co-expressing IL-23a and IL-12b in *Il4i1*^{cre}DTR mouse skin treated with IMQ for 3 days, and DT-mediated killing of CCR7^{+} DC resulted in deletion of IL-23A^{+}IL-12B^{+} cells (revised **Fig. S7C**).

7. …and then did knocking down IL-23 production by the IL4i1cre DC abrogate disease in the IMQ mice!!!? I was surprised this wasn't mentioned / shown, making me wonder if the mice still developed disease. If they did develop disease, is it because the DC of interest are still producing low level IL-23 or does it mean that IL-23 production by the CCR7 DC is not necessary for disease?

Re: As we confirmed that CCR7^{+} DC abrogation resulted in IL-23 heterodimer to decrease to baseline (**Fig. 3J**), we further evaluated the disease severity of IMQ-treated mice with CCR7^{+} DC deleted, using IL-23 antibody treatment as a control. Our data showed that CCR7^{+} DC deletion markedly attenuated IMQ-induced skin inflammation, and the phenotype was comparable to that of IL-23 blockage (revised **Fig. S7D, E**). These data reinforced CCR7^{+} DC as the dominant cellular source of pathogenic IL-23 in this model.

8. It would be nice to show that the Il4i1-IL23OE DC are indeed inducing IL17 production by T cells and that production is leading to disease ie to confirm the functionality of the pathway being proposed, as opposed to off target/bystander effects of overexpression that is generating disease. Only Suppl. Figure 8B shows IL-17 production by TCRb T cells and it's a bar graph. While comparatively the TCRb staining is shown by flow plots. Why? Can the authors show the flow plots for the IL-17? Or else could they show it in sections/microscopy?

Re: We thank you for prompting us to validate the functional specificity of CCR7^{+} DC in driving IL-17 production. The flow plots of TCRβ and IL-17a are now presented in revised **Fig. S8B**, showing that IL-17 was mostly produced by αβ T cells in *Il4i1-Il23a*^{OE} mice.

We first found that in our *Il4i1-Il23a*^{OE} mouse model, IL-17-producing T cells were predominantly CD8^{+} and DN T cells (revised **Fig. S8C**). We then isolated splenic CD4^{-}

central memory T (Tcm) cells ($CD44^+CD62L^+$) from these mice, assuming that these Tcm cells contained memory T cells producing IL-17 in response to autoantigens and IL-23 from myeloid cells of *Il4i1-Il23a*^{OE} mice. We co-cultured the Tcm cells respectively with cDC1, cDC2, DC3, LC, macrophages, and $CCR7^+$ DC isolated from the skin and lymph nodes of *Il4i1-Il23a*^{OE} mice and found that $CCR7^+$ DC, but not other myeloid cell subsets, reactivated memory T cells to produce IL-17 (revised **Fig. 6E**). Thus, $CCR7^+$ DC-derived IL-23 is both necessary and sufficient to reactivate memory T17 cells, directly linking this cellular axis to disease pathogenesis. These findings were extensively discussed in the revised manuscript, and we sincerely thank you for helping us improve the manuscript (revised Manuscript Text **Pages 17-18, Lines 12-1**).

a. If you neutralize IL-17 and/or use mice lacking ab T cells, do the OE mice still get the skin and joint disease?

Re: Following this constructive suggestion, we injected IL-17a neutralizing antibody or IgG control to *Il4i1-Il23a*^{OE} mice every 3 days from 8- to 20-week age. Anti-IL-17a antibody treatment in *Il4i1-Il23a*^{OE} mice significantly reduced skin disease severity but did not alleviate joint destruction compared to isotype control (revised **Fig. S9**). These data demonstrated that the skin pathology in *Il4i1-Il23a*^{OE} mice was dependent on IL-17, but the mechanisms driving joint pathology go beyond just the IL-23/IL-17 axis.

This aligns with clinical observations that IL-17 inhibition was more effective for skin, while TNF inhibition showed better efficacy in controlling joint inflammation, but neither adalimumab (anti-TNF) nor secukinumab (anti-IL-17) could reverse structural joint damage such as enthesophytes and bone erosions[7].

This inability to relieve structural joint damage through targeting IL-17 highlights the value of the *Il4i1-Il23a*^{OE} mouse model, as it faithfully recapitulates this clinically relevant limitation. Notably, earlier intervention with IL-17 blockade may potentially prevent bone erosion - a hypothesis that can now be rigorously tested using this model, which also provides a valuable preclinical platform for evaluating new therapeutic strategies aimed at preventing or reversing the structural joint complications of psoriatic disease (revised Manuscript Text **Page 16, Lines 18-27**).

Minor comments:

9. Please clarify in the manuscript results section text or title that the figure 6 data are in human – it is unclear in the main body of the text as the preceding figures were first human then mice.

Re: Because the revised Fig. 6 contains data from mice (revised **Fig. 6E**), we made revisions in the text accordingly to ensure clarity.

10. I might be mistaken but the two normal samples shown for spatial transcriptomics look like scalp (both with numerous hair follicles/adnexal structures) though the table says 1 trunk / 1 scalp – I noticed it b/c both have a large burden of KRT17 cells despite being healthy/non psoriasis, which at first is eye-catching. Then on deeper examination, the KRT17 cells are all down in the adnexae rather than epidermis in both samples while the psoriasis KRT17 cells are in the epidermis. This makes sense biologically I suppose, but readers not familiar with skin histopath may be confused / question why KRT17 is so prevalent in healthy controls.

Re: Thank you for catching this important nuance. Indeed, both controls showed normal *KRT17* expression in hair follicles, while psoriasis samples showed abnormal *KRT17* in the epidermis. To avoid confusion, we added text: "Normal skin physiologically expresses *KRT17* in hair follicles, contrasting with pathogenic epidermal expression in psoriasis" (revised Manuscript Text **Page 8, Lines 16-17**).

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Point-by-Point Response to the Reviewers' Comments

Reviewer #2:

The manuscript claims that CCR7^{+} (LAMP3^{+}) dendritic cells are the primary producers of IL-23 in psoriatic skin. reanalysis of the dataset cited from Nakamizo et al. (2021) reveals no detectable IL12B expression in either LAMP3^{+} or LAMP3^{-} DC subsets, which undermines the core conclusion regarding IL-12/IL-23 co-expression. PS074 express IL-23A and IL12B in their LAMP3+ and LAMP3- DCs, which PS-76 there is no expression of IL-12B at all. Not even in the LAMP3+ DCs, ie their conclusion is not supported.

Re: We appreciate your close examination of data from Nakamizo et al. *IL12B* expression values in Pso-74 are universally zero (rendered as pale pink in feature plots), indicating no detectable expression in any DC subset.

[FIGURE REDACTED]

Fig. R1 Feature-plots of indicated genes in psoriatic samples from Nakamizo et al. 2021

The discrepancy in *IL12B* detection likely stems from technical and biological factors:

1. Nakamizo et al. used Smart-seq2 (full-length, low-throughput scRNA-seq), while our study employed 10× Genomics (3' end-focused, high-throughput). The wider cell coverage of our data (5,371 psoriatic myeloid cells vs. Nakamizo's 1,032 psoriatic myeloid cells) enhances detection of *IL12B* expressed by limited numbers

of cells.

2. As noted in Pso-74 vs. Pso-76, *IL12B* expression varies across patients. Our patient cohort (n = 6 psoriasis) provides greater statistical power to identify rare but biologically critical populations.
3. Crucially, our flow cytometry, ELISA, and spatial co-detection demonstrated CCR7$^+$ DC co-express IL-12p40 and IL-23p19 proteins and secrete bioactive IL-23. This functional evidence supersedes transcript-level limitations in any single dataset.

Furthermore, the group has previously reported that DC3 are the main source of IL-23A in skin (the reviewer understands that its not both IL23A and IL12B), and this inconsistency is not addressed or reconciled, which could create confusion in the field. the relevance of the co expression of these subunits is not clear, obviously, treatments that were directed towards IL-12B compared to IL-23A were not as effective in psoriasis treatment and are not used (p40 inhibitor (ustekinumab) → blocks both IL-12 and IL-23, broader but less effective), p19 inhibitors (guselkumab, risankizumab, tildrakizumab, mirikizumab) → specific to IL-23, better psoriasis targeting.

Re: We appreciate your concern regarding potential confusion between the prior characterization of DC3 expressing *IL23A* and the current identification of CCR7$^+$ DC as bioactive IL-23 producers.

IL-23p19 inhibitors achieve superior efficacy by selectively neutralizing CCR7$^+$ DC-derived IL-23 while sparing protective IL-12 signaling in keratinocytes—a homeostatic mechanism recently elucidated by Zwicky et al.[1]. IL-12p40 blockers inadvertently disrupt both pathogenic IL-23 and keratinocyte-protective IL-12, explaining their reduced clinical impact. Our model thus clarifies why p19 inhibitors represent a precision strategy: they exclusively target the CCR7$^+$ DC subset responsible for functional IL-23 secretion, reconciling cellular heterogeneity with therapeutic outcomes.

if the authors argue that CCR7+ DCs and not DC3 are the main IL-23 producers in psoriasis, leading to Th17 (which also make IL-22) your finding aligning with findings from Ma et al. 2023, Korenfeld et al. 2017, Zaba et al. 2007, Lee et al. 2004 and others and should be discussed.

Re: We thank you for this constructive suggestion. We now explicitly position our work within the established paradigm:

"While Korenfeld et al. described dermal DCs priming cytotoxic T cells and Th22 cells in psoriasis[2], we now pinpoint the specific CCR7$^+$ subset responsible for sustained IL-23 production and direct orchestration of the IL-23/IL-17 axis within the epidermal compartment. Critically, our spatial transcriptomics and immunofluorescence analyses reveal that this epidermal niche enables CCR7$^+$ DC to license CD161$^+$ T cells, driving compartmentalized IL-17A production through a type 17 module – a mechanistic insight supported by Ma et al.'s independent observation of epidermis-confined *IL17A* expression in their spatial dataset[3].

Pioneering studies by Lee et al. and Zaba et al. implicated the crucial role of IL-23 in psoriasis pathogenesis[4, 5]. Afterwards, the identification of IL-23-producing cells in psoriasis has been complicated by conflicting reports implicating monocytes, monocyte-derived DCs, and more recently CD14$^+$ DC3 as primary sources of IL-23A expression[4, 6, 7, 8, 9]. "

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