nature portfolio

Peer Review File

Integration of GWAS and single-cell analysis for psoriasis identifies disease-associated cell subtypes and therapeutic targets

Corresponding Author: Professor Hong-Hee Won

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)
Hyeobin Jo and colleagues report a GWAS meta-analysis of psoriasis, identifying a number of previously unreported susceptibility loci. They proceed to integrate their GWAS results with gene expression data, using various methods applied to both bulk tissue and single-cell RNA-seq data, to propose therapeutic targets and draw inferences about cell-type specific pathobiology.

I commend the authors on a very well written manuscript, which describes a coherent and competently conducted series of analyses combining a large amount of data relevant to the genetic and functional genomic basis of psoriasis. The methods are largely appropriate and conclusions are measured. My main concern with the study is whether the findings are sufficiently novel.

The authors queried GWAS Catalog for known psoriasis susceptibility associations in October 2023, but since then further psoriasis meta-analyses have been published (e.g. PMID: 38763176, PMID: 39885523, PMID: 40021644). The last of these in particular, which they cite as reference 11, found and discussed many of the genes that are described here as previously unreported. Furthermore, these previous studies all describe various downstream analyses considering heritability and genetic correlation with other traits. It is not clear where the current manuscript provides novel insights.

Likewise, the authors re-analyse single-cell RNA-seq data from two previous studies (Ma et al 2023, Francis et al 2024), including a weighted gene coexpression network analysis (on the Ma data? – not explicitly stated). While these analysis are clearly and logically laid out, and thorough, I am again unclear as to where the findings here highlight new biology beyond the original papers. I think the integration of GWAS and scRNA-seq using the single-cell disease relevance score is a new and potentially interesting angle; however, I think a clearer description is needed of what this score represents (lines 189-196). I also found the description of within cell type scDRS heterogeneity hard to follow (lines 196-202) and am not sure what conclusion to take from it.

Overall, I do think the GWAS meta-analysis is probably a modest improvement on recent studies in terms of power, and this work represents an excellent synthesis of genetic and transcriptomic data on psoriasis to highlight candidate druggable targets. But the authors should state more clearly where previous studies have fallen short and left unanswered questions, and more carefully indicate how their findings contribute to answering them.

Minor comments on detail:

1. Line 108: A total of 198 SNPs were identified – I think clearer to state that these are independent association signals. Presumably there are many more individual SNPs that are associated. I think it also worth mentioning the significance threshold here.

2. Local heritability analysis: around 106 COJO signals are in Bonferroni significant regions of local heritability, while 42 are not. Does this tell us anything about these 42 signals? The authors suggest that regions with high local heritability without significant loci may harbour the potential for novel discoveries. Many of the most significant regions matching this description

are adjacent to psoriasis susceptibility loci and presumably residual LD drives the high local heritability here. Would it be worth distinguishing in Table S5 regions where this is or is not the case? Can the non-adjacent high heritability regions be characterised in any way?

3. The finding that psoriasis heritability is most significantly enriched in genomic regions actively expressed in synovial fluid is a little counterintuitive, given the established immune models of psoriasis as a systemic disease with fairly well understood drivers. The authors should elaborate further on why this could represent a true finding, or else disclose limitations of their analysis which could explain why more relevant tissues or cell types are missed. It is also true that many of the psoriasis cases in their GWAS analysis will also have psoriatic arthritis and therefore their results may conflate genetic signals for psoriatic arthritis alongside those specific to cutaneous psoriasis. So their claim "this finding recapitulates the association for psoriasis susceptibility variants and increased risk of psoriatic arthritis" (line 136) is not well supported. Previous studies have investigated the genetic differences between PsA and cutaneous psoriasis (e.g. PMID: 35507331). Relatedly, I also feel the claims about shared mechanisms with IBD (line 140) are a little speculative (how do we know these digestive system tissues are most enriched in those traits?). And in methods (line 413) the description "estimates the relative genetic heritability of psoriasis based on the expression of associated genes" is not precise and should be reworded.

4. Line 156 – genetic correlation with atopic dermatitis: why is the p-value only marginally significant when the magnitude of correlation is similar to the "notable positive correlations" described earlier?

5. Line 185 – "… follows distinct lineages compared to that of normal tissues." – not sure what this means?

6. Line 215 – "opposing causal effects" – does this mean the direction of causality is opposite or that the direction of causality is always from gene expression to psoriasis but the direction of change in gene expression is opposite with respect to increasing psoriasis risk?

7. Line 230 – "module M1 exhibited the highest module eigengene in basal layer cells" – needs to be more specific. Presumably this means highest *average* eigengene across cells in the module? (Or across samples?)

8. Line 237 – "within the MYE-M2 network, IL23A displayed notably high connectivity" – to me it is unclear what this actually means and could benefit from elaboration. Is this gene in multiple modules within the MYE-M2 network?

9. Line 242 – "during immunotherapy" – needs to be more specific to what has actually been studied; we don't expect that results will generalises to other immunomodulators

10. Discussion line 287 – "identified subpopulations of keratinocytes and ECs that may be involved in psoriasis development" – if I understand correctly, it is not established whether these mechanisms are causal or a consequence of psoriatic inflammation. Could this be clarified?

11. In the discussion of the longitudinal scRNA-seq data (lines 307-325) there is a major focus on IL-23 pathway mechanisms. But these longitudinal data are based on treatment with an IL-23 inhibitor, IL-23 pathway mechanisms would be expected to be identified. Again, I think it needs to be more clear here what the novel findings around these pathways are.

12. Line 331 – "In scDRS analysis, we observed marginal significance…" – what does this mean? Are these associations that do not survive multiple testing correction?

13. Lines 334-336 – "this study did not identify any fibroblast subpopulations…" – several of the scRNA-seq papers have highlighted the importance of fibroblasts in psoriatic inflammation. The authors should reconcile more fully the lack of fibroblast mechanisms identified in their results. Are there study limitations that precluded their discovery? Or do the authors have another hypothesis?

14. Methods line 460 – NS, PN and PP are not defined in this section.

15. Line 483 – COJO lead SNP colocalization – perhaps clarify whether these analyses used inferred conditional association statistics for each independent signal within a locus? Or just the marginal summary statistics for the lead signal at each locus?

16. In the equation in line 526, I am not clear whether this is calculation is done at the single-cell level or the "meta-cell" level. What is ME_c_i in the sum? Should the sum be over all c in C_i rather than all c in C? And I think technically, C must represent the set of all cells and |C| the number of all cells?

17. In the equation in line 533, I think the numerator should have index i, not j. But I have a more fundamental question about this specificity score. Is it valid to transform values that could be positive or negative to a [0,1] scale and calculate specificity as the relative share of a cell type on this scale? Surely this means that the largest negative eigengene values, which could be directionally meaningful, are transformed close to zero and are assigned minimal specificity. While eigengene values that are originally close to zero, indicating little activity in a cell type, get transformed to a value midway between 0 and 1 and are assigned some "medium" amount of specificity? I don't understand the intuition here.

18. Fig 4 legend – line 804 – ratio of observed and expected: it's unclear where the expected value comes from.

19. Fig 6F – there is a small table containing P1 to P5 rows. It is unclear what table entries represent.

20. Error in Table S3: SNP rs10754016 on chr 1 is annotated with RGS14 but this gene is on chr 5 and found elsewhere in the table.

21. In Table S8, it is not clear where some of the GWAS summary statistics derive from, for example “UK Biobank (SAIGE)” or “Psychiatric Genomics Consortium”. A little more detail is needed for readers to be able to identify the underlying study and understand the traits represented.

Reviewer #2

(Remarks to the Author)
This manuscript presents a comprehensive genome-wide meta-analysis of psoriasis integrated with single-cell transcriptomic data. The authors leverage large-scale datasets and employ a multiomic framework to dissect the cellular and molecular basis of psoriasis susceptibility. The integration of scRNA-seq, GWAS summary statistics, and co-expression network analyses adds considerable value. However, several aspects require clarification, additional analysis, methodological transparency, and biological validation to strengthen the conclusions and reproducibility of the study. Below are specific points for major revision:

1. The manuscript briefly states that Duffy’s method was used to correct for self-reported cases in UK Biobank and 23andMe, but it would benefit from a clearer explanation of how this adjustment was validated. Specifically, how robust are the 73.85% and 37.5% "true positive" estimations for UK Biobank and 23andMe? The relatively low true-positive rates raise major concerns about the quality of the data and impact on downstream analyses. Was sensitivity analysis performed to test how these estimates affect meta-analysis results?

2. For the UK Biobank analysis, it would be informative to stratify by diagnosis source (self-report vs. ICD codes) to assess heterogeneity. Also, clarify whether Tsoi et al.'s European ancestry samples were used exclusively for RAF estimation, and provide ancestry PCA comparisons between reference and study populations.

3. A demographic summary for the single-cell cohort is lacking. Please include a table summarizing the sample composition, including age, sex, disease status, and whether matched lesional and non-lesional skin samples were obtained from the same individuals. Common risk factors would be important to know as well, such as BMI and smoking.

4. The integration of scRNA-seq data from lesional, non-lesional, and normal skin samples is well-executed. However, the sample size is modest, especially when making claims about tissue-specific or disease-specific expression patterns. Given the availability of publicly accessible psoriasis single-cell datasets, incorporating and comparing to external will improve statistical power and benchmarking.

5. The interpretation of pseudotime as reflecting the epidermal layer structure is compelling, but it's not clearly explained how pseudotime was inferred and validated. Further analysis and validation is needed to determine if the inferred pseudotime aligns with known keratinocyte differentiation trajectories.

6. The scDRS integration adds a powerful layer of interpretation, linking GWAS signal to specific cell types. However, could the results of granular layer keratinocytes from normal skin show high disease relevance reflect shared genetic programs or some baseline inflammatory priming? Do higher scDRS scores indicate causality, increased susceptibility, or response to inflammation?

7. Related to the scDRS, “The scDRS score was weighted using the MAGMA z-scores and inversely weighted using technical noise in single-cell gene expression. The mitochondrial UMI percentages and tissue types (NS, PN, and PP) were used as covariates.” Traditionally, mitochondrial UMIs are quality control metrics and it’s being used as a covariate just as the tissue types and not part of the technical noise? Please clarify their role in the model.

8. The integrative pipeline (MAGMA, TWAS, eQTL colocalization, scRNA expression, SMR) is a rigorous strategy for gene prioritization. However, it’s unclear how these criteria were weighted or ranked when selecting the top genes.

9. “While all myeloid and T cells exhibited significant associations, Langerhans cells (LCs) and cycling myeloid cells displayed higher disease relevance scores”. When the authors say exhibited significant associations, is this a logistic model or other statistical tests? Any adjustments for any covariates (which would be nice to see in a demographic table mentioned above). Specifically, in Fig 4D there is a great visual of effect size of ration of observed/expected non-lesion/lesion cells in each cell type. However, was just in the figure legend and not mentioned in the methods. This seems to not be a standard statistical test, there’s no hypothesis testing, no variance estimates, no adjustment for potential confounders, no p-values, and no control for multiple comparisons.

10. The association of keratinocyte modules and the mapping of immune modules to specific myeloid and T cell subtypes are interesting. Do any modules uncover novel biology or candidate regulators not previously implicated in psoriasis?

11. The identification of IL23A as a highly connected node in the MYE-M2 module is notable. What specific downstream immune pathways are co-expressed or co-regulated with IL23A in the MYE-M2 module? Is there any evidence of LC–T cell

interaction, especially with Tc17 or Th17 cells, in the dataset that would support functional IL23 signaling? Is IL23A hub status driven by lesion-specific expression or broader co-regulatory structure? I believe further in-depth analysis and/or experimental validation would be needed.

12. The clustering of keratinocyte, myeloid, and T cell modules could reveal evidence of cross-cell-type communication. Can the authors extend this analysis to infer ligand-receptor interactions to validate and expand these findings?

Reviewer #3

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
I thank the authors for carefully considering my comments on the initial submission and providing a very thorough response; I appreciate the substantial additional work this has entailed. In many cases my concerns have been satisfactorily resolved. However, there are several places where important issues remain.

The authors have partially taken on board my comment that their manuscript ignores three recent large psoriasis GWAS meta-analyses, e.g. adjusting the number of unreported loci that they claim to find. However, their Results section (lines 132-136) still claims “Previously unreported loci were mapped to genes implicated in immune-related functions (IRF2BP2, CXCR4, NRROS, ULBP3, IL7R, CRTAM, SOCS3, and NFATC2), mitochondrial functions (SDHD, ACOXL, and BCO2), and cell growth and differentiation (CDC7, POU2F3, PIK3R5, and MED15)”. Considering the meta-analysis published in Nature Communications last year (PMID: 40021644): not only are some of the listed genes located in loci identified by that study, but some actually appear in the main text of that paper. This raises serious doubts about the authors’ appreciation of the existing body of knowledge concerning psoriasis genetics.

I also feel that the authors have not fully understood or addressed my point regarding the functional enrichment in synovial fluid. They have added (line 171) “This suggests a high genetic overlap between psoriasis and psoriatic arthritis”. I still disagree with this conclusion. Psoriasis cases in their study will be very strongly enriched for the presence of psoriatic arthritis compared to controls; their GWAS meta-analysis is to some extent therefore also a psoriatic arthritis GWAS and they cannot make comparisons as if it were a distinct disease. On the same point I still disagree with the statement in Discussion: “which may explain the progression to psoriatic arthritis observed in some patients”. And at the end of the Discussion: “Comparative studies on cutaneous psoriasis and psoriatic arthritis may offer additional insight into shared and distinct disease mechanisms” – as pointed out in my review of the initial submission, such genetic studies do already exist.

Minor comments:

1. Results lines 107-113: “we compared polygenic risk scores (PRS) between ICD-diagnosed psoriasis cases, self-reported cases, and controls […] 73.85% for UK Biobank and 37.5% for 23andMe of cases are likely true positives” – it has been shown that the PRS distribution is higher on average in severe psoriasis cases than in mild psoriasis cases (PMID: 41408349). So the authors’ inference about true vs false positive cases in UKB/23andMe is not valid: it may be that there is just lower average psoriasis severity in these groups.

2. Results line 121: “identified a total of 198 independent SNPs from conditional and joint (COJO) association analysis across 125 independent loci” – I still feel the use of “independent” twice here is confusing and unclear. The meaning should be made more explicit, or preferably use another word (e.g. distinct loci).

3. Results lines 150-52: “We compared 106 independent SNPs located in 72 LD blocks that showed statistically significant heritability with 42 independent SNPs within the 152 non-significant LAVA regions identified in the GWAS.” - The authors have not stated any findings here. In the next sentence they say that SNPs were categorised into four classes; I think this classification is sufficiently bespoke that they should direct the reader to the Methods here.

4. Results line “These patterns suggests that the genetic architecture of psoriasis may diverge from that of allergic diseases” – I disagree that the revised p-value of 0.0005 for atopic dermatitis supports this conclusion.

5. Results line 270-73: “Notably, RGS14, NEK6, PRSS53, and PRDX5 exhibited opposite directions of effects on psoriasis risk between the blood and skin expression levels. For example, RGS14, NEK6, and PRDX5 exhibited negative effects in the skin eQTL datasets, whereas their effect directions were positive in the blood eQTL datasets.” – I still feel the wording here needs to be more carefully considered, as it remains imprecise. What I believe the authors are actually describing is that the same alleles that increase psoriasis risk are associated with lower expression of these genes in skin eQTL datasets,

but higher expression in blood eQTL datasets.

6. In the same part of the results, I also feel that the authors should treat with more caution the genes where HEIDI p-values are found to be <0.05 and therefore the analysis is less convincing that the causal variants are shared.

7. Results lines 306-07: “Pathway analysis of genes with decreased connectivity in lesional samples revealed the enrichment of lipoxin (LX) biosynthesis pathway” – needs further interpretation. What does lower connectivity actually mean?

8. Line 331-32: “were differentially active between non-lesional and lesional skin, as well as between lesional and treatment conditions” – for clarity should the latter comparison be between *baseline* lesional and treatment conditions?

9. PASI = Psoriasis Area and Severity index – acronym is stated incorrectly in line 343 and Supp Fig. 17 legend.

10. Discussion line 370 – for clarity, consider “lacked *genome-wide* significant signals”.

11. Discussion lines 382-84: “With the increased statistical power of our GWAS, we detected stronger genetic associations with conditions such as myocardial infarction, asthma, and anxiety disorders.” – are they actually stronger (larger magnitude)? Or similar magnitude to earlier estimates but now with lower p-values?

12. Discussion lines 389-91: “The observed positive genetic correlation between sedentary behavior and increased cardiovascular disease risk in individuals with psoriasis may therefore reflect the shared genetic factors underlying these phenotypes” – I don’t believe the authors have looked at CVD risk in individuals with psoriasis; I think the authors intend a different meaning and this sentence needs to be reworded to be clearer.

13. Discussion lines 403-05: “Cells in the outermost nucleated epidermal layer, which is characterized by pronounced cornification features, showed the strongest association with psoriasis” – if I understand the proposed novelty of this correctly, it is about the potentially causal role of the later-stage epidermal cells (and that these cells are not just ‘passengers’ in the process – they are to some extent causal and not just consequential). Perhaps this point can be made more clearly.

14. Discussion lines 472-73: “One possible hypothesis is that immune cells, keratinocytes and endothelial cells may already possess a genetically determined susceptibility to psoriasis.” – since cells cannot have psoriasis, I think this should be reworded.

15. Methods: the stated date of accession of GWAS Catalog remains October 22, 2023. Does this remain correct following the revisions, where the authors have included newer studies?

16. Methods line 571: “We investigated the two cases in the contingency table of the local heritability” – do the authors mean ‘two classes of LD block’ here?

17. Since I feel it is a limitation of the analysis approach, I feel that “original effect estimates” (line 681) should have added the word “marginal” as the authors disclosed in their rebuttal. I think it would also be prudent to disclose that one of the assumptions of the coloc method is of a single causal variant underlying the tested association signals, and in the authors’ use case this may not always hold.

18. Methods lines 765-66: “In this study, we compared non-lesional, lesional, and treatment conditions using IL-23 inhibition treatment datasets focusing on keratinocytes, myeloid cells, and T cells.” – I feel more detail is needed on the analysis approach. For example, are these pairwise or multivariable comparisons?

19. In the newly added Supplementary Figure 7 – part C, suggest clarifying the legend (“Significant”, “NS”) to indicate what is meant by significant here.

20. In the newly added Supplementary Figure 13 – part A, it’s not clear to me which are the high heterogeneity cell types or where the difference between lesional and non-lesional tissues is significant. Perhaps these features could be added to the plot? In part F, what do the line and point colours mean?

21. The authors state in their response (response document line 411) that local heritability block classifications have been added to Supplementary Table 6 but I do not see these in the submitted version of this table.

22. I thank the authors for correcting my misunderstanding in relation to minor comment 11 on the original submission (response document line 681). The comment related to the consideration of gene modules enriched for IL23 pathway mechanisms, when the data came from patients treated with IL23 inhibitors. If I now understand correctly, the modules were already defined in the Ma dataset, and not dervied directly from the Francis data. Perhaps this point should be made more explicitly.

Reviewer #2

(Remarks to the Author)

We thank the authors for their thoughtful responses to the initial comments and the substantial additions to the manuscript. However, several critical concerns remain that must be addressed before this work can be considered for publication.
1: We appreciate the detailed explanation of the Duffy approach and agree that Supplementary Figure 3 substantially strengthens the manuscript.
2: Supplementary Figure 1 is an excellent addition that enhances the paper's clarity.
3 (Critical): While we acknowledge the inherent limitations of using publicly available datasets, we remain deeply concerned about the lack of demographic information, particularly regarding sex and age. These variables are standard confounders routinely adjusted in any type of transcriptomic analyses. Furthermore, the datasets employed appear heavily skewed toward male participants, raising serious questions about the generalizability of your findings to female populations and across age groups. Please address this limitation explicitly in the main text and discuss how sex and age differences might impact your result.
4: Lines 1092-1094 remain unclear. When stating that "scDRS analysis robustly and consistently revealed significant enrichment in dendritic cell and T cell clusters," what is the specific comparison being made? Are these cells enriched when comparing pre- to post-treatment samples, or when comparing diseased versus healthy tissue? Additionally, Supplementary Figure 18's heatmap is difficult to interpret due to the confusing notation of ligands (L), receptors (R), and left/right (L or R) comparisons. Please explicitly define all terms in the figure legend and clarify what each comparison represents.
5: We appreciate the added clarity. We recommend incorporating Supplementary Figure 12b into a main figure, as it provides important context for understanding the results.
6-8: Thank you for the additional descriptions.
9: We appreciate the clarification. Please also add the p-value threshold to the Methods section, as currently only the expected value calculation is described.
10-12 (Critical): While we appreciate the new computational analyses, they do not provide the mechanistic validation necessary to support the biological insights currently being claimed. The identification of cell-cell communication represents hypothesis generation rather than hypothesis testing. To substantiate your claims, functional validation experiments are essential. Specific examples from the manuscript:
CD80-CTLA4 interaction
Basal keratinocyte-cDC2A-T cell trio
Please provide experimental validation demonstrating the functional relevance of these pathways in your disease context. What is the mechanistic basis for these interactions? Does disrupting one component alter the disease phenotype or treatment response? Computational prediction alone is insufficient to establish biological significance. Without such validation, the manuscript remains primarily descriptive.

Reviewer #3

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have done an excellent job of responding to my comments.

However, I think there is one missing figure panel: the proposed new Supp Fig 13 panel B sounds like it should fully address my concern in minor comment 20, but is missing in the resubmission.

If I were being very picky, I would also note that:
- In response to minor comment 1, I don't think it is quite correct to say that 73.85% of the UK Biobank cases "met the criteria for severe psoriasis" while the remainder may "represent milder disease severity" (and similar for 23andMe). If this were the case, I believe the estimate would come out higher than 73.85%. Isn't the point that the total genetic liability across cases is *consistent with* 73.85% severe psoriasis and the remainder *unaffected* (misclassified)? But it doesn't mean that the distribution actually looks like this; in reality it could be that a much higher proportion are true cases but with lower average genetic liability (perhaps due to lower severity).
- In response to minor comment 16, I still find the revised phrasing "We investigated the two classes in the contingency table" to be unclear.

Beyond these minor points, I have no remaining major concerns. Congratulations on a through, insightful and well-written exploration of psoriasis genetics and mechanisms.

Reviewer #2

(Remarks to the Author)
The authors address my comments. No more comments.

Manuscript Number: NCOMMS-25-41335

Manuscript: Genome-wide association study of 1 million people and single-cell analysis for psoriasis identify disease-associated cell subtypes and novel therapeutic targets

Responses from the Authors for Review Comments:

In response to the reviewers' comments, we have conducted additional and thorough analyses and validation, which have enabled us to make clear and novel contributions. The responses to the reviewers' comments are highlighted in blue and the revised text in the manuscript is highlighted in red.

Reviewers' Comments:

Reviewer #1:

Hyeobin Jo and colleagues report a GWAS meta-analysis of psoriasis, identifying a number of previously unreported susceptibility loci. They proceed to integrate their GWAS results with gene expression data, using various methods applied to both bulk tissue and single-cell RNA-seq data, to propose therapeutic targets and draw inferences about cell-type specific pathobiology.

I commend the authors on a very well written manuscript, which describes a coherent and competently conducted series of analyses combining a large amount of data relevant to the genetic and functional genomic basis of psoriasis. The methods are largely appropriate and conclusions are measured. My main concern with the study is whether the findings are sufficiently novel.

Response: We greatly appreciate the reviewer's thoughtful evaluation of our study and the helpful comments. Point-by-point responses to the reviewer's comments have been provided.

The authors queried GWAS Catalog for known psoriasis susceptibility associations in October 2023, but since then further psoriasis meta-analyses have been published (e.g. PMID: 38763176, PMID: 39885523, PMID: 40021644). The last of these in particular, which they cite as reference 11, found and discussed many of the genes that are described here as previously unreported. Furthermore, these previous studies all describe various downstream analyses considering heritability and genetic correlation with other traits. It is not clear where the current manuscript provides novel insights.

Response: Thank you for the reviewer's valuable comment. As the reviewer suggested, we have updated the GWAS Catalog to the latest version (as of October 2, 2025), which includes the significant loci of three papers. We have redefined the novel loci based on previously reported psoriasis-susceptibility loci. Consequently, 17 loci still remained as previously unreported loci. We have updated the **Results**, **Supplementary Tables**, and the related **Figures**.

[Updated to the Abstract, page 3, lines 35–37]

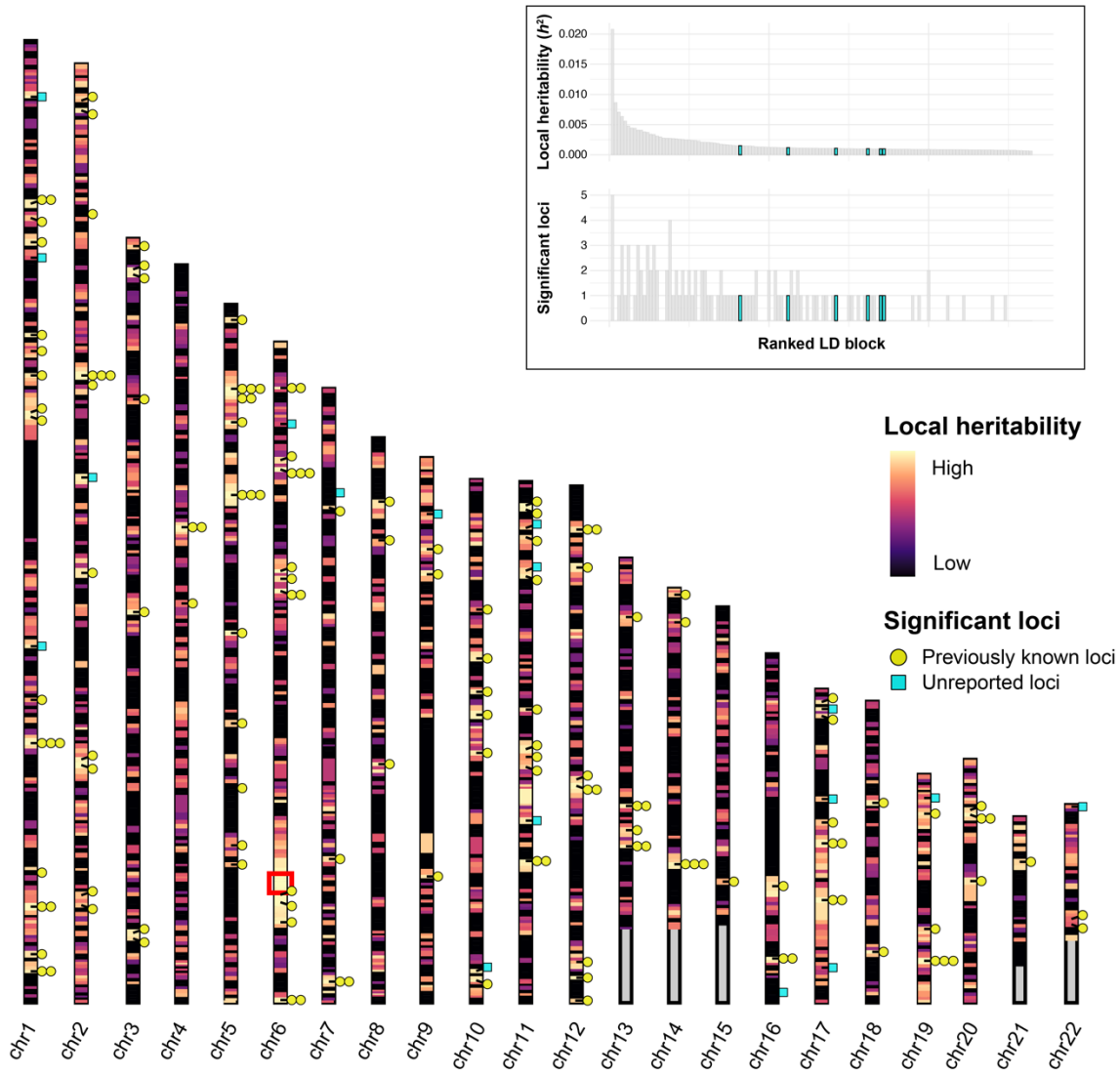
We conducted a large-scale genome-wide meta-analysis of individuals of European ancestry (n = 1,131,685) and identified 125 independent susceptibility loci associated with psoriasis, including 17 previously unreported loci.

[Updated to the Results, page 8, lines 120–124]

Based on the genome-wide significance threshold of $P < 5 \times 10^{-8}$, our meta-analysis identified a total of 198 independent SNPs from conditional and joint (COJO) association analysis across 125 independent loci, including 17 previously unreported loci (**Fig. 2 and Supplementary Fig. 4**).

[Modified Figure 2]

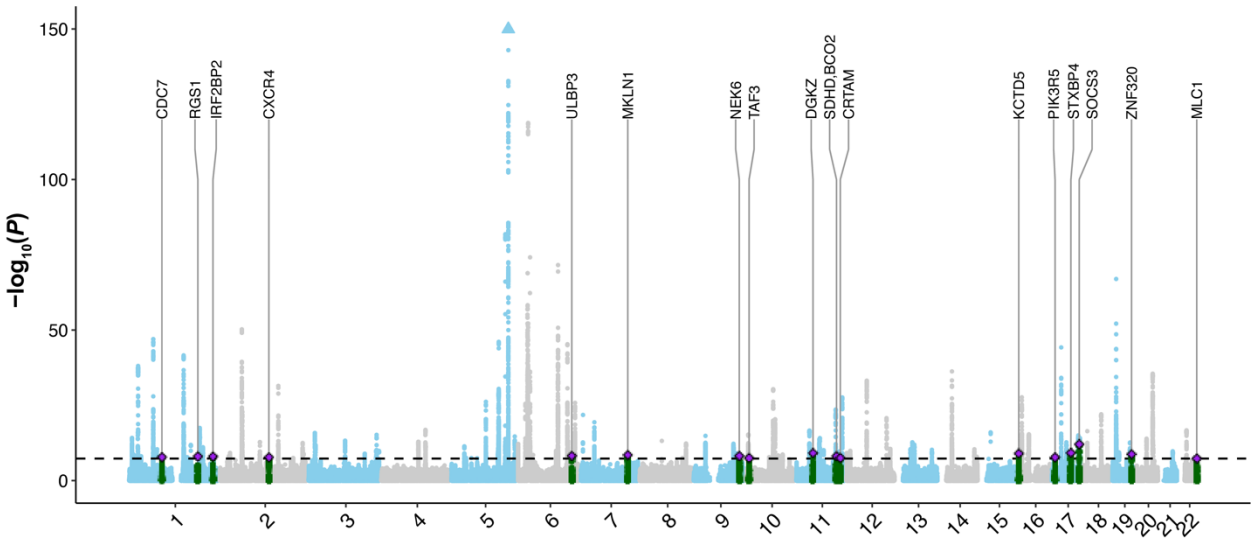
Figure 2. A karyotype plot for the psoriasis meta-GWAS result.



[Modified Supplementary Figures 4 and 5]

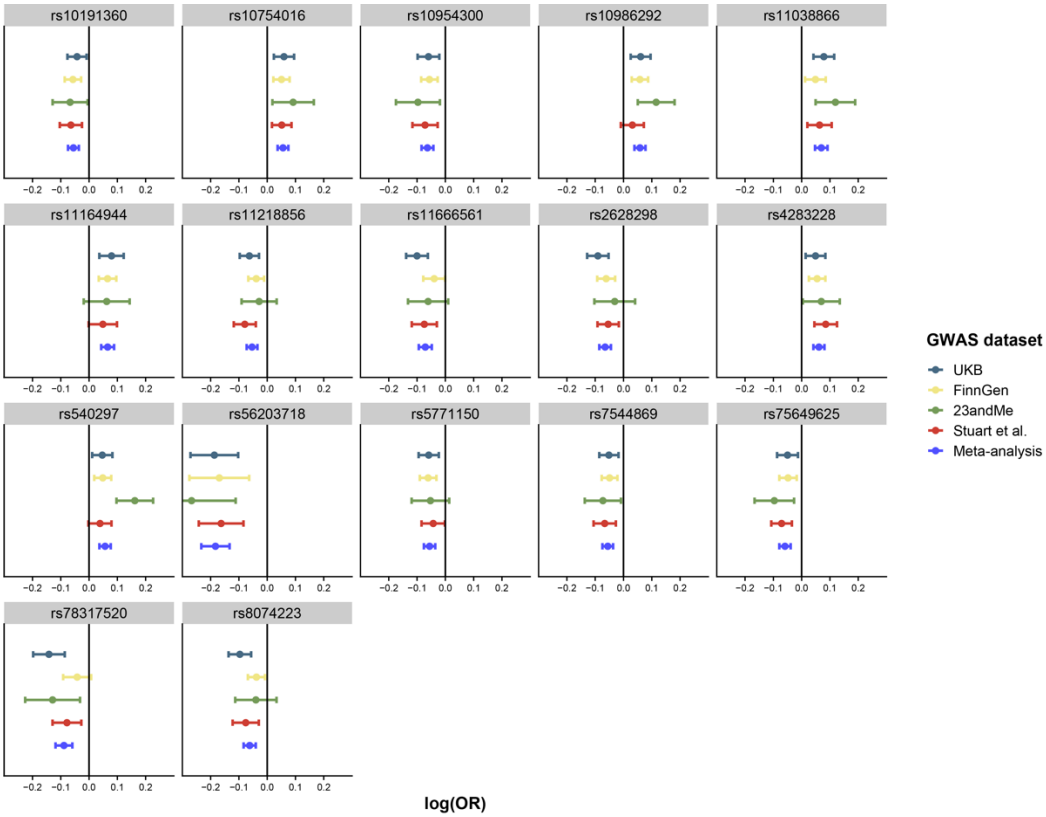
Supplementary Figure 4. Manhattan plot of psoriasis meta-GWAS

Newly identified loci are shown as dark green points. The lead SNP in each region is shown as a purple diamond, and SNPs with *p*-values $< 1 \times 10^{-150}$ are clipped and shown as triangles. SNPs in the MHC region were excluded from the analysis. The mapped genes represent the genes nearest to the lead SNP.



Supplementary Figure 6. Forest plots of effect sizes for newly identified loci.

Newly identified 17 lead SNPs from individual genome-wide association study (GWAS) data and the results of our meta-analysis are shown. OR, odds ratio.



[Updated Supplementary Table 3]

Table S3. List of independent significant SNP from GCTA-COJO

SNP	CHR	BP	ALT	REF	FRQ	BETA	SE	P	Direction	HetISq	HetPVal	Mapped genes	Loci
rs80278967	1	8209572	G	A	0.9096	0.1347	0.0175	1.39E-14	----	60.6	0.05465	*ERRFI1*	Reported
rs11121131	1	8272231	G	A	0.7327	-0.0788	0.0101	6.09E-15	++++	32.7	0.2162	*SLC45A1*	Reported
rs4846085	1	12050634	T	C	0.3205	-0.0596	0.0099	1.74E-09	----	39.6	0.1745	*MFN2*	Reported
rs10794648	1	24518206	T	C	0.2591	-0.0878	0.0106	1.20E-16	----	84.5	0.0002295	*IFNLR1*	Reported
rs7542079	1	25294607	T	C	0.4655	-0.1185	0.0091	9.18E-39	----	0	0.4839	*RUNX3*	Reported
rs12040146	1	33589426	T	C	0.2647	0.0599	0.0104	8.43E-09	++++	0	0.8492	*ADC*	Reported
rs11209006	1	67619259	T	G	0.6638	-0.0671	0.0096	2.76E-12	----	60.8	0.05368	*C1orf141*	Reported
rs11805303	1	67675516	T	C	0.2825	0.0896	0.01	3.25E-19	++++	77.2	0.00432	*C1orf141,IL23R*	Reported
rs11581607	1	67707690	G	A	0.9406	0.3037	0.0209	7.70E-48	----	85.3	0.0001437	*IL23R*	Reported
rs34517439	1	78450517	C	A	0.8857	-0.0938	0.0142	3.96E-11	++++	57.1	0.07193	*DNAJB4,GIPC2*	Reported

(The remaining rows are omitted.)

We agree with the reviewer's comment regarding the downstream analyses of the GWAS, which were similar to those described in three previous studies. However, we also provided novel findings that were not addressed in earlier studies and improved methodological approaches for drug target prioritization. Our contributions are summarized as follows:

(1) Increased statistical power of GWAS meta-analysis and downstream analyses.

Our meta-GWAS analysis included the largest sample size among the psoriasis GWAS studies published to date. A larger sample size provided increased statistical power and identified previously unreported psoriasis susceptibility loci. The higher statistical power resulting from the increased sample size enabled us to achieve strong statistical significance for these loci and the subsequent downstream analyses of associated traits.

For example, our GWAS with increased power achieved more precise estimates and stronger significance for the same traits such as myocardial infarction, inflammatory bowel disease,

asthma, and anxiety disorders in the genetic correlation analysis. The comparative results with those of previous studies are summarized in **Response Table 1**.

Response Table 1. Comparison of genetic correlation analysis with other studies.

Trait	Study PMID	Genetic correlation r_g (standard error)	*p*-value
Myocardial infarction	40021644	0.116 (0.046)	0.012
	Ours	0.161 (0.041)	8.30E-05
Inflammatory bowel disease	39885523	N.A.	< 0.001
	38763176	0.272 (N.A.)	1.19E-08
	Ours	0.284 (0.041)	2.34E-11
Asthma	40021644	0.234 (0.094)	0.013
	39885523	N.A.	N.S.
	Ours	0.166 (0.048)	5.0E-4
Anxiety disorder	40021644	-0.001 (0.051)	0.992
	Ours	0.223 (0.053)	2.68E-05
HDL cholesterol	40021644	-0.147 (0.039)	1.44E-04
	Ours	-0.126 (0.028)	7.82E-06
ADHD	Ours	0.220 (0.037)	4.27E-09
Urate	Ours	0.116 (0.024)	1.35E-06
Health satisfaction	Ours	0.244 (0.036)	1.09E-11
Time watching television	Ours	0.144 (0.025)	5.34E-09

N.A., not applicable; N.S., not significant.

(2) Expanded genetic correlation analysis

Our genetic correlation analysis also included several traits that have not been investigated in previous studies (**Response Table 1**). Notably, we identified significant positive associations between psoriasis and traits such as ADHD, urate levels, health satisfaction, and time spent watching television, which had not been previously reported. Specifically, the time spent watching television is a type of leisure sedentary behavior that was previously shown to be causally associated with cardiovascular diseases [**Ref. 1**]. The observed positive genetic correlation between increased cardiovascular disease risk and sedentary behavior in patients with psoriasis may indicate shared genetic factors underlying these phenotypes. Investigating the

shared genetic architecture between lifestyle factors and psoriasis is an important research direction, and we have added the relevant content to the **Discussion** section.

References

Ref. 1: van De Vegte YJ, Said MA, Rienstra M, van Der Harst P, Verweij N. Genome-wide association studies and Mendelian randomization analyses for leisure sedentary behaviours. Nature Communications 11, 1770 (2020).

[Added to the Discussion, page 20, lines 379–391]

Significant genetic overlap with diverse diseases suggests that a shared genetic architecture influences multiple conditions. Compared to previous GWAS-based studies, our analyses enabled a more precise and expanded assessment of the genetic relationships between psoriasis and other traits. With the increased statistical power of our GWAS, we detected stronger genetic associations with conditions such as myocardial infarction, asthma, and anxiety disorders. Additionally, our genetic correlation analysis included a broader range of traits that have not been examined in earlier studies. We identified novel genetic correlations with traits, such as attention deficit/hyperactivity disorder (ADHD), urate levels, health satisfaction, and time spent watching television. Time spent watching television is a form of leisure-time sedentary behavior that has been previously shown to be causally associated with cardiovascular disease. The observed positive genetic correlation between sedentary behavior and increased cardiovascular disease risk in individuals with psoriasis may therefore reflect the shared genetic factors underlying these phenotypes.

(3) Integration of GWAS and single-cell data for drug target prioritization

Finally, our analysis integrated GWAS findings with single-cell transcriptomic data, thereby enabling the identification of psoriasis-associated cell types. For drug target prioritization, we combined GWAS, eQTL, and single-cell information and performed rigorous filtering based on multiple lines of genetic evidence. Similar drug target analyses were performed by Zhang et al. (PMID: 39885523) and Dand et al. (PMID: 40021644); however, Zhang et al. did not utilize single-cell information, and Dand et al. only utilized TWAS and gene expression for candidate

gene discovery. Additionally, we have expanded our analyses to investigate the underlying disease mechanisms using spatial transcriptomics, cell-cell interactions, and module analysis through the revision process.

We have added concise summaries of previous findings, their limitations, and how our study addresses these points in the **Introduction** section.

[Added to the Introduction, page 5, lines 76–82]

In summary, although GWAS facilitate the exploration of broad genetic susceptibility across large populations, integrating single-cell data enables a more in-depth examination of causal genetic variant effects. Recent studies have attempted to understand psoriasis-related mechanisms and identify novel therapeutic target genes. However, most studies have analyzed single-cell gene expression data and genetic information separately, and have not fully integrated multiple data modalities, including spatial context and cell-cell interactions, to understand the underlying biological mechanisms.

> Likewise, the authors re-analyse single-cell RNA-seq data from two previous studies (Ma et al 2023, Francis et al 2024), including a weighted gene coexpression network analysis (on the Ma data? – not explicitly stated). While these analysis are clearly and logically laid out, and thorough, I am again unclear as to where the findings here highlight new biology beyond the original papers. I think the integration of GWAS and scRNA-seq using the single-cell disease relevance score is a new and potentially interesting angle; however, I think a clearer description is needed of what this score represents (lines 189-196). I also found the description of within cell type scDRS heterogeneity hard to follow (lines 196-202) and am not sure what conclusion to take from it.

Response: We thank the reviewer for the constructive comments that have strengthened our manuscript. We constructed a weighted gene co-expression network using the Ma et al. dataset and then projected the network structure onto the Francis et al. dataset to examine the changes across non-lesional, lesional, and treatment conditions. The corresponding description has been clarified and revised in the **Methods**.

[Rephrased to the Methods, page 35, lines 707–708]

We performed a weighted gene co-expression network analysis (WGCNA) on within each cell type using the hdWGCNA package with Ma et al.'s single-cell data.

The scDRS algorithm projects GWAS-derived genetic signals onto single-cell expression data, enabling us to prioritize cell types genetically implicated in psoriasis and to quantify their disease relevance with statistical significance. We have added a description of these implications to the **Methods** section.

[Added to the Methods, page 32, lines 643–648]

To quantify the relative disease enrichment of psoriasis risk genes at the single-cell level, scDRS analysis (version 1.0.2) was performed. The scDRS algorithm translates the GWAS-based genetic susceptibility to psoriasis into cell-level disease relevance scores by projecting GWAS signals onto single-cell expression profiles. This allowed us to quantify the cells and cell types that showed stronger genetic associations with psoriasis. This method uses MAGMA z-scores from psoriasis GWAS with preprocessed scRNA-seq data to calculate aggregated disease scores.

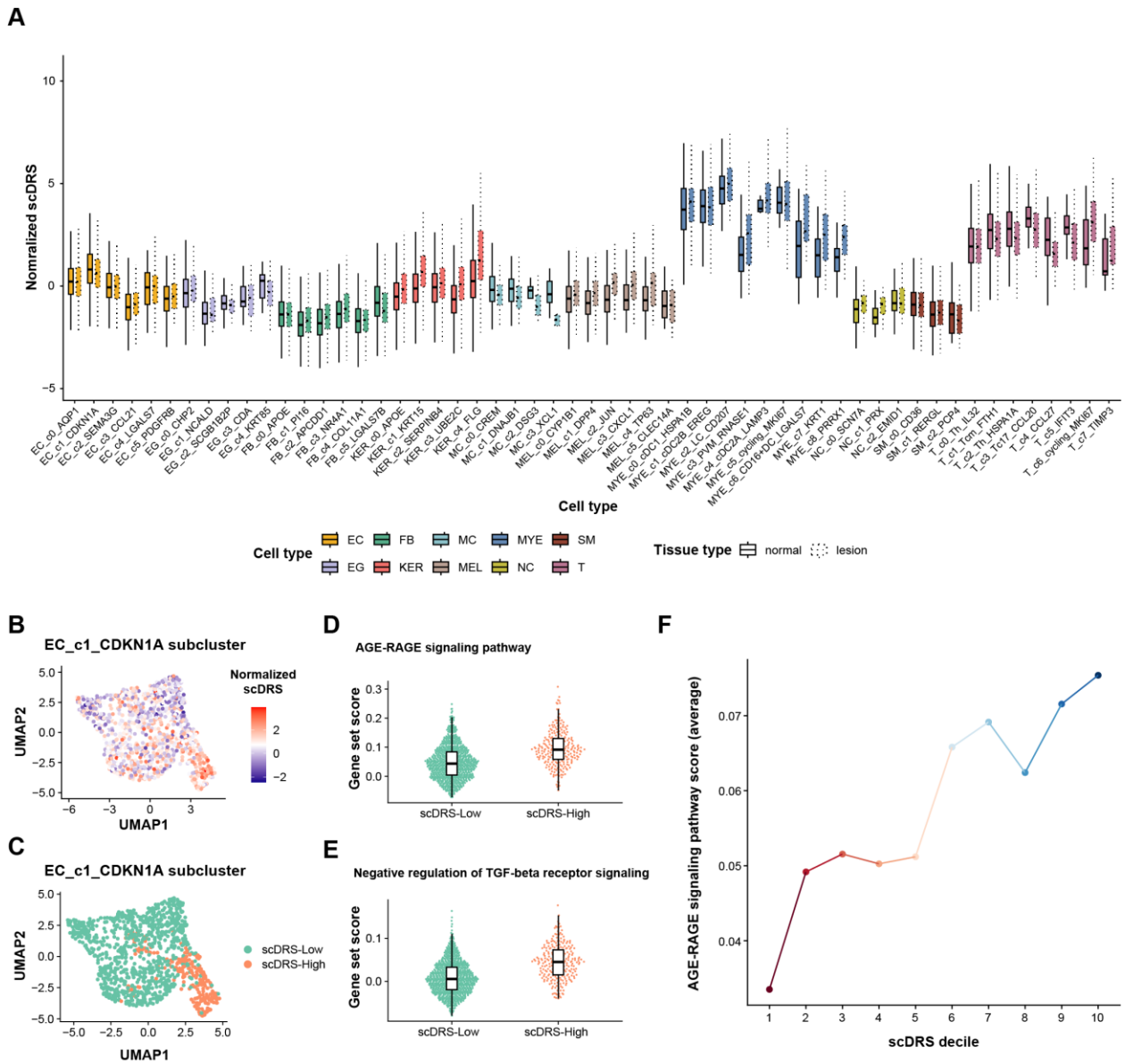
Within-cell-type heterogeneity reflects how scDRS scores differ among cells of the same cell type. This variation may arise from multiple biological factors, including differences between normal and lesional skin as well as the presence of cells at distinct differentiation stages within a given cell type. We have conducted an additional analysis to examine the potential implications of heterogeneity (**Supplementary Fig. 13**).

First, the distribution of scDRS scores showed a larger difference between normal and lesional skin in the cell types with significant scDRS heterogeneity (**Supplementary Fig. 13A**). In particular, the KER_c4_FLG keratinocyte cluster exhibited increased disease-relevance scores in the lesional state. This observation suggests that granular keratinocytes in psoriatic lesions tend to exhibit a higher overall expression of susceptibility genes identified by GWAS.

Secondly, heterogeneity suggests that disease relevance may be driven by a specific subpopulation of cells. For example, a significant number of cells were preferentially located in the outermost layers of the skin (**Fig. 4F**). In addition, we have examined the endothelial cell (EC) cluster EC_c1_CDKN1A, which showed marginal scDRS significance. Among the CDKN1A-enriched ECs, we identified a cluster of cells with particularly high scDRS scores (**Supplementary Fig. 13B, C**). Cells in this high-scDRS group showed increased expression of genes involved in the AGE-RAGE signaling pathway and the negative regulation of TGF-beta receptor signaling (**Supplementary Fig. 13D, E**). Notably, the enrichment of these functional signatures gradually increased with higher scDRS scores (**Supplementary Fig. 13F**). Collectively, these results indicate that cell populations with high heterogeneity provide opportunities for fine-grained analysis. Accordingly, we have added descriptions of these results to the **Results** section.

[Added Supplementary Figure 13]

Supplementary Figure 13. scDRS heterogeneity-based analysis. **(A)** Distributions of scDRS scores for each cell type stratified by the tissue type (normal and lesion). **(B)** scDRS score distribution of the endothelial cluster EC_c1_CDKN1A in the UMAP space. **(C)** Subclustering of the endothelial cluster EC_c1_CDKN1A. Distributions of gene set scores of two EC_c1_CDKN1A subclusters representing **(D)** the AGE-RACE signaling pathway and **(E)** negative regulation of the TGF-β receptor signaling pathway. **(F)** Average gene set scores per scDRS decile of cells in EC_c1_CDKN1A.



[Added to the Results, page 13, lines 244–257]

Potential implications of within-cell type heterogeneity

We further examined the within-cell-type heterogeneity of the scDRS scores across epidermal cell types. Cell types with significant scDRS heterogeneity showed larger differences in scDRS score distribution between normal and lesional skin (**Supplementary Fig. 13A**). Among the keratinocyte populations, the KER_c4 cluster exhibited higher scDRS scores in lesional skin than in normal skin, indicating increased disease relevance in the lesional state. We observed that cells with significant scDRS scores were preferentially localized in the outermost layers of the

skin (**Fig. 4F**), consistent with the heterogeneity within the defined cell populations. Among the ECs, the EC_c1 cluster showed marginal scDRS significance at the cluster level. Within this cluster, a subset of cells displayed particularly high scDRS scores (**Supplementary Fig. 13B, C**). These high-scDRS cells showed increased expression of genes involved in the AGE-RAGE signaling pathway and negative regulation of TGF-β receptor signaling (**Supplementary Fig. 13D, E**) and the enrichment of these pathways increased progressively with higher scDRS scores (**Supplementary Fig. 13F**).

[Added to the Discussion, pages 20–21, lines 402–417]

Notably, keratinocytes exhibited a continuous expression pattern from the basal to granular layers, reflecting the spatial organization of the epidermis. Cells in the outermost nucleated epidermal layer, which is characterized by pronounced cornification, showed the strongest association with psoriasis. The EC_c1_CDKN1A cluster, which was enriched in normal skin tissue, showed marginal significance in psoriasis (FDR = 0.069). These cells were characterized by the expression of *NFKBIA* and *SOCS3*, which suppress cytokine responses and regulate inflammatory signaling via negative feedback mechanisms (**Supplementary Table 13**). These findings suggest that dysfunction of this specific subset of endothelial cells may contribute to the pathogenesis of psoriasis. Within this cell cluster, cells with higher scDRS scores formed a distinct subcluster. This subpopulation showed higher expression of genes involved in the AGE-RAGE signaling pathway and negative regulation of TGF-β receptor signaling. Previous studies have reported that AGE-RAGE interactions are implicated in psoriasis and can suppress downstream TGF-β-mediated functions. Given the marginal statistical significance and increased analytical complexity, we excluded endothelial cells from downstream analyses. Further research will provide deeper insights into the role of endothelial cells in modulating immune cell function in psoriasis.

[Added to the Methods, page 31, lines 629–631]

To calculate the gene-set-based score for each cell, we used the *AddModuleScore* function with parameter nbins=12 and other default parameters.

Finally, in addition to single-cell-level genetic relevance and hdWGCNA analyses, we have performed cell-cell interaction analyses. We identified two novel findings related to the pathogenic mechanisms of psoriasis, which are described in response to subsequent comments.

> Overall, I do think the GWAS meta-analysis is probably a modest improvement on recent studies in terms of power, and this work represents an excellent synthesis of genetic and transcriptomic data on psoriasis to highlight candidate druggable targets. But the authors should state more clearly where previous studies have fallen short and left unanswered questions, and more carefully indicate how their findings contribute to answering them.

Response: We thank the reviewer for providing important comments regarding the contributions of our study. Although the increase in the sample size in our GWAS was modest, we believe that it provides more power for downstream analyses that integrate transcriptomic data. Accordingly, we have added results summarizing the recent advances and trends in psoriasis GWAS with improvements to our own GWAS analysis. We systematically surveyed all non-redundant European-ancestry GWASs on psoriasis (**Supplementary Table 4**). We summarized the association between the effective sample sizes and independent loci counts (**Supplementary Fig. 5**). Notably, compared with a previous psoriasis GWAS (Dand et al., 2025; PMID: 40021644), our study identified 17 previously unreported loci with an increased sample size and enabled the prioritization of additional psoriasis-associated genes.

[Added the Supplementary Table]

Table S4. Psoriasis summary statistics of sample sizes and independent loci counts.
Independent loci were defined based on the ±1-Mb window size around each independent COJO SNP (see **Methods**). If the full GWAS summary statistic file was not accessible, the number of independent loci was estimated from the paper description and the GWAS Catalog.
Neff, Effective sample size.

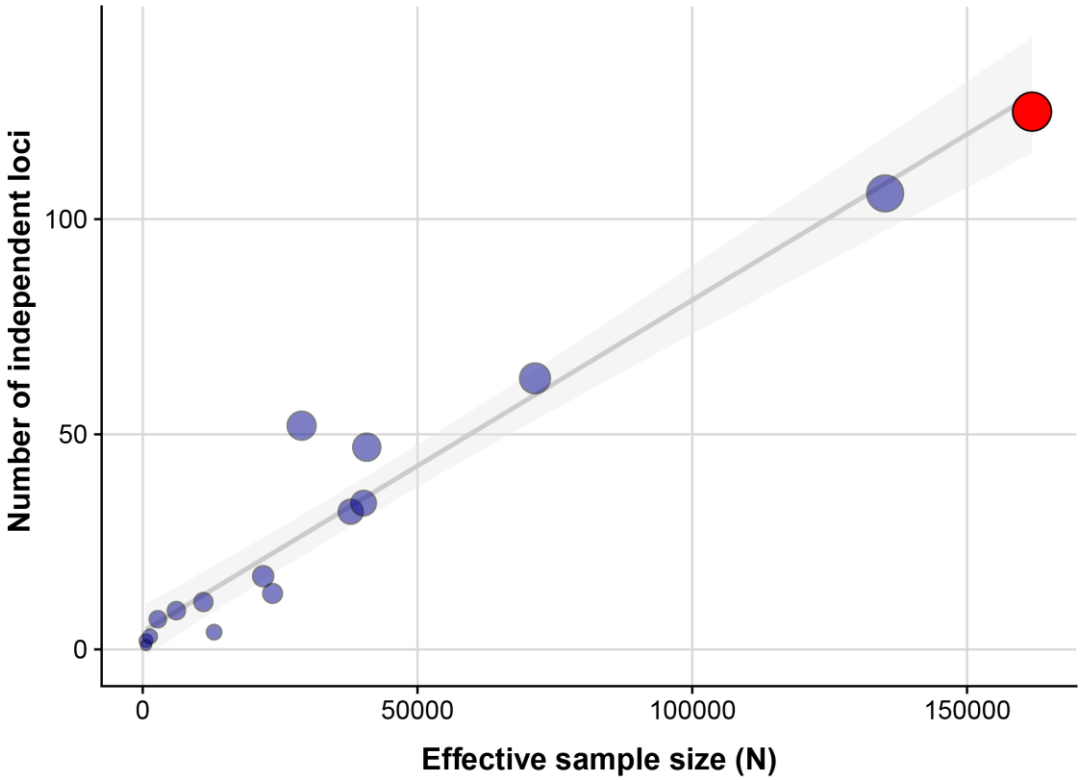
Source	PMID	Ncase	Ncontrol	Neff	Independent loci	URL
Capon et al., Human Molecular Genetics, 2008	18364390	318	288	605	2	https://www.ebi.ac.uk/gwas/studies/GCST000165
Liu et al., PLoS Genetics, 2008	18369459	218	519	614	1	https://www.ebi.ac.uk/gwas/studies/GCST000173

Ellinghaus et al., Nature Genetics, 2010	20953188	472	1,146	1,337	3	https://www.ebi.ac.uk/gwas/studies/GCST000836
Nair et al., Nature Genetics, 2009	19169254	1,359	1,400	2,758	7	https://www.ebi.ac.uk/gwas/studies/GCST000322
Strange et al., Nature Genetics, 2010	20953190	2,178	5,175	6,131	9	https://www.ebi.ac.uk/gwas/studies/GCST000833
Baurecht et al., American journal of human genetics, 2015	25574825	4,212	8,032	11,052	11	https://www.ebi.ac.uk/gwas/studies/GCST002738
Stuart et al., Nature Genetics, 2010	20953189	5,895	7,231	12,990	4	https://www.ebi.ac.uk/gwas/studies/GCST000834
Ellinghaus et al., Nature Genetics, 2016	26974007	6,530	34,213	21,934	17	https://www.ebi.ac.uk/gwas/studies/GCST005537
23andMe (Duffy's method adjusted)	-	6,045	264,984	23,641	13	https://research.23andme.com/dataset-access
Tsoi et al., Nature genetics, 2012	23143594	10,588	22,806	28,924	52	https://www.ebi.ac.uk/gwas/studies/GCST005527
UK Biobank (Duffy's method adjusted)	-	9,695	398,960	37,860	32	Performed in this study
FinnGen r10	-	10,312	397,564	40,205	34	https://www.finngen.fi/en/access_results
Stuart et al., HGG Advances, 2021	34927100	15,967	28,194	40,776	47	https://www.ebi.ac.uk/gwas/studies/GCST90019016
Tsoi et al, Nature Communications, 2017	28537254	19,032	286,769	71,390	63	https://www.ebi.ac.uk/gwas/studies/GCST004346
Dand et al., Nature communications, 2024	40021644	36,466	458,078	135,109	106	https://www.ebi.ac.uk/gwas/studies/GCST90472771
Our meta-GWAS	-	42,019	1,089,702	161,835	125	Performed in this study

[Added Supplementary Figure 5]

Supplementary Figure 5. Identified independent loci counts corresponding to effective sample size in psoriasis GWAS. The red circle represents the GWAS result from our study. Dot sizes are proportional to the effective sample sizes. Information on the psoriasis GWASs

included in this plot is summarized in **Supplementary Table 4**.



[Added to the Results, page 8, lines 125–128]

This GWAS included the largest sample size for psoriasis among the published studies, and a comparison of our GWAS with previous psoriasis GWASs based on the effective sample size (**Supplementary Table 4**) revealed an approximately linear increase in the number of significant independent loci (**Supplementary Fig. 5**).

As suggested by the reviewer's comment, we have structured our contributions around three key findings.

First, our results suggest that fibroblasts are unlikely to play a primary role in the initiation of psoriasis. Although several recent single-cell studies have reported fibroblast alterations in skin lesions and highlighted their potential importance, our integrated GWAS and single-cell analysis identified dendritic cells as the most strongly implicated cell type, followed by T cells and specific subsets of basal and granular keratinocytes. In contrast, fibroblasts showed limited enrichment of GWAS-associated genetic signals. We further validated these findings using

multiple independent single-cell datasets in which fibroblasts consistently exhibited low scDRS associations.

Second, our study demonstrated that lipid metabolism in Langerhans cells and granular-layer keratinocytes is a key component of psoriasis pathogenesis. While previous mouse studies have suggested that the dysfunction of lipid-related pathways in Langerhans cells may contribute to psoriasis, to the best of our knowledge, our study is the first to demonstrate this mechanism using human single-cell data. Through additional module-based connectivity analyses, we observed a reduction in the biosynthesis of lipoxins in Langerhans cells in lesional skin.

Third, during the revision process, we have incorporated cell-cell communication and spatial transcriptomics analyses to propose a novel hypothesis involving enhanced physical adhesion between basal keratinocytes and cDC2A, together with the localized suppression of T cell activity. Taken together, these analyses support the existence of epidermal layer-specific pathogenic mechanisms in psoriasis (**Fig. 6J**). This perspective provides an important conceptual framework for developing future therapeutic strategies.

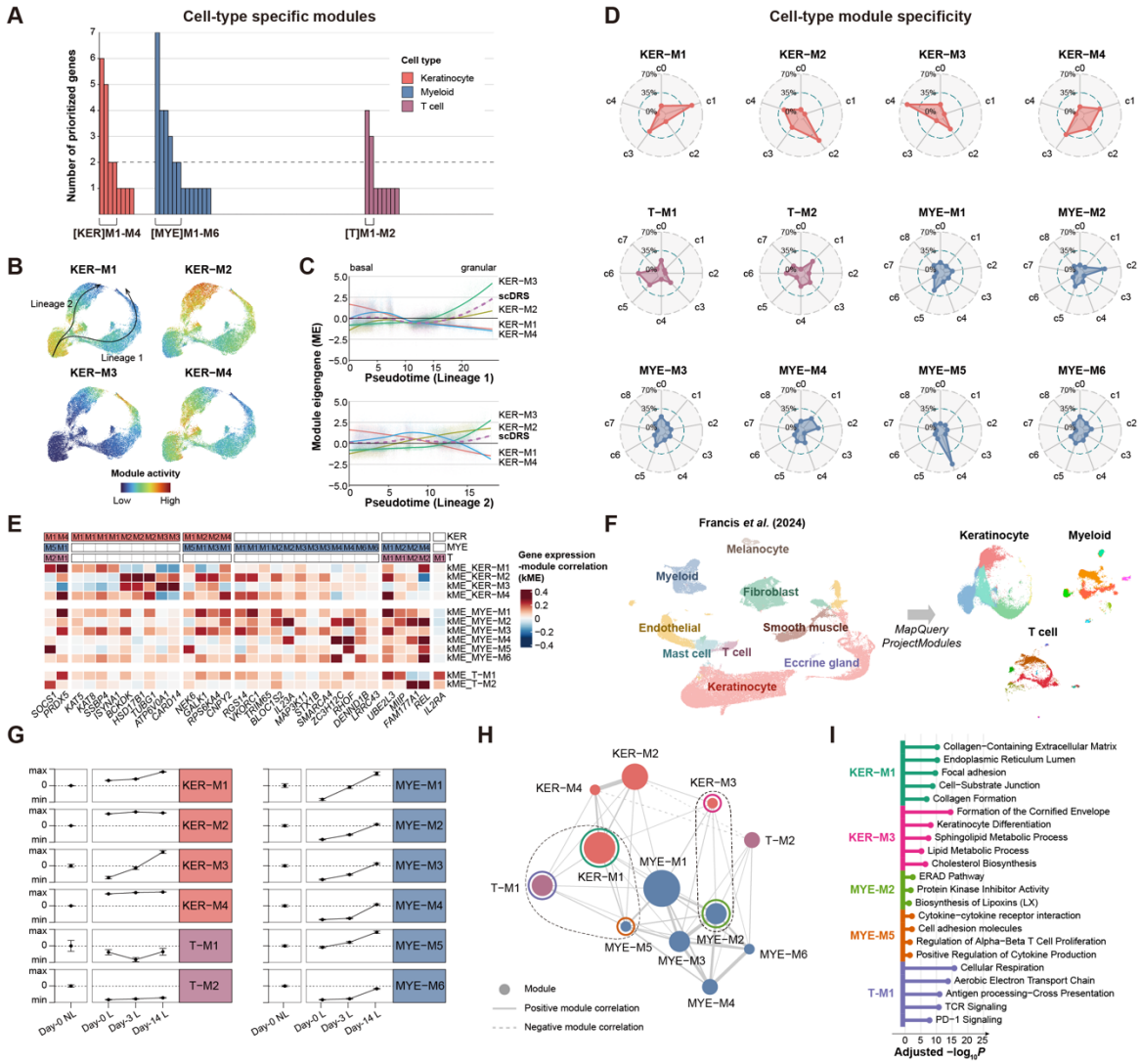
To clearly state our findings, we have summarized these results in a single paragraph in the **Discussion** section and added the panel **Fig. 6J** as a schematic illustration.

[Added to the Discussion, page 23, lines 453–459]

In summary, we propose two epidermal layer-specific pathogenic mechanisms in psoriasis based on integrative analyses of various IL-23 inhibition-based multimodal datasets (**Fig. 6J**). In the basal epidermal layer, enhanced physical interactions between keratinocytes, cDC2A, and T cells may be associated with localized immune suppression, which may contribute to the excessive proliferation of basal keratinocytes. Disrupted interactions between keratinocytes and Langerhans cells may be associated with the dysregulation of lipid metabolic processes in both cell types.

[Added Figure 6J panel]

Fig. 6: **Cell type-specific gene co-expression modules associated with risankizumab treatment. (A)** Cell type specific co-expression gene network identification using hdWGCNA, which identified 13, 49, 30 modules for keratinocyte, myeloid, and T cell, respectively. Each expressed gene was assigned to single module. The bar plot represents the number of assigned genes that were defined as therapeutic targets. **(B)** Module eigengenes (MEs) of keratinocyte module 1 to module 4 (KER-M1 to KER-M4). **(C)** MEs of keratinocyte modules change over the pseudotime of two keratinocyte trajectories. Dots represent the ME score of single cells. Smoothed curves with 95% confidence interval were fitted by the LOESS algorithm. scDRS scores of keratinocyte cells were plotted together (Dashed purple curve). **(D)** Cell type enrichment of 12 modules having two or more prioritized genes. The enrichments represent the proportion of average MEs for all cell subtypes in the major cell type. **(E)** Module memberships and connectivities of prioritized genes. Genes assigned to at least one of the 12 modules are shown. **(F)** Overview of procedure about risankizumab treatment data for psoriasis from Francis et al. **(G)** Temporal enrichment changes of MEs for non-lesions and lesion skins over treatment. NL, Non-lesion; L, Lesion. **(H)** A correlation network for 12 modules from 20 samples of Francis et al. (I) Gene-set pathway analysis for five modules of interest. Representative pathways with adjusted *p*-values < 0.10 were illustrated. **(J)** A proposed schematic illustration of two layer-specific mechanisms of psoriasis pathogenesis.



[Rephrased the Abstract, page 3, lines 40–42]

Finally, we prioritized 50 potential therapeutic target genes using multiple robust approaches and identified their cell subtype-specific activity patterns across non-lesional, lesional, and treatment conditions, thereby revealing layer-specific cross-cell-type interactions in psoriasis.

Minor comments on detail:

> 1. Line 108: A total of 198 SNPs were identified – I think clearer to state that these are independent association signals. Presumably there are many more individual SNPs that are associated. I think it also worth mentioning the significance threshold here.

Response: We thank the reviewer for the helpful comment. In our GWAS, we used GCTA-COJO to identify 198 independent SNPs (COJO SNPs). We then performed clumping in 1-Mb windows around these COJO SNPs to define independent loci. We have clarified these procedures in the manuscript and added a genome-wide significance threshold ($P < 5 \times 10^{-8}$).

[Revised to the Results, pages 7–8, lines 120–125]

Based on the genome-wide significance threshold of $P < 5 \times 10^{-8}$, our meta-analysis identified a total of 198 independent SNPs from conditional and joint (COJO) association analysis across 125 independent loci, including 17 previously unreported loci (**Fig. 2** and **Supplementary Fig. 4**). Excluding the major histocompatibility complex (MHC) region, 159 independent SNPs were mapped to 122 independent loci (**Supplementary Table 3**).

> 2. Local heritability analysis: around 106 COJO signals are in Bonferroni significant regions of local heritability, while 42 are not. Does this tell us anything about these 42 signals? The authors suggest that regions with high local heritability without significant loci may harbour the potential for novel discoveries. Many of the most significant regions matching this description are adjacent to psoriasis susceptibility loci and presumably residual LD drives the high local heritability here. Would it be worth distinguishing in Table S5 regions where this is or is not the case? Can the non-adjacent high heritability regions be characterised in any way?

Response: We thank the reviewer for the valuable comment. We agree that considering residual LD is important for this analysis. We examined the relationship between heritability of local LD blocks and the presence of significant SNPs within the block (**Response Table 2**). To further clarify the meaning of components 1 and 2 in Response Table 2, we additionally performed in-depth analyses.

Response Table 2. Contingency table for local heritability and genome-wide significance in LD blocks.

	GWS SNP exist	GWS SNP does not exit
LAVA significant	72 (106 independent SNPs exist in 72 regions)	(*ii*) 60
LAVA not significant	(*i*) 42	1,073

GWS, Genome-wide significant. Bonferroni-adjusted *p*-values were calculated based on multiple testing correction for a total of 2,495 LD blocks. *p*-value threshold = 0.05 / 2495. Only 1,247 LD blocks with calculated latent heritability are shown.

First, we confirmed that the local LD significance was associated with increased likelihood of observing significant SNPs, as supported by the chi-square test result ($P = 2.42 \times 10^{-80}$).
We examined 42 cases in which LD local heritability was not significant but contained significant SNPs (case (*i*) in the **Response Table 2**).

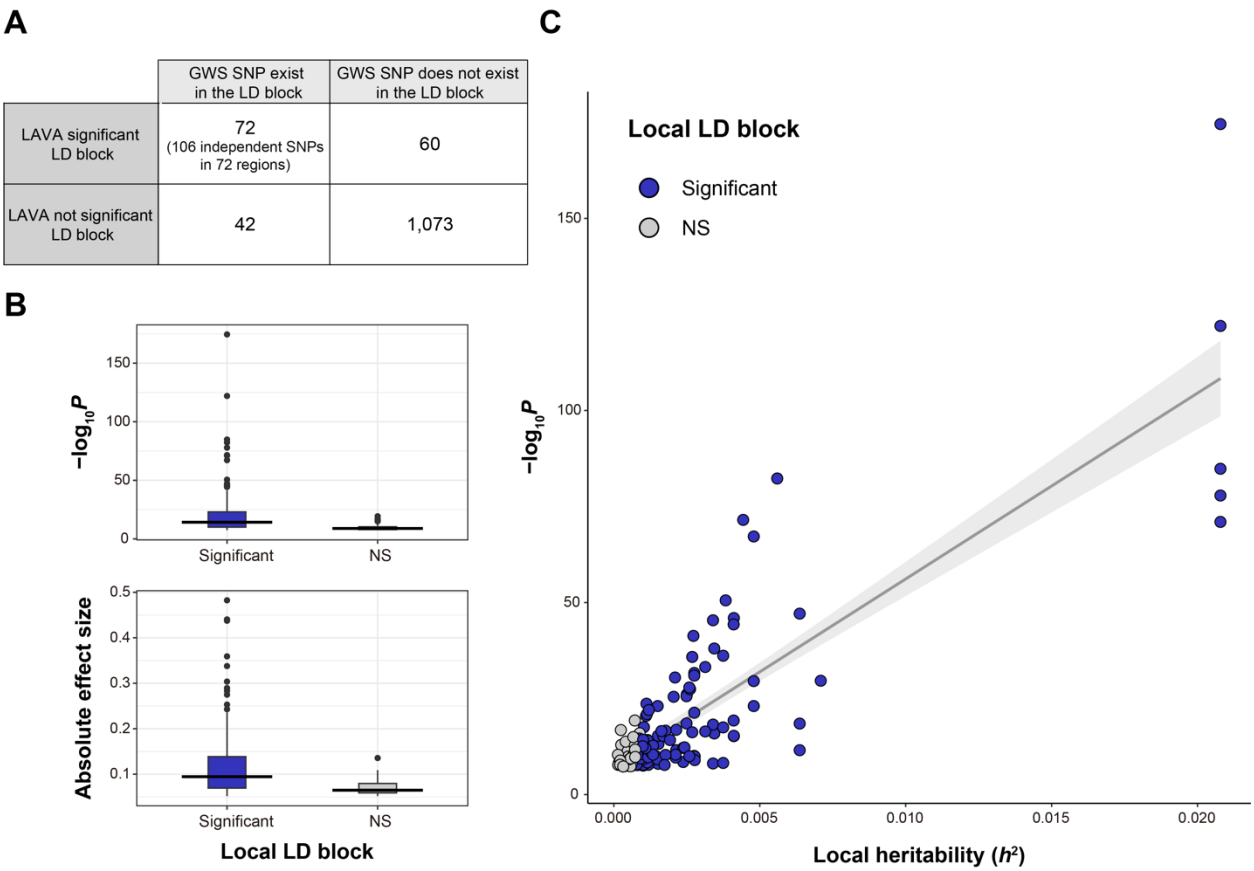
We compared 106 independent SNPs located in 72 LD blocks that showed statistically significant heritability with 42 independent SNPs within the non-significant LAVA regions identified in the GWAS (**Supplementary Fig. 7B, C**). The SNPs in case (*i*) generally exhibited smaller effect sizes and lower statistical significance than the SNPs located in the LAVA-significant regions.

[Added Supplementary Figure 7]

Supplementary Figure 7. The relationship between local heritability and genome-wide association significance.
(A) Contingency table of the heritability and presence of genome-wide significant (GWS) SNPs.
(B) Distribution of statistical significance and effect sizes for variants in significantly heritable LD blocks compared with variants in non-significantly (NS) heritable LD blocks.
(C) Scatter plot showing the relationship between local heritability and psoriasis association *p*-value of COJO independent SNPs.

A

	GWS SNP exist in the LD block	GWS SNP does not exist in the LD block
LAVA significant LD block	72 (106 independent SNPs in 72 regions)	60
LAVA not significant LD block	42	1,073



Next, we examined the 60 LAVA-significant regions where there were no genome-wide significant (GWS) SNPs (case (*ii*) in the **Response Table 2**). We defined the concept of potential for novel discovery by considering the inflated heritability due to residual LD, as suggested by the reviewer (**Supplementary Fig. 8A**). First, LD blocks located within ±10 Mb of the MHC region were classified as "Near MHC region". Next, we classified each LD block based on its local heritability and the presence of nearby significant SNPs within a 5-Mb region: blocks for which another block within the 5-Mb region showed higher heritability were classified as "Likely residual LD"; blocks with previously identified significant SNPs in other blocks within the 5-Mb region were classified as "Nearby GWS"; otherwise, blocks that exhibited the highest heritability in the region and no nearby GWS had been reported were classified as having "high potential for novel discovery". We have added this classification to the local heritability results table (**Supplementary Fig. 8B and Supplementary Table 6**).

We further investigated whether psoriasis-associated variants in these LD blocks had previously been reported in the GWAS Catalog and identified 18 independent loci from seven studies (**Supplementary Fig. 8C and Supplementary Table 7**). These results suggest that although such variants were not detected in the current GWAS, they have the potential for future discovery. Additionally, we have added details of the local heritability estimation in LAVA and related analyses to the **Methods** section. Moreover, the LD block with the highest local heritability among regions not yet associated with psoriasis (chr10:118,416,050–119,528,730) has previously been reported to be associated with metabolic traits such as HDL cholesterol, BMI, systolic blood pressure, prostate-specific antigen, and type 2 diabetes.

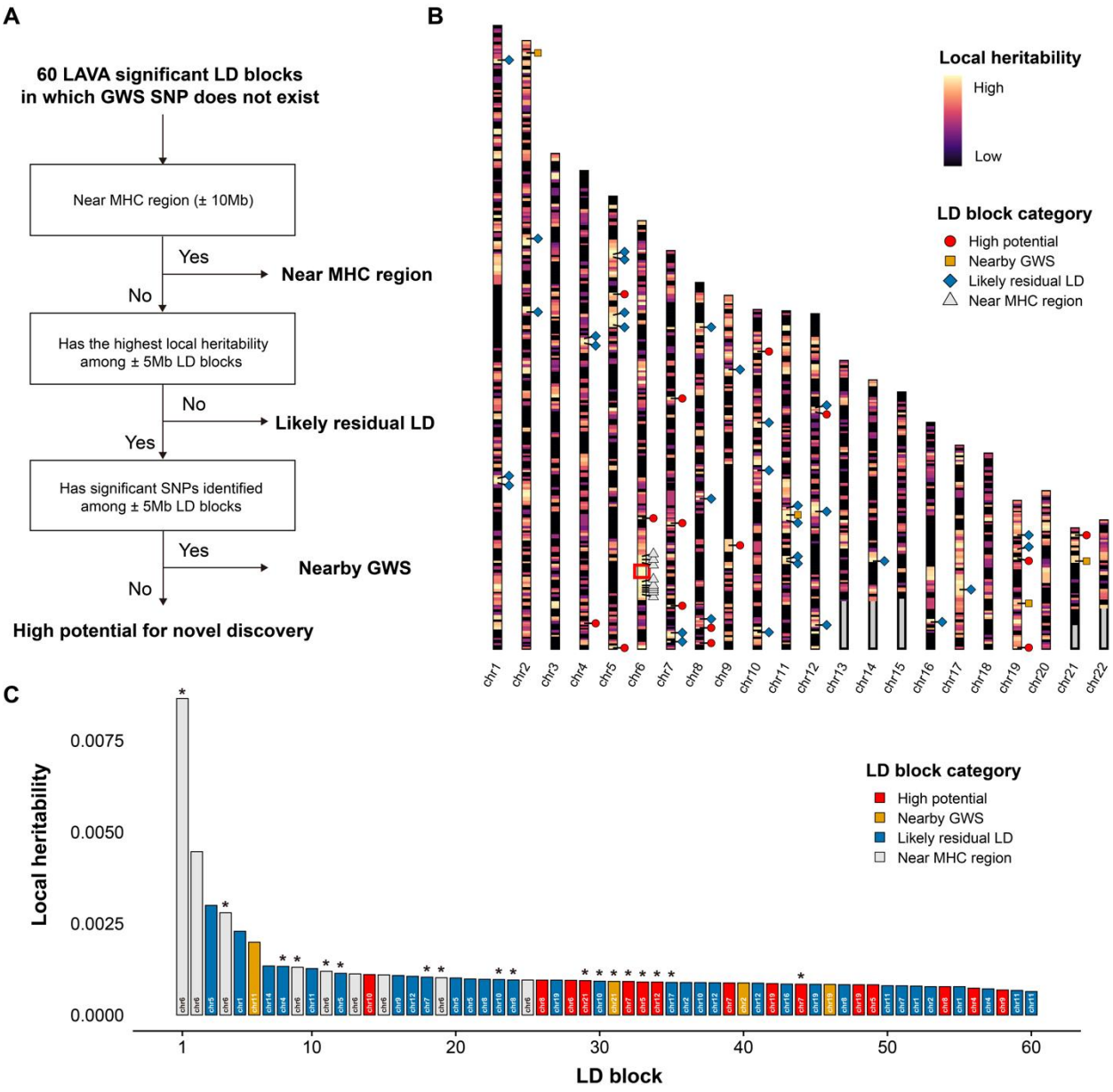
[Added Supplementary Figure 8]

Supplementary Figure 8. Estimating the potential novel discovery of LD blocks that have not reached genome-wide significance in current GWAS.

(A) Flow diagram categorizing LAVA significant regions where no genome-wide significant SNPs were identified.

(B) Karyotype plot for the psoriasis-associated SNP discoverability with four categories defined from (A).

(C) Ranked LD blocks with local heritability. Upper asterisk of each block denotes previously reported psoriasis-susceptibility regions.



[Added to the Results, pages 8–9, lines 142–164]

Estimating genomic discovery potential in GWAS

Local heritability analysis was performed to assess the identification of unreported loci associated with psoriasis. Regions with high local heritability predominantly contained previously identified loci. Notably, significant SNPs were also identified in the regions with low local heritability (**Supplementary Table 6**). We examined the relationship between the heritability of local LD blocks and the presence of significant SNPs within the blocks (**Supplementary Fig. 7A**). The local LD significance was associated with increased likelihood

of observing significant SNPs (chi-square test $P = 2.42 \times 10^{-80}$). We first examined 42 cases in which LD local heritability was not significant but contained significant SNPs. We compared 106 independent SNPs located in 72 LD blocks that showed statistically significant heritability with 42 independent SNPs within the non-significant LAVA regions identified in the GWAS (**Supplementary Fig. 7B, C)**. Next, 60 significant LAVA regions where no genome-wide significant SNPs were identified were categorized into four classes for each LD block to explore the potential for novel discoveries (**Supplementary Fig. 8A**). Except for nine MHC region-adjacent blocks, 13 of 51 (25%) blocks were previously reported as psoriasis-susceptibility loci in the GWAS Catalog across seven studies (**Supplementary Fig. 8B, C** and **Supplementary Table 7**). Notably, LD blocks classified as having high discovery potential showed a higher likelihood of harboring psoriasis-associated variants (5 of 15 (33%)). The LD block with the highest local heritability among regions not yet associated with psoriasis (chr10:118,416,050–119,528,730), which contains *RPL21P16*, *PLPP4*, and *WDR11* genes, has previously been reported to be associated with metabolic traits such as HDL cholesterol, BMI, systolic blood pressure, prostate-specific antigen, and type 2 diabetes. These findings suggest that regions with high local heritability for psoriasis yet lacking significant loci may have the potential for novel discoveries.

[Added to the Methods, pages 28–29, lines 570–585]

Evaluation of potential for novel discovery in local LD blocks

We investigated two cases in the contingency table of the local heritability significance and genome-wide significance in LD blocks (**Supplementary Fig. 7A**). Local heritability was calculated using the LAVA tool with default parameters across 2,495 genomic loci. The statistical significance of each local LD block was defined as a Bonferroni-corrected *p*-value below 0.05 (0.05 / 2,495). Each LD block was classified based on the significance of LAVAs and the presence of genome-wide significant SNPs. The 60 LD blocks that were LAVA-significant but lacked genome-wide significant SNPs were further categorized these blocks according to their potential for novel discovery, considering the inflated heritability due to residual LD. First, LD blocks located within ±10 Mb of the MHC region were classified as "Near MHC region". Next, we classified each LD block based on its local heritability and the presence of nearby significant SNPs within a 5-Mb region: blocks for which another block

within the 5-Mb region showed higher heritability were classified as “Likely residual LD”; blocks with previously identified significant SNPs in other blocks within the 5-Mb region were classified as “Nearby GWS”; otherwise, blocks that exhibited the highest heritability in the region and no nearby GWS had been reported were classified as having “high potential for novel discovery”.

3. The finding that psoriasis heritability is most significantly enriched in genomic regions actively expressed in synovial fluid is a little counterintuitive, given the established immune models of psoriasis as a systemic disease with fairly well understood drivers. The authors should elaborate further on why this could represent a true finding, or else disclose limitations of their analysis which could explain why more relevant tissues or cell types are missed. It is also true that many of the psoriasis cases in their GWAS analysis will also have psoriatic arthritis and therefore their results may conflate genetic signals for psoriatic arthritis alongside those specific to cutaneous psoriasis. So their claim “this finding recapitulates the association for psoriasis susceptibility variants and increased risk of psoriatic arthritis” (line 136) is not well supported. Previous studies have investigated the genetic differences between PsA and cutaneous psoriasis (e.g. PMID: 35507331). Relatedly, I also feel the claims about shared mechanisms with IBD (line 140) are a little speculative (how do we know these digestive system tissues are most enriched in those traits?). And in methods (line 413) the description “estimates the relative genetic heritability of psoriasis based on the expression of associated genes” is not precise and should be reworded.

Response: We appreciate the reviewer’s comment and would like to clarify this point based on the data used in our study. In our analysis, individuals diagnosed with psoriasis in each cohort are likely to include a proportion of patients who also have psoriatic arthritis (PsA). As previously reported (PMID: 35507331), approximately 30% of patients with psoriasis may also be diagnosed with PsA. Our study, as well as previously published psoriasis GWAS, was designed to include all individuals with psoriasis and did not specifically focus on cutaneous psoriasis (PsC) without psoriatic arthritis. Therefore, the genetic signals related to PsA may have been partially captured within the psoriasis-associated signals identified in our study.

The study referenced by the reviewer (PMID: 35507331) highlights the differences between cutaneous-only psoriasis (PsC) and PsA. However, as noted in that work, there is a large overlap between PsC and PsA at the SNP level (**Response Note 1**).

Response Note 1. Text from Soomro et al. (2022) article [**Ref. 1**].

While the individual SNP analysis showed large overlap between PsC and PsA, pathway analysis revealed important differences, including enrichment of PsA-significant SNPs found in key pathways such as NF-κB and Wnt signaling.

We replicated the previously reported findings of prioritized genes (*ICAM1*, *IRF1*, *STAT2*, and *TYK2*) and target pathways (IFN and interleukin signaling) for psoriasis, further supporting the notion that psoriasis in patients with PsA is genetically and biologically similar to psoriasis in patients with PsC.

In addition, single-cell transcriptomic data derived from synovial fluid have shown that the vast majority of cells within the samples are immune cells [**Ref. 2**]. This observation suggests that the high comorbidity of PsA in patients with psoriasis may be driven by shared immune cell-based mechanisms.

Investigating the differences between PsA and the diverse clinical phenotypes of psoriasis is beyond the scope of the present study, and therefore we did not include detailed analyses to address this question. Nevertheless, we agree that the comparative analyses of cell types obtained from synovial fluid tissues and their relevance to psoriasis and PsA are important directions for future research. We have added this perspective to the **Discussion** section. In addition, to reduce the confusion raised by the reviewer, we have rephrased the relevant text in the manuscript.

References

Ref. 1: Soomro M, et al. Comparative genetic analysis of psoriatic arthritis and psoriasis for the discovery of genetic risk factors and risk prediction modeling. Arthritis & rheumatology 74, 1535-1543 (2022).

Ref. 2: Edalat SG, et al. Molecular maps of synovial cells in inflammatory arthritis using an optimized synovial tissue dissociation protocol. Iscience 27, (2024).

[Rephrased the Results, page 10, lines 171–172]

~~This finding recapitulates the association between psoriasis-susceptibility variants and increased risk of psoriatic arthritis through inflammatory responses in the synovial fluid.~~

This suggests a high genetic overlap between psoriasis and psoriatic arthritis.

[Added to the Discussion, page 24, lines 488–491]

Finally, comparative studies on cutaneous psoriasis and psoriatic arthritis may offer additional insights into shared and distinct disease mechanisms. Integrating single-cell data from synovial fluid with skin-based analyses may further clarify immune cell-driven processes across subtypes of psoriatic diseases.

Regarding the last point, we thank the reviewer for highlighting the issue in the **Methods** section, and we have revised it accordingly.

[Added to the Methods, page 29, lines 588–590]

This approach estimates the enrichment of psoriasis heritability in specific tissues and cell types using expression-derived functional annotations.

4. Line 156 – genetic correlation with atopic dermatitis: why is the p-value only marginally significant when the magnitude of correlation is similar to the “notable positive correlations” described earlier?

Response: Thank you for noting this interesting point. Statistically, the genetic correlation between atopic dermatitis and psoriasis is relatively high with a large standard error, which likely reflects underestimated heritability due to the limited power of the atopic dermatitis GWAS. We have replaced the data with the recently published GWAS summary statistics from Oliva et al, 2025 (PMID: 40164604) [**Ref. 1**]. Using the new data, the liability-scale heritability increased

from 0.053 to 0.093. In the genetic correlation analysis, the genetic correlation coefficient r_g increased from 0.230 to 0.272, and the reduced standard error yielded a more significant p-value (p = 0.0005; Bonferroni-adjusted p-value = 0.057). Although this still does not pass the Bonferroni-corrected threshold, a larger GWAS would reduce the standard error.

[Updated Supplementary Table 10]

Table S10. Phenotypes used in the genetic correlation analysis

Phenotypes	Category	rg	se	z	p	Bonferroni adj p	h2_obs	h2_obs_se	h2_int	h2_int_se	gcov_int	gcov_int_se	Source	Source URL
Inflammatory Bowel Disease	Autoimmune	0.28339	0.04225	6.68229	2.34E-11	2.65E-09	0.31333	0.00314	1.12278	0.01065	0.03348	0.00104	de Lange et al., 2017 [1]	https://ftp.sanger.ac.uk/pub/project/humgen/summary_statistics/human/2016-11-07/
(Omitted)														
Atopic dermatitis	Immune	0.2722	0.07779	3.49088	0.00005	0.05065	0.02744	0.00335	1.05117	0.01142	0.07794	0.00104	Oliva et al., 2025 [5]	https://www.ebi.ac.uk/gwas/studies/GCST90503108
Asthma	Immune	0.16557	0.04788	3.46555	5.00E-04	0.05065	0.03441	0.003	1.03553	0.01114	0.01186	0.00669	PheCode 495, UK Biobank (SAIGE) from Lee Lab. [2]	https://www.leelabsg.org/resources

(The remaining rows are omitted)

References

Ref. 1: Oliva, M. et al. Integration of GWAS, QTLs and keratinocyte functional assays reveals molecular mechanisms of atopic dermatitis. Nature Communications 16, 3101 (2025).

5. Line 185 – "… follows distinct lineages compared to that of normal tissues." – not sure what this means?

Response: We thank the reviewer for this comment. In response, we have revised this paragraph to provide a more detailed explanation of the results.

[Rephrased to the Results, page 12, lines 220–225]

Additionally, KER_c2 was enriched with cells derived from lesion tissues, whereas KER_c4 was enriched with cells from normal tissues (**Fig. 4D**). These findings suggest that keratinocytes derived from psoriatic lesions show a higher proportion of differentiation toward a distinct lineage compared with normal-derived keratinocytes (**Fig. 4F**). ~~Additionally, the two clusters exhibited relative enrichment in lesional and non-lesional tissues, respectively (**Fig. 4D**), suggesting that keratinocyte differentiation from the basal to the granular layers follows distinct lineages compared to that of normal tissues.~~ The continuous trajectory observed in the UMAP plot, interpreted as pseudotime, reflects the spatial organization within the epidermal layers (**Supplementary Fig. 11**).

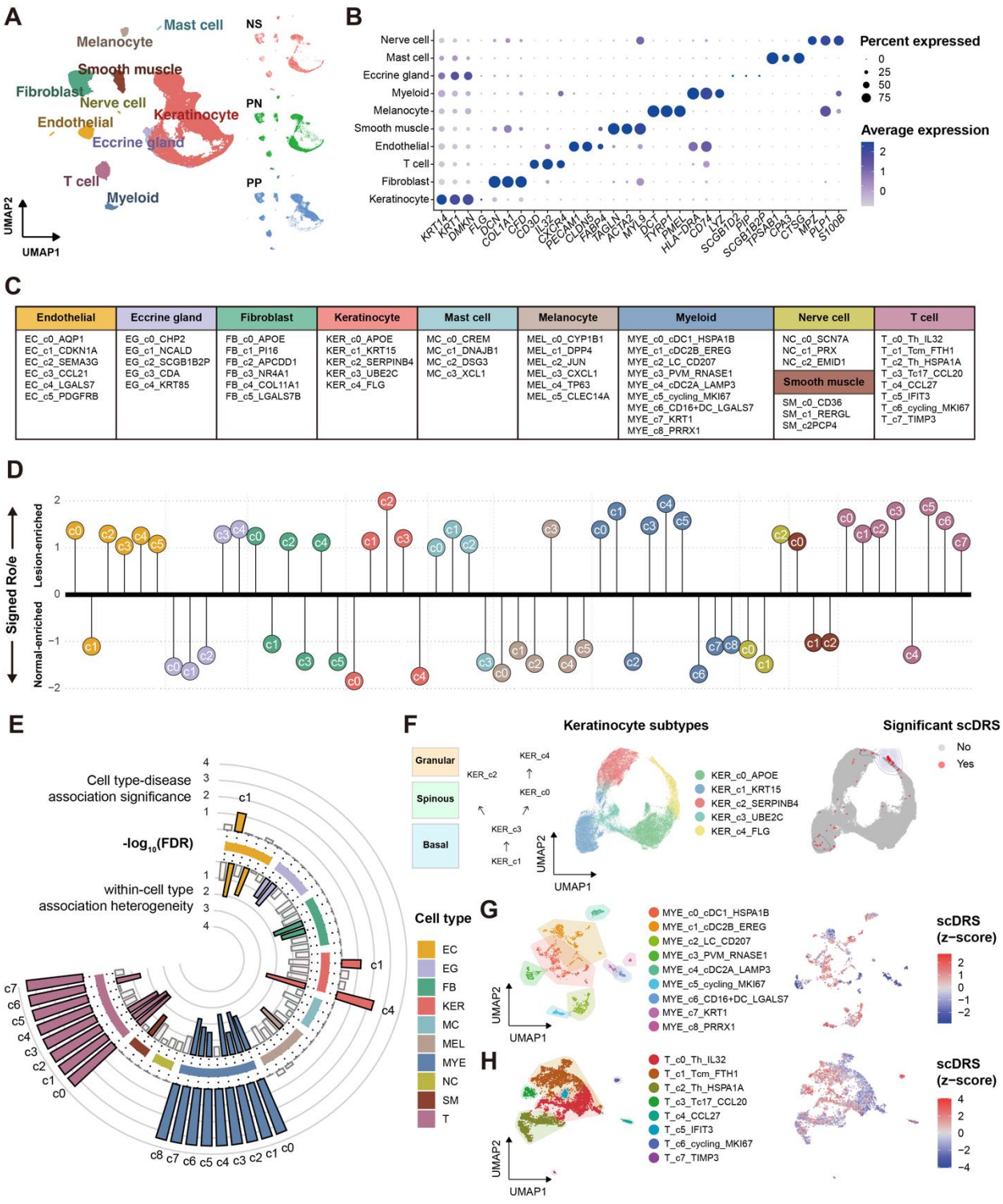


Fig. 4: **Joint analysis of single-cell transcriptomics and GWAS data.** (**A**) UMAP plot of processed single-cell RNA-seq data including non-lesions from healthy controls (NS), non-lesions from psoriasis patients (PN), and psoriatic lesions (PP). (**B**) Dot plot for showing expression of representative cell markers. (**C**) Identification of cell subtypes for each major cell

type. (**D**) Lollipop plot displaying relative tissue enrichment. We compared the number of non-lesion cells (NS+PN) and lesion cells (PP) in each cell subtype and calculated the ratio of observed and expected (Ro/e). If the Ro/e values for the non-lesion have higher than 1, the Ro/e values for the cell subtype were defined as -1×(Non-lesion Ro/e). Otherwise, Ro/e were defined as (Lesion Ro/e). (**E**) The plot illustrates the cell subtype-level scDRS result. The outward bars represent false discovery rate (FDR)-adjusted *p*-values of the cell subtype-disease association. The inward bars represent FDR-adjusted within-cell subtype association heterogeneity. Bars with FDR<0.10 were drawn with darker colors and thicker boundaries. (**F**) Two keratinocyte lineages (left), UMAP visualization (center), and FDR-adjusted Monte Carlo *p*-values of cells (right) are shown. UMAP and z-scaled scDRS for myeloid (**G**) and T cell (**H**) are shown.

6. Line 215 – "opposing causal effects" – does this mean the direction of causality is opposite or that the direction of causality is always from gene expression to psoriasis but the direction of change in gene expression is opposite with respect to increasing psoriasis risk?

Response: We thank the reviewer for the comment. Our results indeed indicated that the direction of change in gene expression was opposite to that of increasing psoriasis risk. We have revised the relevant sentences accordingly.

[Rephrased to the Results, page 14, lines 270–277]

Notably, *RGS14*, *NEK6*, *PRSS53*, and *PRDX5* exhibited opposite directions of effects on psoriasis risk between the blood and skin expression levels. For example, *RGS14*, *NEK6*, and *PRDX5* exhibited negative effects in the skin eQTL datasets, whereas their effect directions were positive in the blood eQTL datasets. These results highlight the importance of tissue-specific gene regulation (**Supplementary Table 16**). ~~Notably, *RGS14*, *NEK6*, *PRSS53*, and *PRDX5* exhibited opposing causal effects on psoriasis depending on their expression levels in blood versus skin tissues, highlighting the importance of tissue-specific gene regulation.~~ Among these prioritized genes, four (*IL23A*, *VKORC1*, *TYK2*, and *IL2RA*) were associated with FDA-approved drugs, and approximately 50% of them are currently under investigation in the preclinical or clinical stages (**Fig. 5** and **Supplementary Table 17**).

7. Line 230 – “module M1 exhibited the highest module eigengene in basal layer cells” – needs to be more specific. Presumably this means highest *average* eigengene across cells in the module? (Or across samples?)

Response: We thank the reviewer for pointing this out. As shown in Figures 6B and 6C, the highest average eigengene values were observed across cells in the basal layer (earlier pseudotime). As suggested, we have revised the corresponding description to make this point more specific as suggested.

[Rephrased in the Results, page 15, lines 284–290]

In keratinocytes, the four modules were enriched in specific epidermal layers respectively. For instance, module M1 exhibited the highest average module eigengene score (module activity) across cells in the basal layer, whereas module M3 showed elevated activity in the outermost cell subpopulation undergoing differentiation from the granular to the corneal layer **(Fig. 6B**–**D**).

8. Line 237 – “within the MYE-M2 network, IL23A displayed notably high connectivity” – to me it is unclear what this actually means and could benefit from elaboration. Is this gene in multiple modules within the MYE-M2 network?

Response: We thank the reviewer for the comment. High connectivity in a gene co-expression network can be interpreted as an indication of a hub-like role of genes within a given module. IL23A showed a specific module membership in MYE-M2, suggesting that genes functionally related to IL23A were enriched within this module.

[Rephrased in the Results, page 15, lines 297–298]

Importantly, within the MYE-M2 network, IL23A displayed notably high connectivity, as defined by strong Pearson correlations with other genes in the module, indicating its role as a hub gene. This finding suggests that IL-23–related pathways may play a pivotal role in disease

pathogenesis through interactions between LCs and their adjacent cells.

9. Line 242 – "during immunotherapy" – needs to be more specific to what has actually been studied; we don't expect that results will generalises to other immunomodulators

Response: Thank you for the comment. We have clarified the statement in the context of IL-23 inhibitor treatment.

[Rephrased to the Results, page 16, line 312]

Changes in module activity during IL-23 inhibitor treatment were investigated by analyzing temporal scRNA-seq data from five patients undergoing risankizumab treatment.

10. Discussion line 287 – "identified subpopulations of keratinocytes and ECs that may be involved in psoriasis development" – if I understand correctly, it is not established whether these mechanisms are causal or a consequence of psoriatic inflammation. Could this be clarified?

Response: Thank you for this comment. Using scDRS, we identified the cell types most affected by genetic risk associated with psoriasis. While this algorithm does not establish definitive causal relationships, the scDRS provides a cell-level disease relevance score. Based on this score, we proposed candidate cell types that are potentially involved in the development of psoriasis. In addition to immune cells, we also observed statistically significant enrichment in non-immune cells, keratinocytes, and a subset of endothelial cells. We have added a clarification in the **Discussion** section regarding the specific characteristics of the endothelial cell cluster showing marginal significance and explained why it was not included in the downstream analyses.

[Added to the Discussion, pages 20–21, lines 397–417]

The scDRS algorithm enabled the identification of specific cell types potentially involved in the development of psoriasis by calculating a genetically driven disease relevance scores for each

cell type. Using the scDRS algorithm, strong associations between myeloid cells and T cells were consistently observed. We also identified subpopulations of keratinocytes and ECs that may be involved in psoriasis development. Notably, keratinocytes exhibited a continuous expression pattern from the basal to granular layers, reflecting the spatial organization of the epidermis. Cells in the outermost nucleated epidermal layer, which is characterized by pronounced cornification features, showed the strongest association with psoriasis. The EC_c1_CDKN1A cluster, which was enriched in normal skin tissue, showed marginal significance in psoriasis (FDR = 0.069). These cells were characterized by the expression of *NFKBIA* and *SOCS3*, which suppress cytokine responses and regulate inflammatory signaling via negative feedback mechanisms (**Supplementary Table 13**). These findings suggest that dysfunction of this specific subset of endothelial cells may contribute to the pathogenesis of psoriasis. Within this cell cluster, cells with higher scDRS scores formed a distinct subcluster. This subpopulation showed higher expression of genes involved in the AGE-RAGE signaling pathway and negative regulation of TGF-β receptor signaling. Previous studies have reported that AGE-RAGE interactions are implicated in psoriasis and can suppress downstream TGF-β-mediated functions. Given the marginal statistical significance and increased analytical complexity, we excluded endothelial cells from downstream analyses. Further research will provide deeper insights into the role of endothelial cells in modulating immune cell function in psoriasis.

11. In the discussion of the longitudinal scRNA-seq data (lines 307-325) there is a major focus on IL-23 pathway mechanisms. But these longitudinal data are based on treatment with an IL-23 inhibitor, IL-23 pathway mechanisms would be expected to be identified. Again, I think it needs to be more clear here what the novel findings around these pathways are.

Response: We thank the reviewer for the comment. MYE-M2 is a key module that includes IL23A and shows the strongest association with Langerhans cells. Therefore, we performed an in-depth analysis of this module (**Supplementary Fig. 14**). Among the 895 MYE-M2 member genes, those co-expressed with *IL23A* (TOM pairwise similarity > 0.10) included established Langerhans cell markers (*CD207* and *HLA-DQB2*) as well as genes involved in lipid metabolism (*ACOT7* and *LPAR3*) (**Supplementary Fig. 14A, B**). Next, we calculated module connectivity

separately in normal and lesion samples and compared the fold changes between the two conditions (**Supplementary Fig. 14C**). Pathway analysis of genes with increased connectivity in lesion samples revealed enrichment of the lipoxin (LX) biosynthesis pathway. This finding indicates that the co-regulatory relationships among the genes involved in LX biosynthesis were disrupted in psoriatic lesions. Notably, 5-lipoxygenase is a key enzyme involved in the LX biosynthesis pathway in Langerhans cells [**Ref. 1**], and lipoxins are known to play important roles in immunoregulation and wound healing [**Ref. 2**].

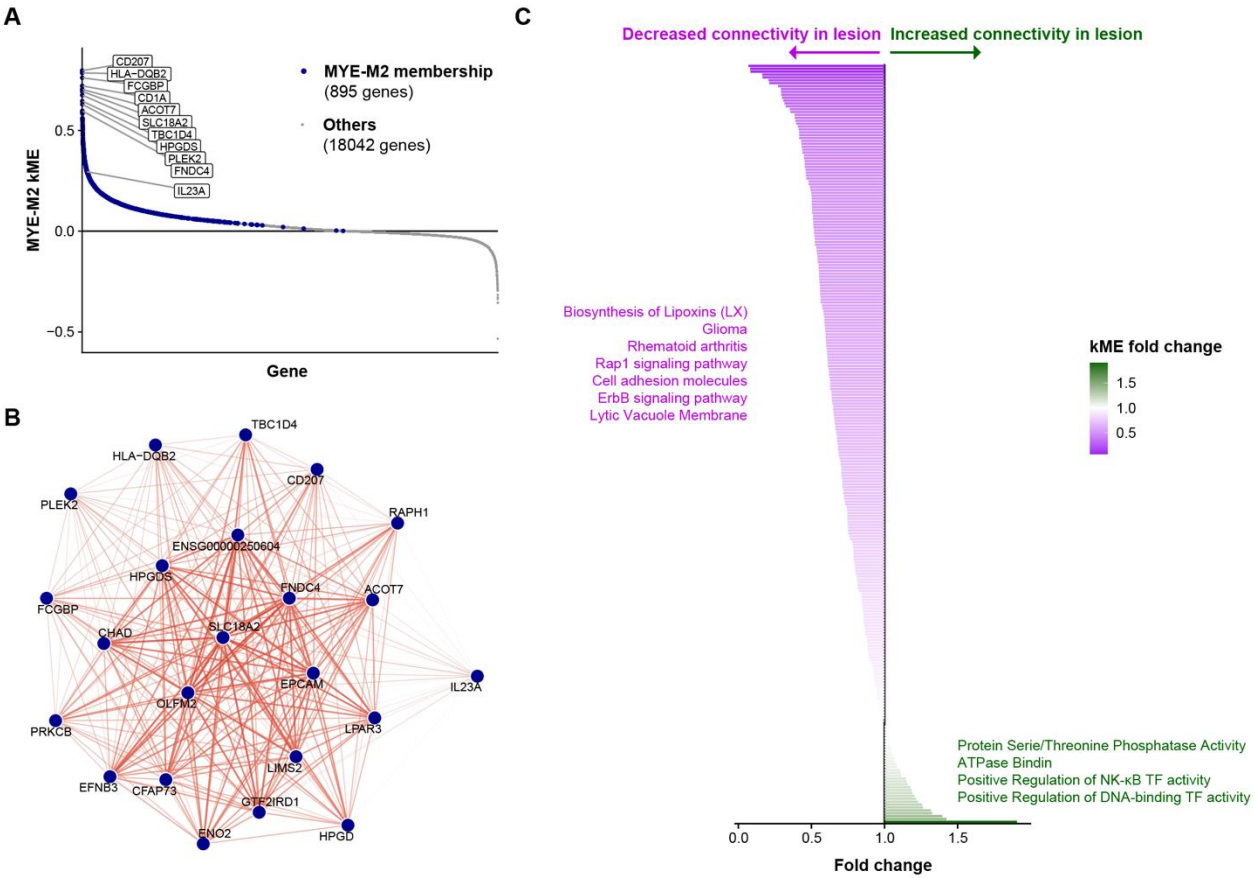
[Added to the Results, pages 15–16, lines 300–308]

We further analyzed the MYE-M2 module to characterize lesion-specific functional changes (**Supplementary Fig. 14**). Among the 895 MYE-M2 member genes, those co-expressed with *IL23A* (Topological overlap matrix pairwise similarity > 0.10) included established Langerhans cell markers (*CD207* and *HLA-DQB2*) as well as genes involved in lipid metabolism (*ACOT7* and *LPAR3*) (**Supplementary Fig. 14A, B**). Next, we calculated module connectivity separately in normal and lesional samples and compared the fold changes between the two conditions (**Supplementary Fig. 14C**). Pathway analysis of genes with decreased connectivity in lesional samples revealed enrichment of the lipoxin (LX) biosynthesis pathway, which is known to play an important role in immunoregulation and wound healing (**Supplementary Table 19**).

[Added Supplementary Figure 14]

Supplementary Figure 14. MYE-M2 module connectivity analysis.

(A) Scatter plot of genes ranked by module connectivity (kME) and kME scores. The top-10 genes with the highest kME values and *IL23A* are annotated. **(B)** Network plot showing genes co-expressed with *IL23A*. Genes were filtered based on a TOM pairwise similarity > 0.10 with *IL23A*. **(C)** Comparison of gene connectivities between lesional samples and healthy control samples, ranked by fold change. Genes with fold changes below 1.0 indicate decreased connectivity in lesional samples. Only genes with kME > 0.25 were included in the analysis. Gene sets with increased and decreased connectivity were both used for pathway analysis, and representative pathways with adjusted p-value < 0.10 are shown.



[Added Supplementary Table 19]

Table S19. Associated pathways with differential connectivity between normal and lesional samples in MYE-M2 module genes.

The enrichR R library was used for pathway analysis. Only pathways with adjusted *p*-values < 0.10 and at least two member genes are shown.

Category	Term	Overlap	P	adjP		Geneset
Decreased connectivity in lesion	Transcriptional misregulation in cancer	'10 / 192'	7.39E-05	1.71E-02		KEGG_2021_Human
Decreased connectivity in lesion	Biosynthesis of Lipoxins (LX)	'3 / 6'	2.85E-05	2.04E-02		Reactome_Pathways_2024
Decreased connectivity in lesion	Glioma	'6 / 75'	2.15E-04	2.49E-02	…	KEGG_2021_Human
Decreased connectivity in lesion	Rheumatoid arthritis	'6 / 93'	6.86E-04	3.75E-02		KEGG_2021_Human
Decreased connectivity in lesion	Rap1 signaling pathway	'9 / 210'	7.07E-04	3.75E-02		KEGG_2021_Human
Decreased connectivity in lesion	Thyroid cancer	'4 / 37'	8.09E-04	3.75E-02		KEGG_2021_Human

Decreased connectivity in lesion	Non-small cell lung cancer	'5 / 72'	1.39E -03	5.08E -02		KEGG_2021_Human
Decreased connectivity in lesion	Cell adhesion molecules	'7 / 148'	1.57E -03	5.08E -02		KEGG_2021_Human
Decreased connectivity in lesion	Chronic myeloid leukemia	'5 / 76'	1.76E -03	5.08E -02		KEGG_2021_Human
Decreased connectivity in lesion	Leukocyte transendothelial migration	'6 / 114'	1.97E -03	5.08E -02		KEGG_2021_Human
Decreased connectivity in lesion	Anion Binding (GO:0043168)	'14 / 402'	2.24E -04	6.21E -02		GO_Molecular_Function_2025
Decreased connectivity in lesion	ErbB signaling pathway	'5 / 85'	2.88E -03	6.69E -02		KEGG_2021_Human
Decreased connectivity in lesion	Lytic Vacuole Membrane (GO:0098852)	'11 / 314'	1.00E -03	7.17E -02		GO_Cellular_Component_2025
Decreased connectivity in lesion	Sex Chromosome (GO:0000803)	'2 / 5'	1.26E -03	7.17E -02		GO_Cellular_Component_2025
Decreased connectivity in lesion	External Side of Apical Plasma Membrane (GO:0098591)	'2 / 5'	1.26E -03	7.17E -02		GO_Cellular_Component_2025

(The remaining rows are omitted)

References

Ref. 1. Spanbroek R, et al. 5-Lipoxygenase expression in Langerhans cells of normal human epidermis. Proceedings of the National Academy of Sciences 95, 663-668 (1998).

Ref. 2. Fu T, et al. Therapeutic potential of lipoxin A4 in chronic inflammation: focus on cardiometabolic disease. ACS pharmacology & translational science 3, 43-55 (2020).

12. Line 331 – "In scDRS analysis, we observed marginal significance…" – what does this mean? Are these associations that do not survive multiple testing correction?

Response: Thank you for the comment. Although these cell populations passed the FDR-corrected *p*-value of < 0.1 threshold, we described them as showing marginal significance because of the relatively modest strength of the associations (FDR = 0.065 for KER_c1_KRT15 and FDR = 0.069 for EC_c1_CDKN1A). We have added the names of the clusters and their corresponding FDR-corrected *p*-values to the revised manuscript. As the interpretation of these marginal significant cell types is related to Comment #10, we have updated the content accordingly to avoid redundancy.

[Rephrased to the Discussion, page 23, lines 463–467]

Increasing the scale of single-cell datasets may improve statistical power and enable a more comprehensive characterization of disease-associated cell types through integration with GWAS

results, including those showing marginal significance in scDRS analysis, such as keratinocytes in the basal layer (KER_c1_KRT15, FDR = 0.065) and a subset of endothelial cells (EC_c1_CDKN1A, FDR = 0.069).

> 13. Lines 334-336 – "this study did not identify any fibroblast subpopulations…" – several of the scRNA-seq papers have highlighted the importance of fibroblasts in psoriatic inflammation. The authors should reconcile more fully the lack of fibroblast mechanisms identified in their results. Are there study limitations that precluded their discovery? Or do the authors have another hypothesis?

Response: We thank the reviewer for pointing out this important issue. We fully agree that it is necessary to provide an explanation and a hypothesis for why the fibroblast associations were not detected in this study. This study fundamentally aimed to investigate the genetic basis of psoriasis using a GWAS-based approach. As described in Minor Comment #10, scDRS was used to identify specific cell types potentially involved in the development of psoriasis. However, no statistically significant signal was observed in fibroblasts, and this lack of significance was consistently confirmed in an independent validation dataset (**Supplementary Fig. 18D**).

One possible hypothesis is that immune cells, keratinocytes and endothelial cells may already possess a genetically determined susceptibility to psoriasis. After disease onset, these genetically susceptible cells may secondarily (i.e., downstream) influence the gene expression profiles of fibroblasts within the skin tissue. In this context, the observed fibroblast-related changes may be a consequence of psoriasis rather than a primary genetic driver.

However, further experimental validation under controlled conditions is required to test this hypothesis. For example, epidermal organoid models can be established to assess the effect of perturbations in genetically susceptible cells on fibroblast transcription. We have added this rationale in the revised manuscript as a direction for future research.

[Added to the Discussion, pages 23–24, lines 467–479]

Additionally, while previous studies have highlighted the role of fibroblasts in psoriasis progression, this study did not identify any fibroblast subpopulations that exhibited significant disease enrichment from the GWAS signals. This trend was consistently confirmed by using an independent validation dataset (**Supplementary Fig. 18D**). Our study fundamentally aimed to investigate the genetic basis of psoriasis using a GWAS-based approach. One possible hypothesis is that immune cells, keratinocytes and endothelial cells may already possess a genetically determined susceptibility to psoriasis. After disease onset, these genetically susceptible cells may secondarily (i.e., downstream) influence the gene expression profiles of fibroblasts within the skin tissue. In this context, the observed fibroblast-related changes may be a consequence of psoriasis rather than a primary genetic driver. Further experimental validation is required to confirm this hypothesis. For example, epidermal organoid models with genetic perturbations could be established to assess the effects of genetically susceptible cells on fibroblast transcriptional programs.

14. Methods line 460 – NS, PN and PP are not defined in this section.

Response: Thank you for the comment. We have added a description of the definitions of the three tissue types in the single-cell methods section.

[Rephrased to the Methods, page 30, lines 605–606]

This dataset included normal skin samples (denoted as NS) from healthy individuals and non-lesional (denoted as PN) and lesional skin samples (denoted as PP) from patients with psoriasis.

15. Line 483 – COJO lead SNP colocalization – perhaps clarify whether these analyses used inferred conditional association statistics for each independent signal within a locus? Or just the marginal summary statistics for the lead signal at each locus?

Response: We thank the reviewer for pointing out the ambiguous words. We conducted a colocalization analysis on the independent SNPs identified using GCTA-COJO. Instead of the inferred conditional association statistics, effect estimates obtained from the marginal (original) GWAS summary statistics were used for the lead signal at each locus. For clarity, we have revised all "COJO lead SNPs" to "independent COJO SNPs". In addition, we have added detailed descriptions of the procedures used to perform the GCTA-COJO analysis.

[Rephrased to the Methods, page 33, lines 679–681]

Fourth, we conducted a Bayesian colocalization analysis to evaluate whether the independent COJO SNPs for psoriasis colocalized with eQTL signals based on original effect estimates from GWAS summary statistics.

[Rephrased to the Methods, page 27, lines 546–549]

Independent COJO SNPs were identified using genome-wide joint conditional analysis implemented in GCTA (v1.94.1) with -cojo-slct option, a genome-wide significance threshold of $P < 5 \times 10^{-8}$ and a 10-Mb window, based on LD estimates from randomly selected 10,000 UK Biobank individuals of European ancestry.

16. In the equation in line 526, I am not clear whether this is calculation is done at the single-cell level or the "meta-cell" level. What is ME_c_i in the sum? Should the sum be over all c in C_i rather than all c in C? And I think technically, C must represent the set of all cells and |C| the number of all cells?

Response: We apologize for the unclear description of the formula. As the reviewer pointed out, we have revised the description to clarify that the average module eigengene values were calculated at the single-cell level for each cell subtype.

[Rephrased to the Methods, pages 35–36, lines 719–731]

We further quantified the enrichment of cell subtypes within modules containing at least two of the 50 prioritized genes. For a given module *M*, the raw average module eigengene (ME) of cells in cell subtype i was computed as:

$$S_i = \frac{\Sigma_{c \in C_i} ME_c}{|C_i|}$$

where ME denotes the module eigengene, S_i represents the average MEs of cell subtype i, and $|C_i|$ represents the number of the cells of cell subtype i. Because S_i values can be negative, min-max scaling was performed to normalize the module scores S_i within the [0, 1] range across all cell subtypes.

$$S_i' = \frac{S_i - \min(S)}{\max(S) - \min(S)}$$

Using these normalized module scores across cell subtypes, the proportion of normalized module scores was defined as the module M enrichment of a given cell subtype i, calculated as:

$$\text{Specificity Score}_i = \frac{S_i'}{\sum_{j=1}^{n} S_j'}$$

where *n* denotes all cell subtypes belonging to the major cell type analyzed.

17. In the equation in line 533, I think the numerator should have index i, not j. But I have a more fundamental question about this specificity score. Is it valid to transform values that could be positive or negative to a [0,1] scale and calculate specificity as the relative share of a cell type on this scale? Surely this means that the largest negative eigengene values, which could be directionally meaningful, are transformed close to zero and are assigned minimal specificity. While eigengene values that are originally close to zero, indicating little activity in a cell type, get transformed to a value midway between 0 and 1 and are assigned some "medium" amount of specificity? I don't understand the intuition here.

Response: We understand and appreciate the reviewer's thoughtful concern about converting the negative module scores into normalized values through min-max scaling. In WGCNA, a module eigengene is defined as the first principal component that best explains the variation in gene expression within a given module. In other words, it can be represented as a weighted sum of the

standardized gene expression in that module. The module eigengene values were centered around zero and were approximately normally distributed as scaled scores. Here, negative module scores do not indicate negative correlations but instead reflect that the genes within the module show overall lower expression levels. Therefore, we applied min-max scaling to each module eigengene to effectively capture the relative activity of each module across individual cells.

18. Fig 4 legend – line 804 – ratio of observed and expected: it's unclear where the expected value comes from.

Response: We thank the reviewer for noting that the relevant methodological details were missing. We have added a detailed description of the formula used to calculate the ratio of observed to expected in the **Methods** section.

[Added to the Methods, pages 31–32, lines 633–642]

For each cell subtype, we estimated the expected cell counts by constructing a contingency table between the tissue group (lesion vs. non-lesion) and cell membership in the subtype of interest. The expected number of cells was calculated using the following formula, employed in the chi-square test:

$$Expected\ cells\ = \frac{(Total\ cells\ of\ the\ subtype)\ \times\ (Total\ cells\ in\ the\ tissue\ group)}{Total\ number\ cells}$$

Finally, the ratio of observed to expected (Ro/e) values was calculated for lesion and non-lesion tissues as follows:

$$Ro/e\ = \frac{Observed\ cells}{Expected\ cells}$$

If the Ro/e value for the non-lesion was greater than 1, the cell subtype was defined as normal-enriched. Otherwise, the subtype was defined as lesion-enriched if the Ro/e value for lesional tissue was greater than 1.

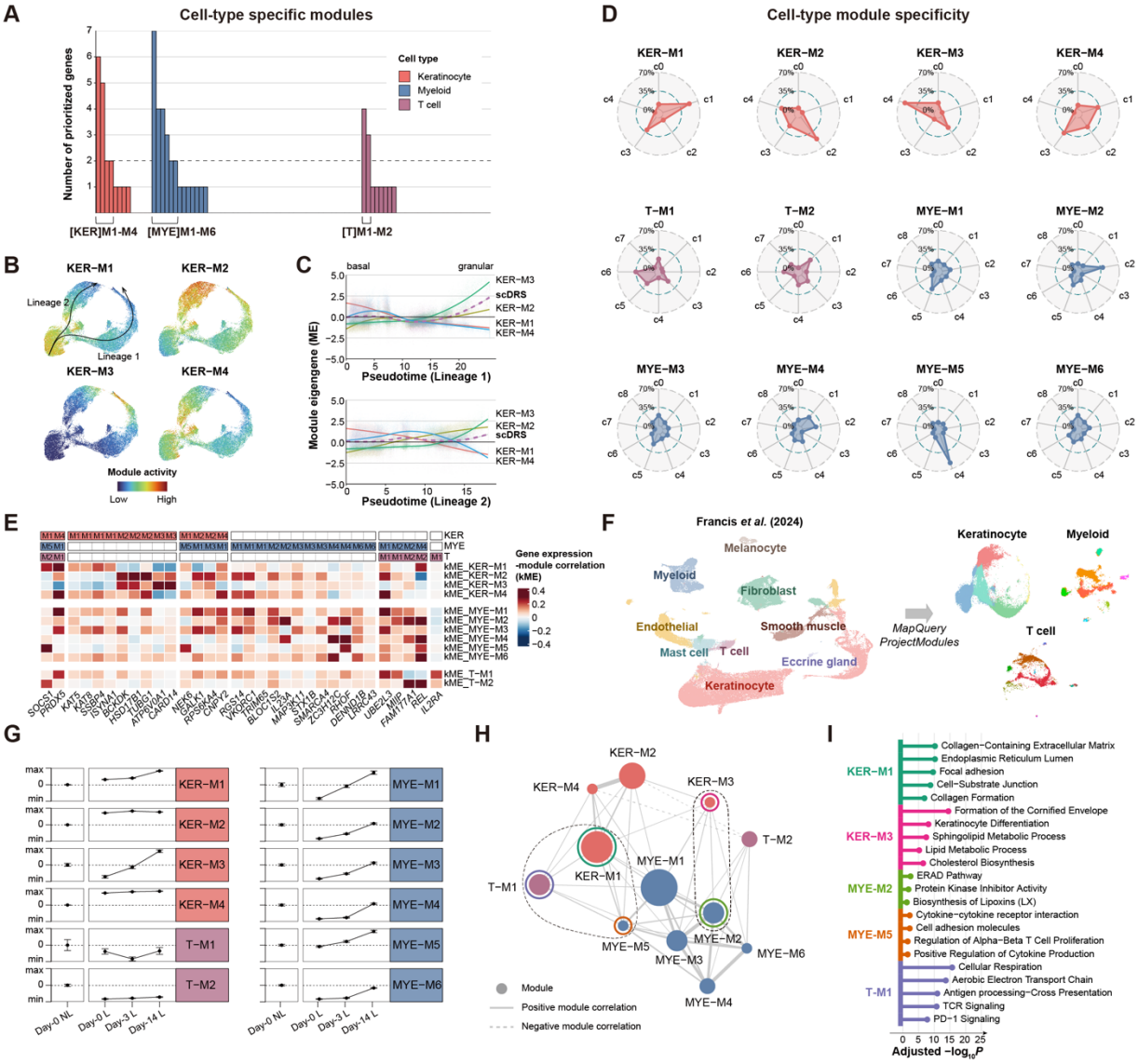
19. Fig 6F – there is a small table containing P1 to P5 rows. It is unclear what table entries represent.

Response: Thank you for the comment. This table provided an overview of the samples from the Francis *et al*. dataset. The dataset included three time points of day 0 (baseline), day 3, and day 14 based on the treatment. They collected non-lesion and lesion skin samples at baseline, and only lesion samples at the later time points. We have added the brief information to the **Results** section and removed the corresponding panel from the main figure to avoid confusion.

[Rephrased to the Results, page 16, lines 310–314]

Changes in module activity during immunotherapy were investigated by analyzing temporal scRNA-seq data from five patients undergoing risankizumab treatment, as reported by Francis et al. (**Fig. 6F** and **Supplementary Fig. 15**). The dataset comprised non-lesional and lesional skin collected at baseline (day 0), as well as lesional skin collected at 3 and 14 days after treatment (days 3 and 14), with each patient contributing samples across all time points.

[Modified Figure 6]


A
Cell-type specific modules
Number of prioritized genes
Cell type
Keratinocyte
Myeloid
T cell
[KER]M1-M4
[MYE]M1-M6
[T]M1-M2
B
KER−M1
KER−M2
KER−M3
KER−M4
Lineage 2
Lineage 1
Module activity
Low
High
C
basal
granular
KER−M3
scDRS
KER−M2
KER−M1
KER−M4
Module eigengene (ME)
Pseudotime (Lineage 1)
Pseudotime (Lineage 2)
D
Cell-type module specificity
KER−M1
KER−M2
KER−M3
KER−M4
T−M1
T−M2
MYE−M1
MYE−M2
MYE−M3
MYE−M4
MYE−M5
MYE−M6
E
KER
MYE
T
Gene expression
-module correlation
(kME)
kME_KER−M1
kME_KER−M2
kME_KER−M3
kME_KER−M4
kME_MYE−M1
kME_MYE−M2
kME_MYE−M3
kME_MYE−M4
kME_MYE−M5
kME_MYE−M6
kME_T−M1
kME_T−M2
F
Francis et al. (2024)
Melanocyte
Myeloid
Fibroblast
Endothelial
Smooth muscle
Mast cell
T cell
Eccrine gland
Keratinocyte
MapQuery
ProjectModules
Keratinocyte
Myeloid
T cell
G
KER−M1
KER−M2
KER−M3
KER−M4
T−M1
T−M2
MYE−M1
MYE−M2
MYE−M3
MYE−M4
MYE−M5
MYE−M6
Day-0 NL
Day-0 L
Day-3 L
Day-14 L
H
KER−M1
KER−M2
KER−M3
KER−M4
T−M1
T−M2
MYE−M1
MYE−M2
MYE−M3
MYE−M4
MYE−M5
MYE−M6
Module
Positive module correlation
Negative module correlation
I
KER−M1
KER−M3
MYE−M2
MYE−M5
T−M1
Collagen−Containing Extracellular Matrix
Endoplasmic Reticulum Lumen
Focal adhesion
Cell−Substrate Junction
Collagen Formation
Formation of the Cornified Envelope
Keratinocyte Differentiation
Sphingolipid Metabolic Process
Lipid Metabolic Process
Cholesterol Biosynthesis
ERAD Pathway
Protein Kinase Inhibitor Activity
Biosynthesis of Lipoxins (LX)
Cytokine−cytokine receptor interaction
Cell adhesion molecules
Regulation of Alpha−Beta T Cell Proliferation
Positive Regulation of Cytokine Production
Cellular Respiration
Aerobic Electron Transport Chain
Antigen processing−Cross Presentation
TCR Signaling
PD−1 Signaling
Adjusted −log10P
J
Layer-specific pathogenesis mechanisms
Langerhans cell – Granular keratinocyte
Langerhans cell
Keratinocyte
Cornification
Lipid metabolism
Dysregulation of lipid metabolisms in granular layer
Stratum corneum
Stratum lucidum
Stratum granulosum
Stratum spinosum
Stratum basale
Basal keratinocyte – cDC2A – T cell trios
Cell-cell adhesion
Type 2A conventional dendritic cell
Keratinocyte
Immune checkpoint
T cell
Local inhibition of inflammation response in basal layer

20. Error in Table S3: SNP rs10754016 on chr 1 is annotated with RGS14 but this gene is on chr 5 and found elsewhere in the table.

Response: We appreciate the reviewer's correction and we apologize for the mislabeling. The SNP rs10754016 (chr1:192528756) was indeed mapped to *RGS1*, not *RGS14*. We have re-examined all independent SNPs and confirmed that there were no further issues.

[Updated Supplementary Table 3]

Table S3. List of independent significant SNP from GCTA-COJO

SNP	CHR	BP	ALT	REF	FRQ	BETA	SE	P	Direction	HetISq	HetPVal	Mapped genes	Loci
rs80278967	1	8209572	G	A	0.9096	0.1347	0.0175	1.39E-14	----	60.6	0.05465	*ERRFI1*	Reported
(Omitted)													
rs10912265	1	172673583	T	C	0.17	0.0862	0.0121	1.05E-12	++++	65.4	0.03405	*FASLG*	Reported
rs10754016	1	192528756	G	C	0.3426	0.0559	0.0097	8.27E-09	----	0	0.7711	*RGS1*	Newly identified
rs17641524	1	197704717	T	C	0.1812	0.1003	0.0115	2.74E-18	++++	33.9	0.2088	*DENND1B*	Reported
rs6675818	1	202783457	T	A	0.2898	0.0603	0.0105	9.31E-09	----	0	0.9796	*KDM5B*	Reported
rs12142086	1	206657802	T	C	0.7594	-0.0723	0.0106	9.06E-12	----	54.1	0.08828	*IKBKE*	Reported
rs3024495	1	206942413	T	C	0.1594	0.076	0.0123	6.46E-10	++++	73.5	0.01008	*IL10*	Reported
rs7544869	1	234734290	T	A	0.5351	0.0556	0.0097	9.93E-09	----	0	0.8559	*IRF2BP2*	Newly identified
rs1077492	2	25286435	T	C	0.5515	-0.0536	0.0092	5.67E-09	----	11.5	0.3351	*EFR3B*	Reported
rs77353560	2	28085544	C	A	0.8206	-0.0774	0.0125	5.94E-10	++++	0	0.8837	*MRPL33, RBKS*	Reported
rs842636	2	61091950	G	A	0.5657	0.1386	0.0092	2.74E-51	----	50.4	0.1091	*REL*	Reported

(The remaining rows are omitted)

21. In Table S8, it is not clear where some of the GWAS summary statistics derive from, for example "UK Biobank (SAIGE)" or "Psychiatric Genomics Consortium". A little more detail is needed for readers to be able to identify the underlying study and understand the traits represented.

Response: We thank you for the helpful suggestions for improving the manuscript. As the reviewer suggested, we have revised Table S8 to include references for the source and trait information. For example, the UK Biobank SAIGE summary statistics were provided by Lee Lab, and the GWAS was conducted based on the PheCode definitions. For the PGC group, we have added references for each published paper corresponding to the individual trait GWAS.

[Revised Supplementary Table 10]

Table S10. Phenotypes used in the genetic correlation analysis

Phenotypes	Category	rg	…	Source	Source URL
Inflammatory Bowel Disease	Autoimmune	0.2839		de Lange et al., 2017 [1]	https://ftp.sanger.ac.uk/pub/project/humgen/summary_statistics/human/2016-11-07
Crohn's Disease	Autoimmune	0.2743		de Lange et al., 2017 [1]	https://ftp.sanger.ac.uk/pub/project/humgen/summary_statistics/human/2016-11-07
Ulcerative Colitis	Autoimmune	0.2308		de Lange et al., 2017 [1]	https://ftp.sanger.ac.uk/pub/project/humgen/summary_statistics/human/2016-11-07
Rheumatoid arthritis / inflammatory polyarthropathies	Autoimmune	0.338		PheCode 714, UK Biobank (SAIGE) from Lee Lab. [2]	https://www.leelabsg.org/resources
Type 1 diabetes	Autoimmune	0.1146		Chiou et al., 2021 [3]	https://www.ebi.ac.uk/gwas/studies/GCST90014023
Systemic Lupus Erythematosus	Autoimmune	0.036		Bentham et al., 2015 [4]	https://www.ebi.ac.uk/gwas/studies/GCST003156
allergy/adverse effect of penicillin	Immune	0.2887		PheCode 960.2, UK Biobank (SAIGE) from Lee Lab. [2]	https://www.leelabsg.org/resources
Atopic dermatitis	Immune	0.272		Oliva et al., 2025 [5]	https://www.ebi.ac.uk/gwas/studies/GCST90503108
Moderate to Severe Asthma	Immune	0.2026		Shrine et al., 2019 [6]	https://www.ebi.ac.uk/gwas/studies/GCST006911
Asthma	Immune	0.1657		PheCode 495, UK Biobank (SAIGE) from Lee Lab. [2]	https://www.leelabsg.org/resources
Other inflammatory spondylopathies	Immune	0.2399		PheCode 715, UK Biobank (SAIGE) from Lee Lab. [2]	https://www.leelabsg.org/resources
Allergy	Immune	0.1027		Ferreira et al., 2017 [7]	https://genepi.qimr.edu.au/staff/manuelf/gwas_results/main.html
Peritoneal adhesions (postoperative) (postinfection)	Immune	0.4329		PheCode 568.1, UK Biobank (SAIGE) from Lee Lab. [2]	https://www.leelabsg.org/resources
Postinflammatory pulmonary fibrosis	Immune	0.1457		PheCode 502, UK Biobank (SAIGE) from Lee Lab. [2]	https://www.leelabsg.org/resources

Inflammatory and toxic neuropathy	Immune	0.1332		PheCode 357, UK Biobank (SAIGE) from Lee Lab. [2]	https://www.leelabsg.org/resources
Inflammatory diseases of Female pelvic organs	Immune	0.4563		PheCode 614, UK Biobank (SAIGE) from Lee Lab. [2]	https://www.leelabsg.org/resources

(The remaining rows are omitted)

Reviewer #2:

This manuscript presents a comprehensive genome-wide meta-analysis of psoriasis integrated with single-cell transcriptomic data. The authors leverage large-scale datasets and employ a multiomic framework to dissect the cellular and molecular basis of psoriasis susceptibility. The integration of scRNA-seq, GWAS summary statistics, and co-expression network analyses adds considerable value. However, several aspects require clarification, additional analysis, methodological transparency, and biological validation to strengthen the conclusions and reproducibility of the study. Below are specific points for major revision:

Response: We thank the reviewer for the thorough evaluation of our manuscript and for the constructive comments that have substantially improved the clarity and impact of our study. We have addressed each comment point-by-point.

1. The manuscript briefly states that Duffy's method was used to correct for self-reported cases in UK Biobank and 23andMe, but it would benefit from a clearer explanation of how this adjustment was validated. Specifically, how robust are the 73.85% and 37.5% "true positive" estimations for UK Biobank and 23andMe? The relatively low true-positive rates raise major concerns about the quality of the data and impact on downstream analyses. Was sensitivity analysis performed to test how these estimates affect meta-analysis results?

Response: We thank the reviewer for the comment. As the reviewer has pointed out, we acknowledge that such a relatively low true-positive rate may raise concerns for downstream analyses and recognize the importance of addressing this issue. Duffy's approach is based on a well-established GWAS meta-analysis of psoriasis [**Ref. 1**]. The authors reported that allele frequencies in control samples from the 23andMe cohort were largely comparable to those in the psoriasis consortium GWAS [**Ref. 2**]. However, notable differences were observed in case samples between the 23andMe cohort and psoriasis consortium. We observed the same patterns in UK Biobank and 23andMe cohorts, which included self-reported cases. Including self-reported cases may introduce a proportion of false positives, leading to an underestimation of odds ratios and standard errors [**Ref. 1**]. The Tsoi's study addressed this problem using a

statistical correction method called the Duffy's method, which could correct for the downward bias of the estimated odds ratios and standard errors at the cost of a substantial decrease in the effective sample size.

We applied the same procedures as those in the original study and implemented the corresponding corrections to our data. Briefly, this approach estimates the proportion of false-positive cases by leveraging the allele frequency differences in well-established psoriasis risk alleles between case and control samples. Assuming that the sensitivity of the binary classification is 1 (i.e., all true cases are correctly reported), the specificity is estimated and used to infer the adjusted effect sizes and standard errors.

To further demonstrate the importance of this correction for downstream analyses, we conducted additional validation analyses. First, we calculated polygenic risk scores (PRS) in the UK Biobank using both the original 23andMe GWAS summary statistics (before Duffy correction) and the corrected GWAS summary statistics (after Duffy correction) to assess the benefit of the correction (**Supplementary Fig. 3**). We observed a modest but consistent improvement in the predictive performance using the corrected GWAS. In addition, in our response to the following Comment #2, we further emphasized the importance of including self-reported cases with appropriate correction using the Duffy's method, based on the comparison of PRS between ICD-diagnosed cases and self-reported cases.

References

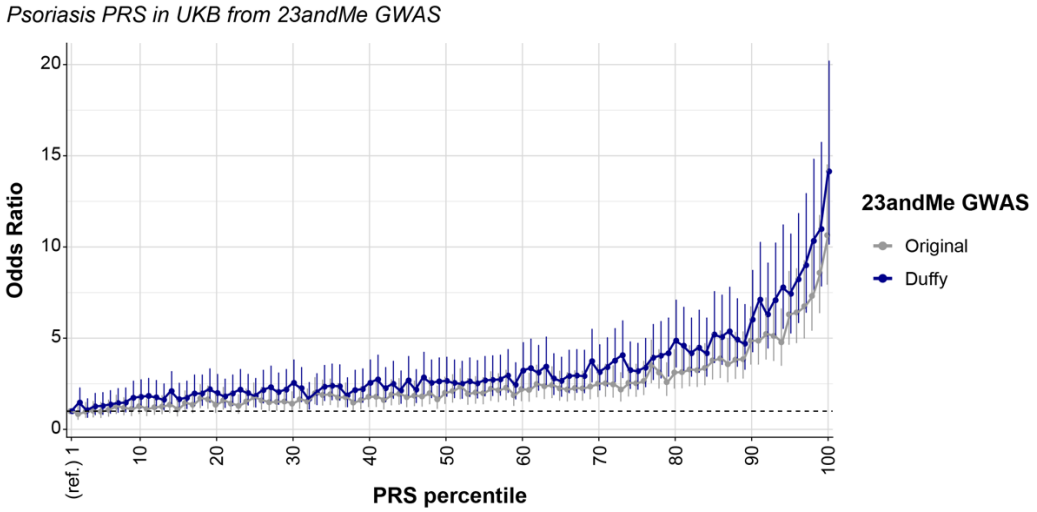
Ref. 1: Tsoi, L. C. et al. Large scale meta-analysis characterizes genetic architecture for common psoriasis associated variants. Nature communications 8, 15382 (2017).

Ref. 2: Tsoi, L. C. et al. Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. Nature genetics 44, 1341-1348 (2012).

[Added Supplementary Figure 3]

Supplementary Figure 3. PRS analysis evaluating the effect of Duffy's adjustment. Polygenic risk scores (PRSs) were calculated for UK Biobank samples using both the original 23andMe GWAS and the Duffy-adjusted 23andMe GWAS summary statistics. The PRS results

were stratified by percentile, and odds ratios were estimated using the lowest percentile group as the reference.



[Added to the Results, page 7, lines 115–118]

To assess the impact of correcting for false-positive cases, we calculated polygenic risk scores (PRS) in UK Biobank samples using 23andMe psoriasis GWAS summary statistics before and after applying Duffy's adjustment (**Supplementary Fig. 3**). The adjusted GWAS statistics resulted in a modest but consistent improvement in the predictive performance.

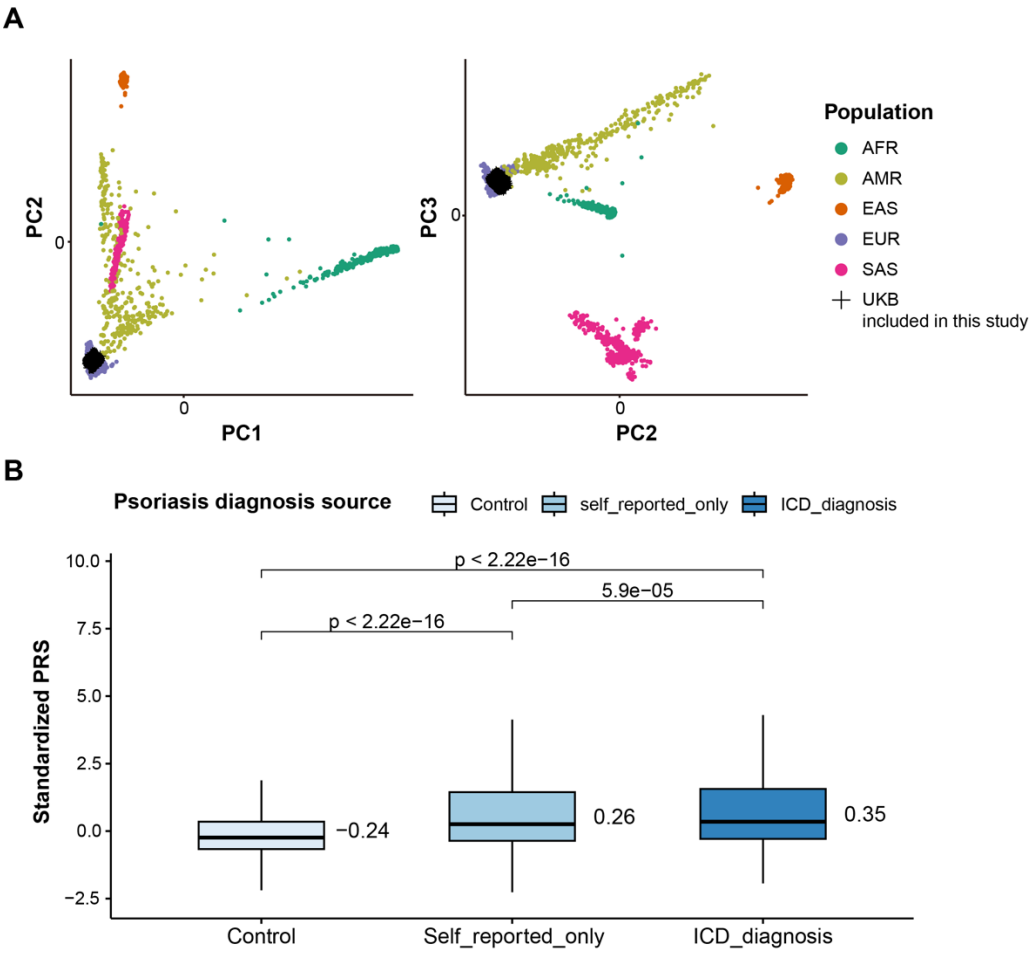
2. For the UK Biobank analysis, it would be informative to stratify by diagnosis source (self-report vs. ICD codes) to assess heterogeneity. Also, clarify whether Tsoi et al.'s European ancestry samples were used exclusively for RAF estimation, and provide ancestry PCA comparisons between reference and study populations.

Response: We thank the reviewer for this comment. We included only UK Biobank participants of European ancestry, which we further verified by projecting them onto the principal components of known populations from the 1000 Genomes Project (**Supplementary Fig. 1A**). To expand on Comment #1, we evaluated how ICD-diagnosed and self-reported psoriasis cases differed in the UK Biobank using polygenic risk scores (**Supplementary Fig. 1B**). For this analysis, we applied summary statistics from a meta-analysis of FinnGen r10 and Stuart et al. (2022) psoriasis GWAS, both of which did not include self-reported cases. Of note, self-reported

cases showed slightly lower scores than ICD-diagnosed cases but still substantially higher scores than controls, indicating a clear enrichment of genetic risk in self-reported cases. These findings support the inclusion of self-reported cases but also indicate the need to correct for false positives using the Duffy's method.

[Added Supplementary Figure 1]

Supplementary Figure 1. UK Biobank samples used in this study.
(A) Principal components of UK Biobank samples projected onto the 1000 Genomes Project reference panel, which includes multi-ancestry samples. The UK Biobank samples used in this study are clustered on the European ancestry group.
(B) Polygenic risk scores (PRSs) of UK Biobank samples stratified by psoriasis status, including ICD-diagnosed cases, or self-reported only cases, and healthy controls. Meta-analysis of FinnGen r10 and Stuart *et al.* GWAS was used to calculate PRSs of the UK Biobank samples.



Although the genotype data from Tsoi et al. (2012) are not publicly accessible, the original paper specified that all five datasets included in that study consisted exclusively of individuals of European ancestry (**Response Notes**).

Response Note 2. Text from the Tsoi et al. (2012) article

The samples used in the three GWAS data sets (Kiel, CASP and WTCCC2) were previously described. Samples in the PAGE and GAPC data sets were collected from subjects of European descent at the participating institutions after obtaining informed consent. Enrollment of human subjects for this study was approved by the ethics boards of the participating institutions in adherence with the Declaration of Helsinki Principles.

Response Note 3. Population details of five datasets used in Tsoi et al. (2012)

Kiel: German from Ellinghaus et al. (2010)

CASP: All white European ancestry from Nair et al. (2009)

WTCCC2: UK, Ireland from Strange et al. (2010)

PAGE Immunochip: USA, Canada, Estonia, Germany

GAPC Immunochip: UK, Finland, Spain, The Netherlands, Austria, Sweden, Ireland

[Added to the Results, page 7, lines 107–111]

Using the UK Biobank samples of European ancestry, we compared polygenic risk scores (PRS) of ICD-diagnosed psoriasis cases, self-reported only cases, and controls. Self-reported only cases showed lower PRS values than ICD-diagnosed cases but higher PRS values than controls, supporting the inclusion of self-reported cases with appropriate correction (**Supplementary Fig. 1**).

[Added to the Discussion, page 19, lines 364–366]

Based on two PRS analyses, we demonstrated the rationale for including self-reported cases and showed that, despite their relatively high false-positive rates, these cases can be appropriately incorporated into GWAS analyses using Duffy's adjustment method.

[Added to the Methods, pages 28, lines 559–568]

Polygenic risk score analysis

To evaluate the impact of adjusting for psoriasis status and effect sizes in GWAS that included self-reported cases, we performed two complementary PRS analyses. First, we constructed GWAS summary statistics by performing a meta-analysis of the FinnGen r10 and Stuart et al. psoriasis GWAS using METAL, both of which did not include self-reported cases. The meta-GWAS summary statistics were used to calculate PRSs in the UK Biobank. Second, we assessed the predictive performance of the PRSs derived from the 23andMe psoriasis GWAS before and after applying the Duffy's adjustment method to the UK Biobank samples. PRSs were calculated using the PRS-CS-auto algorithm with default parameters to infer the posterior SNP effect sizes. European ancestry samples from the 1000 Genomes Project were used as the LD reference panel.

3. A demographic summary for the single-cell cohort is lacking. Please include a table summarizing the sample composition, including age, sex, disease status, and whether matched lesional and non-lesional skin samples were obtained from the same individuals. Common risk factors would be important to know as well, such as BMI and smoking.

Response: We thank the reviewer for this helpful suggestion. Because all single-cell datasets used in our analysis were obtained from publicly available external resources, we apologize that we can only summarize the information that is directly accessible because of limitations in data availability. Considering the reviewer's Comment #4, we incorporated sample-level information for the external single-cell datasets as well as other used data such as the spatial transcriptome dataset in **Supplementary Table 26**.

[Added Supplementary Table 26]

Table S26. Demographics of transcriptomic samples used in this study.

(1) Sample demographics of Ma et al. scRNA-seq data [ref. 1]

Subject ID	Age at biopsy	Sex	Biopsy
AR001	NA	NA	NS
AR003	NA	NA	NS

AR004	NA	NA	NS
AR005	NA	NA	NS
AR006	NA	NA	NS
AR007	NA	NA	NS
AR008	NA	NA	NS
AR009	NA	NA	NS
929	59	M	PP and PN
5851	37	F	PP and PN
8940	50	M	PP and PN
6215A	48	M	PP only
30696	66	M	PP and PN
31170	56	M	PP and PN

(The remaining rows are omitted)

(2) Sample demographics of Francis et al. scRNA-seq data [ref. 2]

Subject ID	Age at baseline	Sex	PASI scores		
			Baseline (Day 0)	Day3	Day 14
P1	Mean: 30.1 SD: 9.6	M	23	20	6
P2		M	40	25	17
P3		M	15	11	7
P4		M	18	15	9
P5		M	23	18	8

(3) Sample demographics of Kim et al. scRNA-seq data [ref. 3]

Sample ID	Subject ID	Age at biopsy	Sex	Timeline	Status	Site	PASI scores
GSM4567877	Control 01	37	M	Baseline	Control	Trunk	0
GSM4567878	Control 02	36	M	Baseline	Control	Trunk	0
GSM4567879	Control 03	65	M	Baseline	Control	Trunk	0
GSM4567880	Control 04	40	M	Baseline	Control	Leg (right)	0
GSM4567881	Control 05	39	M	Baseline	Control	Leg (right)	0
GSM5550124	Control 06	41	M	Baseline	Control	Leg (right)	0
GSM5550125	Control 07	51	F	Baseline	Control	Arm (left)	0
GSM5550126	Control 08	51	F	Baseline	Control	Arm (left)	0
GSM5550127	Control 09	73	M	Baseline	Control	Trunk	0
GSM5550128	Control 10	73	M	Baseline	Control	Trunk	0
GSM8544932	Psoriasis 07	40	M	Baseline	Lesion	Trunk	16.4
GSM8544933	Psoriasis 07	40	M	Baseline	Lesion	Trunk	16.4
GSM8544934	Psoriasis 07	40	M	Baseline	Non-lesion	Trunk	16.4

GSM8544935	Psoriasis 07	40	M	Baseline	Non-lesion	Trunk	16.4
GSM8544938	Psoriasis 10	38	M	Baseline	Lesion	Trunk	17.7
GSM8544939	Psoriasis 10	38	M	Baseline	Lesion	Trunk	17.7
GSM8544940	Psoriasis 10	38	M	Baseline	Non-lesion	Trunk	17.7
GSM8544941	Psoriasis 10	38	M	Baseline	Non-lesion	Trunk	17.7
GSM8544942	Psoriasis 10	39	M	7 months after the first risankizumab injection	Lesion	Trunk	0
GSM8544943	Psoriasis 10	39	M	7 months after the first risankizumab injection	Lesion	Trunk	0

(The remaining rows are omitted)

(4) Sample demographics of Castillo et al. Spatial transcriptome data [ref. 4]

Sample ID	Subject ID	Age at baseline	Sex	Ethnicity	Site	Diagnosis	PASI scores
HV1_TRUNK_R1	Healthy volunteer 1	45	M	Other	Left flank	Healthy	0
HV1_TRUNK_R2	Healthy volunteer 1	45	M	Other	Left flank	Healthy	0
HV2_FA_R1	Healthy volunteer 2	36	F	Asian	Right forearm	Healthy	0
HV2_FA_R2	Healthy volunteer 2	36	F	Asian	Right forearm	Healthy	0
HV2_TRUNK	Healthy volunteer 2	36	F	Asian	Right flank	Healthy	0
HV3_FA_R1	Healthy volunteer 3	36	F	Asian	Left foream	Healthy	0
HV3_TRUNK	Healthy volunteer 3	36	F	Asian	Left flank	Healthy	0
PSO_P1_L	PSO patient 1	57	M	Hispanic	Left flank	Psoriasis	11.2
PSO_P2_L_R1	PSO patient 2	59	F	White	Left foream	Psoriasis	1.8
PSO_P2_L_R2	PSO patient 2	59	F	White	Left foream	Psoriasis	1.8
PSO_P3_L	PSO patient 3	48	M	Hispanic	Right flank	Psoriasis	6
PSO_P4_L	PSO patient 4	68	M	White	Left flank	Psoriasis	32

(The remaining rows are omitted)

4. The integration of scRNA-seq data from lesional, non-lesional, and normal skin samples is well-executed. However, the sample size is modest, especially when making claims about tissue-specific or disease-specific expression patterns. Given the availability of publicly accessible psoriasis single-cell datasets, incorporating and comparing to external will improve statistical power and benchmarking.

Response: We thank the reviewer for the helpful comment. Our study included the two largest available single-cell datasets for psoriasis at the time of analysis. Nevertheless, we acknowledge

the inherent limitation of single-cell studies related to the relatively limited number of samples. In addition, single-cell datasets often exhibit substantial heterogeneity across samples. To strengthen the robustness of our findings, we incorporated an additional independent dataset (Kim et al.) as a replication set [**Ref. 1**]. Similar to the Francis et al. dataset, this dataset included skin scRNA-seq data from controls and patients, as well as samples collected before and after risankizumab treatment. Notably, whereas the Francis et al. dataset captured relatively short-term treatment responses (3 and 14 days post-treatment), the Kim et al. dataset included samples collected at week 28 post-treatment, providing an opportunity to assess long-term single-cell transcriptomic changes.

Owing to differences in data characteristics and increased heterogeneity compared to the two datasets included in the main analysis, direct cell-type matching was challenging (**Supplementary Fig. 18C**). Despite this, we consistently observed significant scDRS enrichment in myeloid and T cells in this independent dataset (**Supplementary Fig. 18D**). In contrast, scDRS significance was not detected in the keratinocytes (basal keratinocytes, p-value = 0.177; granular keratinocytes, p-value = 0.990). We further applied the same cell-cell interaction analysis pipeline introduced during the revision process to this dataset. Unlike the relatively modest associations observed in short-term treatment samples (**Supplementary Fig. 17A**), the long-term dataset revealed a significant shift of gene expression in post-treatment lesional samples toward control levels (**Supplementary Fig. 18E**).

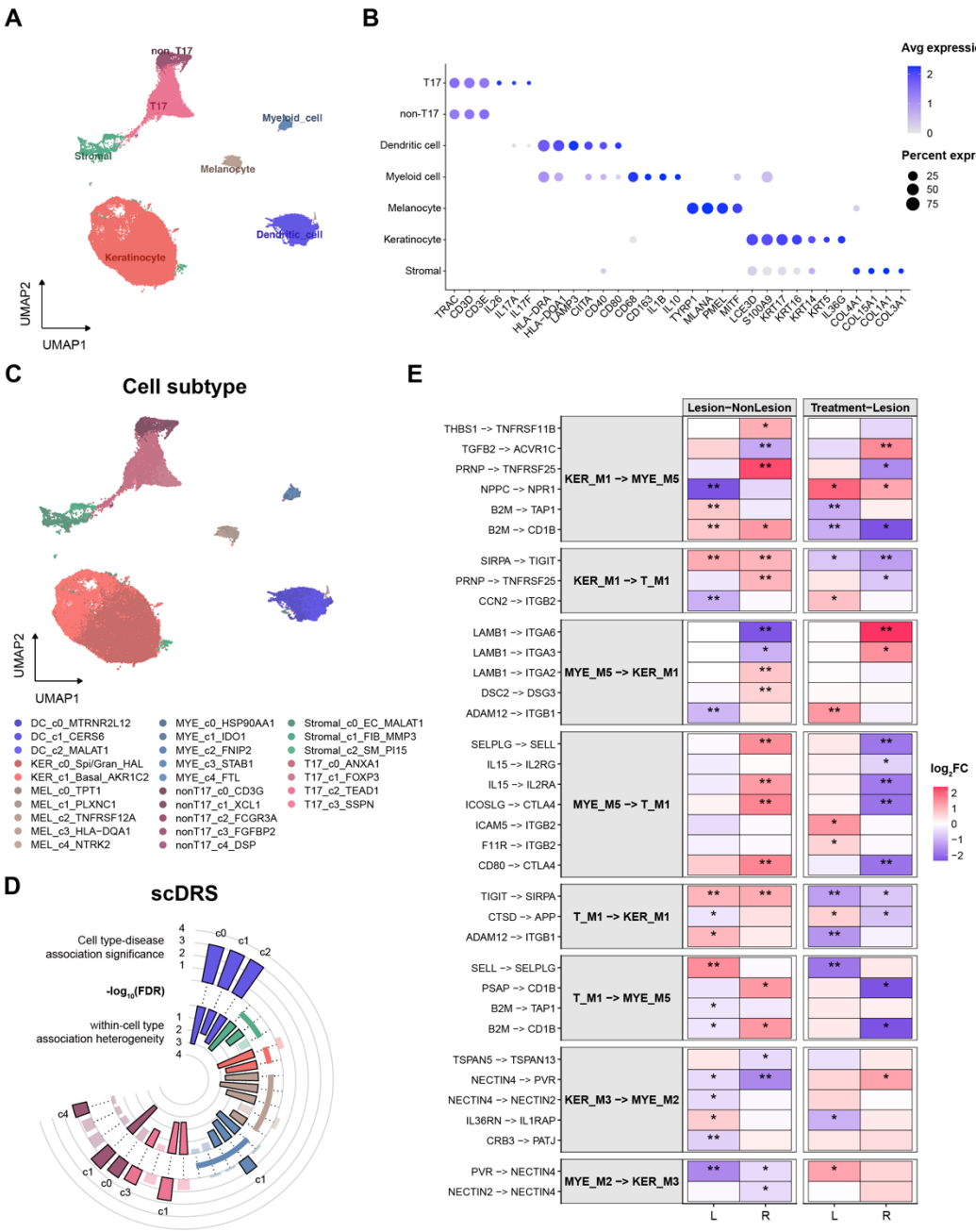
[Added to the Results, page 17, lines 343–350]

To assess the consistency across multiple datasets and the generalizability of the findings, we analyzed the Kim et al. dataset, which included samples of healthy controls and patients with psoriasis collected at week 28 after risankizumab treatment (**Supplementary Fig. 18** and **Supplementary Table 26**). Despite distinct cellular expression profiles compared to other single-cell datasets, including clearer separation of dendritic and other myeloid cells and clustering of endothelial and fibroblast populations, scDRS analysis robustly and consistently revealed significant enrichment in dendritic cell and T cell clusters (**Supplementary Fig. 18A–D**).

[Added Supplementary Figure 18]

Supplementary Figure 18. Replication analysis of single-cell analysis using the Kim et al. dataset.

(A) UMAP plot of single cells with major cell type annotation. **(B)** Dot plot showing expression of representative cell markers. **(C)** UMAP plot of cell subtype annotation of each major cell type. **(D)** Cell-subtype-level scDRS result. Bar plots in the outer circle represent the FDR-adjusted *p*-values of cell-subtype-disease association and bar plots in the inner circle represent the FDR-adjusted *p*-values of within-cell type heterogeneity. **(E)** Ligand (L)-receptor (R) interactions that exhibit differential expression between lesion and non-lesion samples (Left) and treatment and lesion samples (Right). The heatmap shows the log2-transformed fold changes of the gene expression between two conditions. Each panel name represents the directionality of the ligand module to the receptor module. Double asterisks denote the adjusted p-value < 0.05, and single asterisks denote the nominal p-value < 0.05.



[Added to the Methods, pages 36–37, lines 744–751]

Additionally, an independent scRNA-seq dataset from Kim et al. was included as the replication dataset. This dataset included skin scRNA-seq data from healthy controls and patients with psoriasis, as well as samples collected at a long-term post-treatment time point (week 28 after risankizumab treatment). All the preprocessing steps were performed using the same pipeline as described above. Cell type annotation for this dataset was conducted independently using

canonical marker genes reported in the original study rather than reference mapping because substantial heterogeneity in data characteristics driven by distinct expression profiles resulted in reference-based mapping being disproportionately assigned to a single cluster.

References

Ref. 1. Kim, J. et al. Psoriasis harbors multiple pathogenic type 17 T-cell subsets: Selective modulation by risankizumab. Journal of Allergy and Clinical Immunology (2025).

5. The interpretation of pseudotime as reflecting the epidermal layer structure is compelling, but it's not clearly explained how pseudotime was inferred and validated. Further analysis and validation is needed to determine if the inferred pseudotime aligns with known keratinocyte differentiation trajectories.

Response: We thank the reviewer for the comment. Pseudotime inference aims to estimate the relative differentiation states of individual cells by leveraging continuous variation in cell states represented in low-dimensional embeddings, such as PCA or UMAP. Based on this framework, cells can be ordered along inferred trajectories from less-differentiated to more-differentiated states.

In this study, we applied the Slingshot algorithm using single-cell expression profiles and UMAP low-dimensional spaces of keratinocytes to infer the lineage trajectories and pseudotime values for individual cells. Keratinocyte differentiation from the basal layers toward the outer epidermis is well established [**Ref. 1**]. We examined the expression of the known basal keratinocyte markers, *KRT5* and *KRT14*, and confirmed that the KER_c3_UBE2C cluster represented basal keratinocytes (**Supplementary Fig. 10**). Using KER_c3_UBE2C as the initial state (with Slingshot parameter, start.clus = "KER_c3_UBE2C"), Slingshot inferred differentiation trajectories and pseudotime consistent with keratinocyte maturation.

To further validate keratinocyte differentiation, we additionally performed an RNA velocity analysis (**Supplementary Fig. 12**). RNA velocity estimates the future transcriptional states of

cells by modeling the relative abundance of unspliced and spliced transcripts [**Ref. 2**]. Using this approach, we observed the lower proportions of unspliced transcripts in basal layer, with a progressive increase in granular keratinocytes (**Supplementary Fig. 12A)**. Higher proportions of unspliced reads indicated active transcription and were generally associated with less differentiated cellular states. Although the velocity arrows showed some noise due to sample heterogeneity, the overall directionality was consistent with the inferred pseudotime trajectories (**Supplementary Fig. 12B**).

We have added descriptions of trajectory inference, RNA velocity analyses and the corresponding results to the **Results** and **Methods** sections.

References

Ref. 1. Freedberg IM, Tomic-Canic M, Komine M, Blumenberg M. Keratins and the keratinocyte activation cycle. Journal of Investigative Dermatology 116, 633-640 (2001).
Ref. 2. La Manno G, et al. RNA velocity of single cells. Nature 560, 494-498 (2018).

[Added to the Results, page 12, lines 223–227]

Additionally, RNA velocity analysis identified the basal keratinocytes with high unspliced proportions, indicating an active transcription potential in less-differentiated states (**Supplementary Fig. 12A**). Although the velocity arrows showed some noise due to sample heterogeneity, the overall directionality was consistent with the inferred pseudotime trajectories (**Supplementary Fig. 12B**).

[Added to the Methods, pages 32–33, lines 656–666]

Within the keratinocyte population, the cells exhibited a continuous spatial organization along the epidermal layer in the UMAP space. Keratinocyte differentiation from the basal layers toward the outer epidermis is well established. This pattern suggests a gradual differentiation process and applied trajectory inference using a Slingshot library with parameter start.clus = "KER_c3_UBE2C". This analysis allowed us to model pseudotime trajectories, with basal layer keratinocytes serving as the starting point, progressing toward the spinous and granular layers. To further validate keratinocyte differentiation, RNA velocity analysis was performed using

scVelo for the functional validation. RNA velocity estimates future transcriptional states of cells by modeling the relative abundance of unspliced and spliced transcripts in the single-cell expression profiles. Higher proportions of unspliced reads indicated active transcription and were associated with less differentiated cellular states.

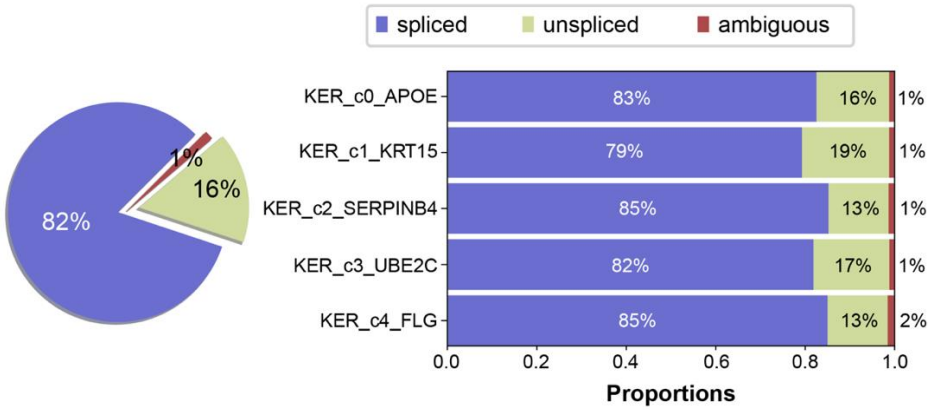
[Added Supplementary Figure 12]

Supplementary Figure 12. RNA velocity analysis of keratinocyte clusters.

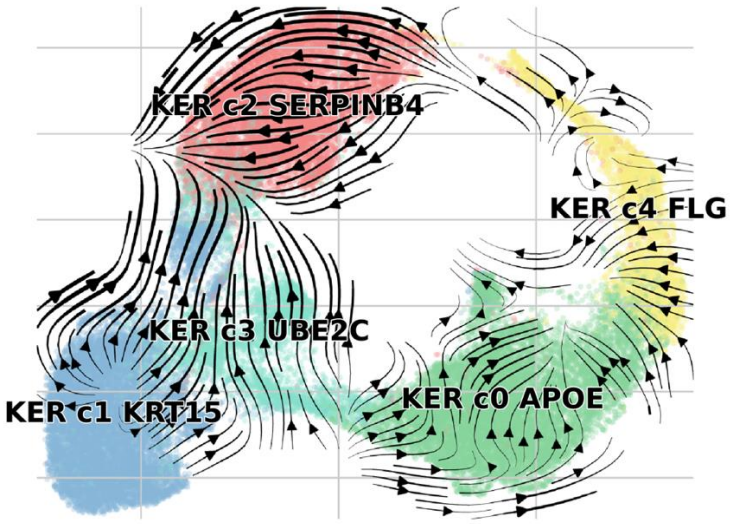
(**A**) The proportion of unspliced and spliced reads per cluster.

(**B**) RNA velocity streams of keratinocytes on UMAP space.

A



B



6. The scDRS integration adds a powerful layer of interpretation, linking GWAS signal to specific cell types. However, could the results of granular layer keratinocytes from normal skin show high disease relevance reflect shared genetic programs or some baseline inflammatory priming? Do higher scDRS scores indicate causality, increased susceptibility, or response to inflammation?

Response: We thank the reviewer for the helpful comment. Using scDRS, we assessed the epidermal cell types that showed stronger enrichment of psoriasis GWAS-associated genetic risk at the single-cell level. Granular keratinocytes exhibited higher scDRS scores than spinous keratinocytes, indicating a greater burden of psoriasis-associated genetic signals. Although these results do not allow us to firmly claim causality, they suggest that granular keratinocytes may be a relevant cell type involved in the earlier stages of psoriasis development. In addition to immune cells, we observed statistically significant enrichment in non-immune cells, keratinocytes, and a subset of endothelial cells. We have added relevant text to the revised **Discussion** section. We have also added a clarification in the **Discussion** section regarding the specific characteristics of the endothelial cell cluster showing marginal significance and explained why it was not included in the downstream analyses.

[Added to the Discussion, pages 20–21, lines 397–417]

The scDRS algorithm enabled the identification of specific cell types potentially involved in the development of psoriasis by calculating a genetically driven disease relevance score for each cell type. Using the scDRS algorithm, strong associations between myeloid cells and T cells were consistently observed. We also identified subpopulations of keratinocytes and ECs that may be involved in psoriasis development. Notably, keratinocytes exhibited a continuous expression pattern from the basal to granular layers, reflecting the spatial organization of the epidermis. Cells in the outermost nucleated epidermal layer, which is characterized by pronounced cornification features, showed the strongest association with psoriasis. The EC_c1_CDKN1A cluster, which was enriched in normal skin tissue, showed marginal significance in psoriasis (FDR = 0.069). These cells were characterized by the expression of *NFKBIA* and *SOCS3*, which suppress cytokine responses and regulate inflammatory signaling via negative feedback mechanisms (**Supplementary Table 13**). These findings suggest that dysfunction of this specific

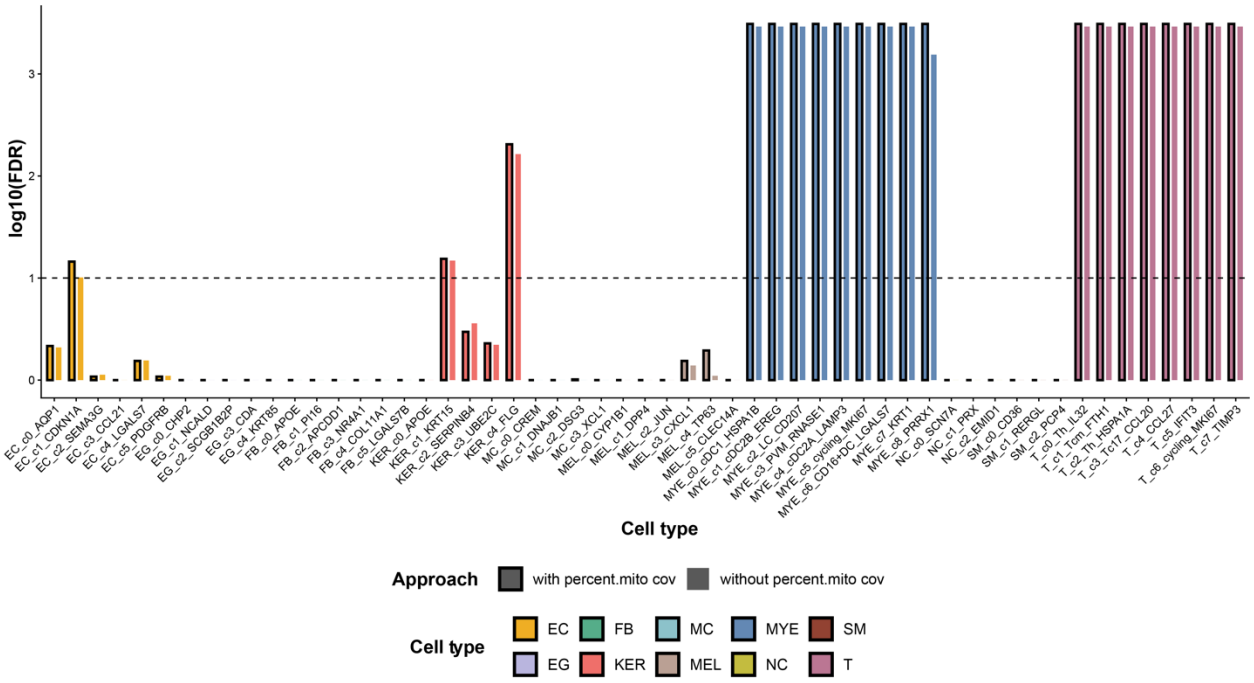
subset of endothelial cells may contribute to the pathogenesis of psoriasis. Within this cell cluster, cells with higher scDRS scores formed a distinct subcluster. This subpopulation showed higher expression of genes involved in the AGE-RAGE signaling pathway and negative regulation of TGF-β receptor signaling. Previous studies have reported that AGE-RAGE interactions are implicated in psoriasis and can suppress downstream TGF-β-mediated functions. Given the marginal statistical significance and increased analytical complexity, we excluded endothelial cells from downstream analyses. Further research will provide deeper insights into the role of endothelial cells in modulating immune cell function in psoriasis.

7. Related to the scDRS, "The scDRS score was weighted using the MAGMA z-scores and inversely weighted using technical noise in single-cell gene expression. The mitochondrial UMI percentages and tissue types (NS, PN, and PP) were used as covariates." Traditionally, mitochondrial UMIs are quality control metrics and it's being used as a covariate just as the tissue types and not part of the technical noise? Please clarify their role in the model.

Response: We thank the reviewer for carefully reviewing our methods and providing the insightful question. In line with the reviewer's comment, mitochondrial UMI percentage is generally used as a per-cell quality metric and can reflect stress responses or other biological states. However, in scDRS, the primary goal is to assess how strongly psoriasis-associated genes identified by GWAS are enriched at the cell-type level rather than to characterize individual cell states. Cells with high mitochondrial gene expression can show artifactual reductions in the relative expression of other genes of interest, thereby inflating the variance in the scDRS scores. To minimize this effect, we included the mitochondrial UMI percentage as a covariate, consistent with previous scDRS-based analysis that was also adjusted for this metric (**Ref. 1**; Github code: https://github.com/zhaoshuoxp/czi_rna).

For sensitivity analysis, we compared the scDRS results with and without mitochondrial UMI percentage as a covariate (**Response Fig. 1**). The analysis showed that including this covariate had only a minimal impact on the overall results.

Response Figure 1. Comparison of the scDRS results with and without the inclusion of mitochondrial UMI percentages (percent.mito) as covariates.
The horizontal dashed line represents the statistical significance threshold (FDR < 0.10).



References

Ref. 1: Zhao Q, et al. A cell and transcriptome atlas of human arterial vasculature. Cell Genomics (2025).

8. The integrative pipeline (MAGMA, TWAS, eQTL colocalization, scRNA expression, SMR) is a rigorous strategy for gene prioritization. However, it's unclear how these criteria were weighted or ranked when selecting the top genes.

Response: We thank the reviewer for the comment. In our pipeline, we defined a prioritization score as the unweighted sum of whether each gene passed the criteria of five different methods, following a previously published GWAS [**Ref. 1**]. Using this score, we identified 110 genes that met the criteria for at least four of the five methods. We then performed summary-data-based Mendelian randomization (SMR) and confirmed that 50 genes were causally associated with gene expression in the blood or skin. We have revised the sentence to improve clarity.

References

Ref. 1. Han X, et al. Large-scale multitrait genome-wide association analyses identify hundreds of glaucoma risk loci. Nature genetics 55, 1116-1125 (2023).

[Rephrased to the Methods, pages 33–34, lines 669–686]

Genes with potential causal effects on psoriasis were prioritized by integrating GWAS and single-cell data. For the initial prioritization, five independent criteria were applied. Genes that met at least four of these criteria were considered as putative susceptibility genes for psoriasis. First, we identified the genes located within 10 kb of the COJO-identified SNPs using the FUMA web tool. Second, we assessed gene-level statistical significance using MAGMA, which calculates significance based on the distribution of *p*-values from GWAS-significant SNPs within coding regions, as implemented in FUMA. (Omitted). Fifth, we selected genes expressed in at least 10% of cells from any of the four cell types: keratinocytes, myeloid cells, T cells, and ECs which showed significant scDRS results (FDR < 0.1). ~~Genes that met at least four of these criteria were considered putative susceptibility genes for psoriasis.~~

9. “While all myeloid and T cells exhibited significant associations, Langerhans cells (LCs) and cycling myeloid cells displayed higher disease relevance scores”. When the authors say exhibited significant associations, is this a logistic model or other statistical tests? Any adjustments for any covariates (which would be nice to see in a demographic table mentioned above). Specifically, in Fig 4D there is a great visual of effect size of ration of observed/expected non-lesion/lesion cells in each cell type. However, was just in the figure legend and not mentioned in the methods. This seems to not be a standard statistical test, there’s no hypothesis testing, no variance estimates, no adjustment for potential confounders, no p-values, and no control for multiple comparisons.

Response: We thank the reviewer for the comment. The statement made by the reviewer indeed refers to the disease relevance scores derived from scDRS analysis. Using group-level statistical associations, we observed significant enrichment across all myeloid and T cell populations (**Fig.**

4E). In the scDRS, statistical significance was evaluated at the single-cell level by constructing control gene sets that were randomly selected to match the mean and variance of the tested gene set. In our analysis, we generated 10,000 control sets. Statistical significance was then calculated using a non-parametric permutation test by comparing the scDRS scores derived from psoriasis-associated genes with the distribution of scDRS scores obtained from the 10,000 control sets using the following formula.

$$p - \text{value} = \frac{\text{The number of control statistics exceeding disease statistic}}{10000 + 1}$$

The panel shown in **Fig. 4D** represents the ratio of observed to expected cell counts (Ro/e), which quantifies whether a given cell type is relatively more abundant in lesional or non-lesional skin. This metric is commonly used in single-cell analyses to assess disease-associated cell-type enrichment. We have added a detailed description of the formula used to calculate the observed-to-expected ratio to the **Methods** section.

[Added to the Methods, pages 31–32, lines 633–642]

For each cell subtype, we estimated the expected cell counts by constructing a contingency table between the tissue group (lesion vs. non-lesion) and cell membership in the subtype of interest. The expected number of cells was calculated using the following formula, employed in the chi-square test:

$$Expected\ cells = \frac{(Total\ cells\ of\ the\ subtype) \times (Total\ cells\ in\ the\ tissue\ group)}{Total\ number\ cells}$$

Finally, the ratio of observed to expected (Ro/e) values was calculated for lesion and non-lesion tissues as follows:

$$Ro/e = \frac{Observed\ cells}{Expected\ cells}$$

If the Ro/e value for the non-lesion was greater than 1, the cell subtype was defined as normal-enriched. Otherwise, the subtype was defined as lesion-enriched if the Ro/e value for lesional tissue was greater than 1.

10. The association of keratinocyte modules and the mapping of immune modules to specific myeloid and T cell subtypes are interesting. Do any modules uncover novel biology or candidate regulators not previously implicated in psoriasis?

Response: We thank the reviewer for the comment. Based on the module and cell-cell interaction analyses, we propose two novel epidermal layer-specific biological mechanisms. To clarify the analytical workflow and progression of our findings, further analyses and results supporting the proposed novel biological mechanisms have been integrated and described in our response to Comment #12.

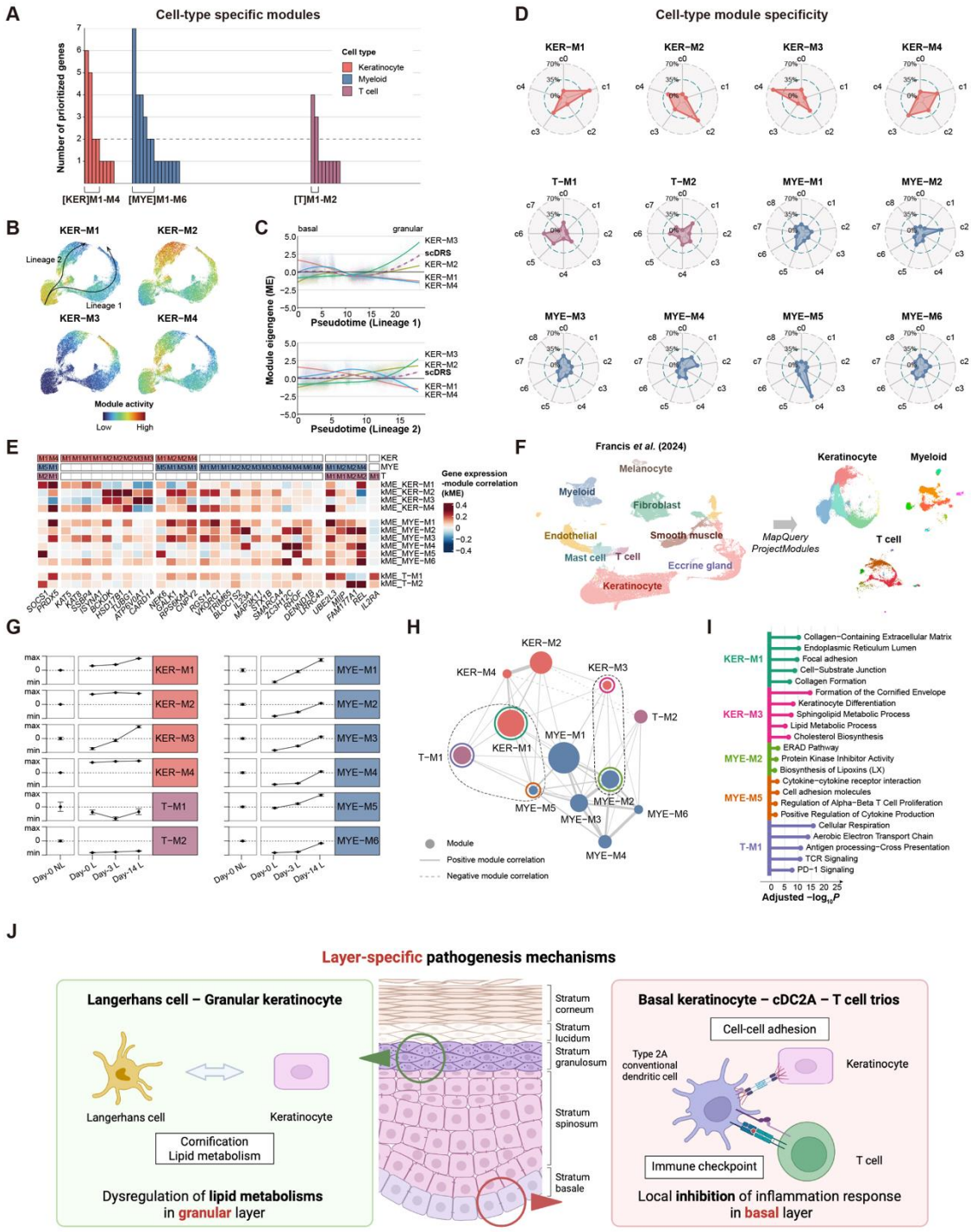
11. The identification of IL23A as a highly connected node in the MYE-M2 module is notable. What specific downstream immune pathways are co-expressed or co-regulated with IL23A in the MYE-M2 module? Is there any evidence of LC–T cell interaction, especially with Tc17 or Th17 cells, in the dataset that would support functional IL23 signaling? Is IL23A hub status driven by lesion-specific expression or broader co-regulatory structure? I believe further in-depth analysis and/or experimental validation would be needed.

Response: We thank the reviewer for highlighting interesting point. Pathway enrichment results for MYE-M2 module genes co-expressed with IL23A are shown in **Fig. 6I**, where we observed the enrichment of pathways related to protein kinase inhibition and biosynthesis of lipoxins. As part of the revision, we identified an issue in which incorrect gene sets were used for the pathway enrichment analysis shown in **Fig. 6I**, which has now been corrected.

In addition, we examined lesion-specific functional differences based on whether the module (MYE-M2)-gene connectivity (correlation) increased or decreased. We also evaluated the physical proximity between Langerhans cells and T17 cells using cell-cell interactions and spatial transcriptomic data. To show the analytical flow of analyses and results, the details of these in-depth analyses are described in our response to Comment #12.

[Modified Figure 6I]

Figure 6. Cell type-specific gene co-expression modules associated with risankizumab treatment.



12. The clustering of keratinocyte, myeloid, and T cell modules could reveal evidence of cross-cell-type communication. Can the authors extend this analysis to infer ligand-receptor interactions to validate and expand these findings?

Response: We thank the reviewer for these helpful comments and for providing suggestions, which substantially improved the direction and biological implications of our study. We have addressed the reviewer's suggestions in comments #10, #11, and #12 as a single related topic. Following these suggestions, we performed additional cross-cell-type communication analyses and validation using spatial transcriptome data from an independent psoriasis study. As a result, we identified and propose two layer-specific biological mechanisms underlying psoriasis.

First, we conducted a cross-cell-type communication analysis using MultiNicheNet [**Ref. 1**], a framework designed to identify ligand-receptor pairs that are differentially active between the conditions of interest (non-lesional, lesional, and treatment conditions in our study). Analyses were performed using IL-23 inhibition treatment data, focusing on keratinocytes, myeloid cells, and T cells. We restricted our analyses to ligand-receptor pairs between two sets of modules highlighted in **Fig. 6H** (KER-M3 and MYE-M2; KER-M1, MYE-M5, and T-M1). For example, we analyzed the interactions between KER-M3 and MYE-M2 by comparing ligand-receptor pairs between keratinocyte genes in the KER-M3 module and myeloid genes in the MYE-M2 module. Differential testing of ligand-receptor pairs across non-lesional, lesional, and treatment samples enabled us to assess changes in ligand-receptor activity associated with psoriasis and its modulation by IL-23 inhibition (**Supplementary Fig. 17A**).

Through this analysis, we identified the basal keratinocyte-DC2A-T cell interaction axis for psoriasis. The MYE-M5 module, which showed the strongest association with cDC2A (**Fig. 6D**), exhibited significantly increased interactions with a module KER-M1, indicating basal keratinocyte function. Between the two cell types, the *DSC2-DSG3* interaction showed increased activity in lesional skin. DSC2 and DSG3 are well known to mediate heterophilic cadherin-based cell-cell adhesion [**Ref. 2**], suggesting that cadherin-mediated physical adhesion between cDC2A and basal keratinocytes is significantly enhanced in psoriatic lesions. In addition, interactions between cDC2A and T cells were also upregulated. Consistent with its reported regulatory and

anti-inflammatory functions, cDC2A showed increased CD80-CTLA4 interactions with T cells in lesional skin.

To validate whether basal keratinocytes and cDC2A are spatially proximal and whether this proximity is associated with disease severity, we leveraged spatial transcriptome data from an independent psoriasis study by Castillo et al. (2023) [**Ref. 3**]. This dataset comprised 14 lesional skin samples from 11 patients with psoriasis and seven normal skin samples from three individuals. We defined cDC2A spots as those co-expressing *LAMP3* and *DSC2*, and basal keratinocyte spots as those co-expressing *KRT5* and *DSG3* and assessed their spatial association using the Lee's L bivariate spatial correlation statistic [**Ref. 4**]. The Lee's L statistic measures whether two spatial values colocalize across neighboring spatial units [**Ref. 4**]. The statistic typically ranges from −1 to 1, where values near 0 indicate spatial randomness, positive values indicate colocalization and negative values indicate spatial segregation. Using this approach, we observed close physical proximity between cDC2A cells and basal keratinocytes specifically in psoriatic tissues (**Supplementary Fig. 17B**). Notably, cDC2A spots were detected in 18 of the 21 samples and all three samples lacking cDC2A spots were all derived from healthy volunteers. Moreover, psoriasis severity measured by PASI scores showed a statistically significant positive correlation with the Lee's L statistics (**Supplementary Fig. 17C**).

[Added to the Results, pages 17–18, lines 329–355]

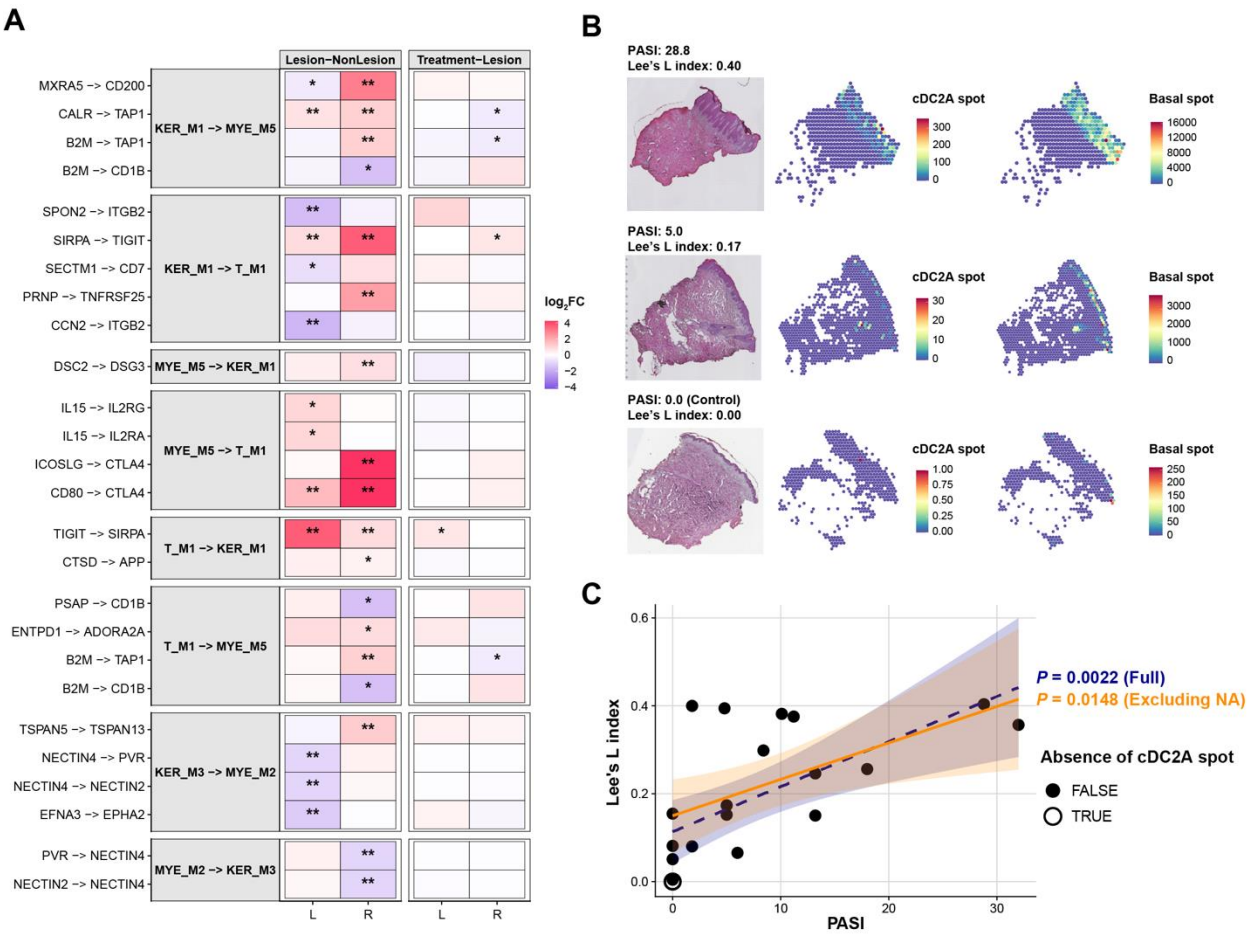
Cross-cell-type communication analysis enables the identification of gene-level interactions between cell types. Using the MultiNicheNet framework, we performed differential analyses of ligand-receptor pairs to identify interactions that were differentially active between non-lesional and lesional skin, as well as between lesional and treatment conditions (**Supplementary Fig. 17A**). The MYE-M5 module, which showed the strongest association with cDC2A, also exhibited a significantly enhanced interaction with the basal keratinocyte-associated module KER-M1. Notably, the DSC2-DSG3 interaction showed increased activity in lesional samples, indicating physical adhesion between cDC2A and basal keratinocytes. Interactions between cDC2A and T cells were also upregulated in lesional skin, including CD80-CTLA4 interactions, representing cDC2A-mediated inhibition of immune activity in the basal epidermal layer. Spatial transcriptomic validation using an independent psoriasis dataset further supported these findings,

revealing close spatial proximity between basal keratinocytes and cDC2A specifically in lesional tissue (**Supplementary Fig. 17B**). cDC2A-associated spots were detected in most samples, and spatial colocalization between basal keratinocytes and cDC2A showed a significant positive association with Psoriasis Area Severity Index (PASI) scores (**Supplementary Fig. 17C**). Additionally, similar module-level patterns were observed between lesional and non-lesional skin in the long-term risankizumab treatment dataset (**Supplementary Fig. 18E**). Notably, unlike the relatively modest associations observed in short-term treatment samples, the long-term dataset revealed a significant shift of gene expression in post-treatment lesional samples toward control levels.

[Added Supplementary Figure 17]

Supplementary Figure 17. **Module-level cell-cell interaction analysis among keratinocytes, myeloids, and T cells.**

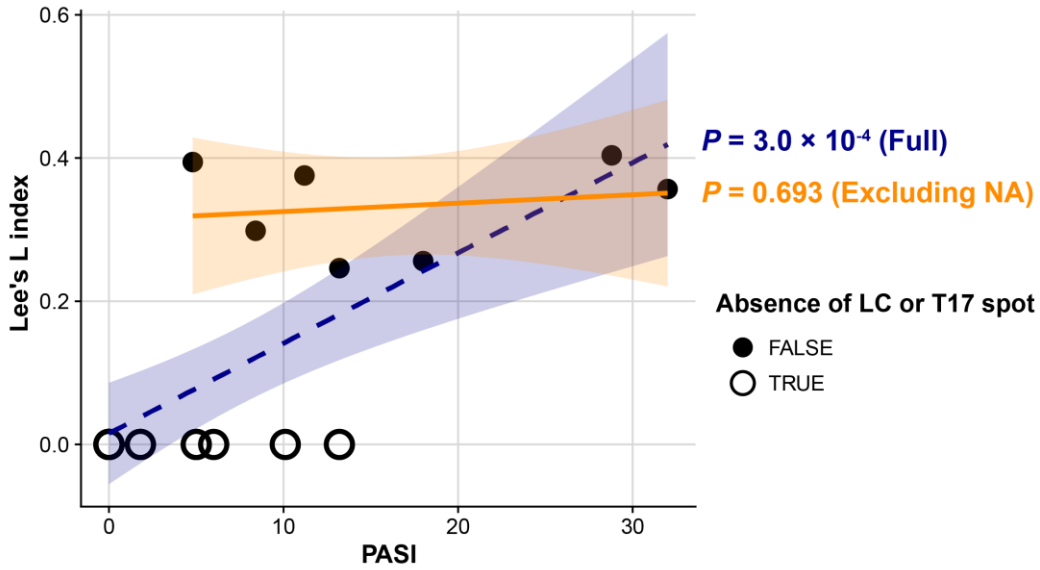
(A) Ligand (L)-receptor (R) interactions that exhibit differential expression between lesion and non-lesion samples (Left) and treatment and lesion samples (Right). The heatmap shows the log2-transformed fold changes of the gene expression between two conditions. Each panel name represents the directionality of the ligand module to the receptor module. Double asterisks denote the adjusted p-value < 0.05, and single asterisks denote the nominal p-value < 0.05. **(B)** Spatial expression plots of cDC2A spots (co-expressing *LAMP3* and *DSC2*) and basal keratinocyte spots (co-expressing *KRT5* and *DSG3*). **(C)** Evaluating associations between psoriasis severity index (PASI) and Lee's L index. Two regression lines were fitted: one excluding samples of non-detected cDC2A spots (orange) and one using all samples with non-detected cDC2A spots imputed as zero (darkblue).



We previously identified the MYE-M2 module representing Langerhans cells that was linked to the granular layer-associated keratinocyte module KER-M3, and suggested interactions related to lipid metabolic processes (**Fig. 6H, I**). Following the reviewer's suggestion in Comment #11, we further examined whether physical proximity between Langerhans cells and T17 cell spots using the same spatial transcriptomics dataset (**Response Fig. 2**). Owing to the limited sequencing depth of spatial transcriptomics, samples simultaneously expressing both Langerhans cells and T17 cells were detected in only seven of 21 samples. Interestingly, all non-detected samples were derived from healthy controls or patients with relatively low PASI scores. When non-detected cases were imputed as zero for the Lee's L statistic, the association of the Lee's L statistic with PASI was statistically significant. However, no significant correlation with PASI was observed when the analysis was restricted to the seven samples with both detections.

Response Figure 2. Spatial autocorrelation between Langerhans cell spots and T17 cell spots.

Langerhans cell spots were defined as those co-expressing *CD1A* and *CD207*. T17 cell spots were defined as those co-expressing CD3 (*CD3D* or *CD3E*) and *IL17A*.



We performed additional analyses to characterize MYE-M2 cells with lesion-specific functional changes (**Supplementary Fig. 14**). Among the 895 genes belonging to MYE-M2, genes co-expressed with *IL23A* (TOM pairwise similarity > 0.10) included established Langerhans cell markers (*CD207* and *HLA-DQB2*) as well as genes involved in lipid metabolism (*ACOT7* and *LPAR3*) (**Supplementary Fig. 14A, B**). Next, we calculated the module connectivity separately in normal and lesional samples and compared the fold changes between the two conditions (**Supplementary Fig. 14C**). Pathway analysis of genes with the decreased connectivity in lesional samples revealed the enrichment of biosynthesis of lipoxin (LX) pathway. This indicates that the co-regulatory relationships involved in lipoxin biosynthesis are disrupted in psoriatic lesions. Notably, 5-lipoxygenase is a key enzyme in the lipoxin biosynthesis pathway in Langerhans cells [**Ref. 5**], and lipoxins are known to play an important role in immunoregulation and wound healing [**Ref. 6**].

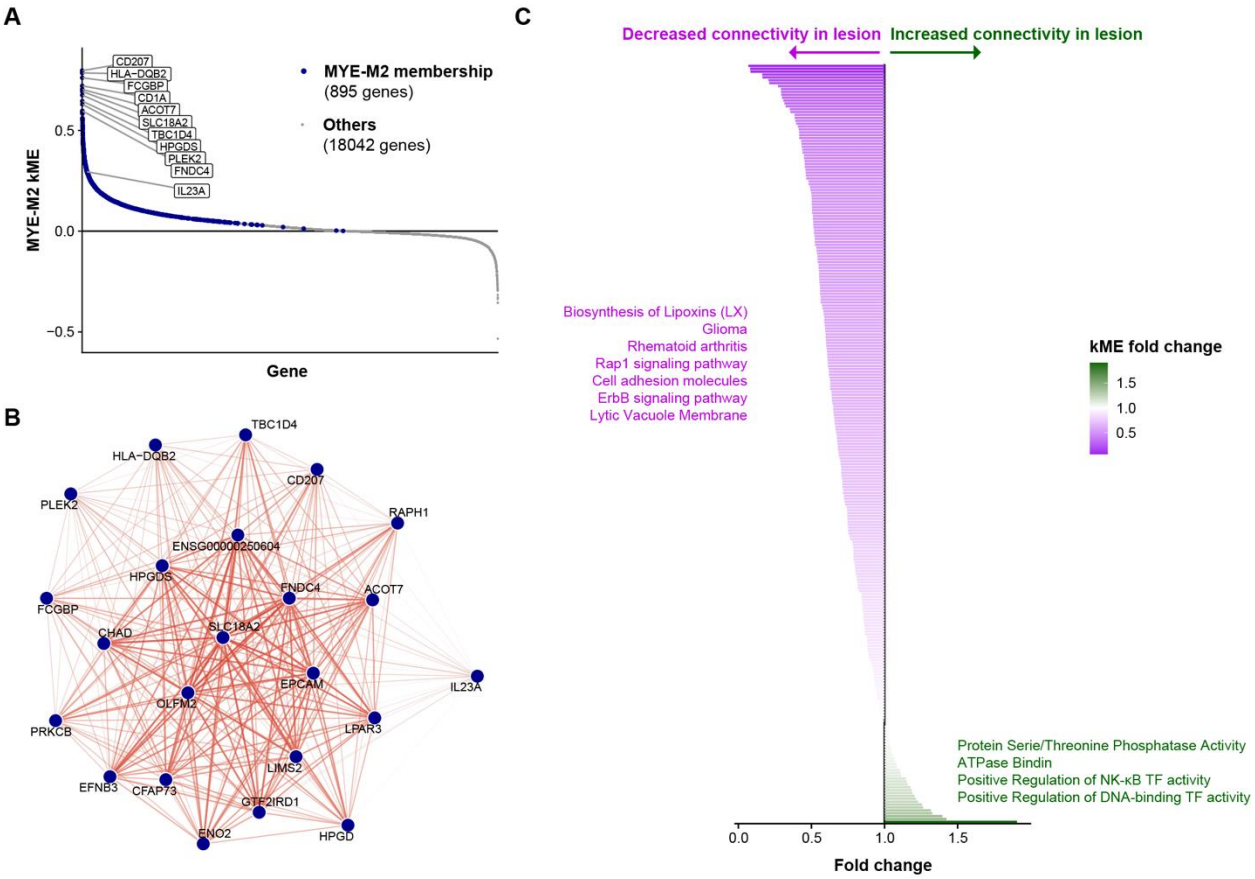
[Added to the Results, pages 15–16, lines 300–308]

We further analyzed the MYE-M2 module to characterize lesion-specific functional changes (**Supplementary Fig. 14**). Among the 895 MYE-M2 member genes, those co-expressed with *IL23A* (Topological overlap matrix pairwise similarity > 0.10) included established Langerhans

cell markers (*CD207* and *HLA-DQB2*) as well as genes involved in lipid metabolism (*ACOT7* and *LPAR3*) (**Supplementary Fig. 14A, B**). Next, we calculated module connectivity separately in normal and lesion samples and compared the fold changes between the two conditions (**Supplementary Fig. 14C**). Pathway analysis of genes with decreased connectivity in lesion samples revealed the enrichment of lipoxin (LX) biosynthesis pathway, which is known to play an important role in immunoregulation and wound healing (**Supplementary Table 19**).

[Added Supplementary Figure 14]

Supplementary Figure 14. MYE-M2 module connectivity analysis.
(A) Scatter plot of genes ranked by module connectivity (kME) and kME scores. The top-10 genes with the highest kME values and *IL23A* are annotated.
(B) Network plot showing genes co-expressed with *IL23A*. Genes were filtered based on a TOM pairwise similarity > 0.10 with *IL23A*.
(C) Comparison of gene connectivities between lesional samples and healthy control samples, ranked by fold change. Genes with fold changes below 1.0 indicate decreased connectivity in lesional samples. Only genes with kME > 0.25 were included in the analysis. Gene sets with increased and decreased connectivity were both used for pathway analysis, and representative pathways with adjusted *p*-value < 0.10 are shown.



[Added Supplementary Table 19]

Table S19. Associated pathways with differential connectivity between normal and lesional samples in MYE-M2 module genes.

The enrichR R library was used for pathway analysis. Only pathways with adjusted *p*-values < 0.10 and at least two member genes are shown.

Category	Term	Overlap	P	adjP		Geneset
Decreased connectivity in lesion	Transcriptional misregulation in cancer	'10 / 192'	7.39E-05	1.71E-02		KEGG_2021_Human
Decreased connectivity in lesion	Biosynthesis of Lipoxins (LX)	'3 / 6'	2.85E-05	2.04E-02		Reactome_Pathways_2024
Decreased connectivity in lesion	Glioma	'6 / 75'	2.15E-04	2.49E-02	…	KEGG_2021_Human
Decreased connectivity in lesion	Rheumatoid arthritis	'6 / 93'	6.86E-04	3.75E-02		KEGG_2021_Human
Decreased connectivity in lesion	Rap1 signaling pathway	'9 / 210'	7.07E-04	3.75E-02		KEGG_2021_Human
Decreased connectivity in lesion	Thyroid cancer	'4 / 37'	8.09E-04	3.75E-02		KEGG_2021_Human
Decreased connectivity in lesion	Non-small cell lung cancer	'5 / 72'	1.39E-03	5.08E-02		KEGG_2021_Human

Decreased connectivity in lesion	Cell adhesion molecules	'7 / 148'	1.57E -03	5.08E -02		KEGG_2021_Human
Decreased connectivity in lesion	Chronic myeloid leukemia	'5 / 76'	1.76E -03	5.08E -02		KEGG_2021_Human
Decreased connectivity in lesion	Leukocyte transendothelial migration	'6 / 114'	1.97E -03	5.08E -02		KEGG_2021_Human
Decreased connectivity in lesion	Anion Binding (GO:0043168)	'14 / 402'	2.24E -04	6.21E -02		GO_Molecular_Function_2025
Decreased connectivity in lesion	ErbB signaling pathway	'5 / 85'	2.88E -03	6.69E -02		KEGG_2021_Human
Decreased connectivity in lesion	Lytic Vacuole Membrane (GO:0098852)	'11 / 314'	1.00E -03	7.17E -02		GO_Cellular_Component_2025
Decreased connectivity in lesion	Sex Chromosome (GO:0000803)	'2 / 5'	1.26E -03	7.17E -02		GO_Cellular_Component_2025
Decreased connectivity in lesion	External Side of Apical Plasma Membrane (GO:0098591)	'2 / 5'	1.26E -03	7.17E -02		GO_Cellular_Component_2025

(The remaining rows are omitted)

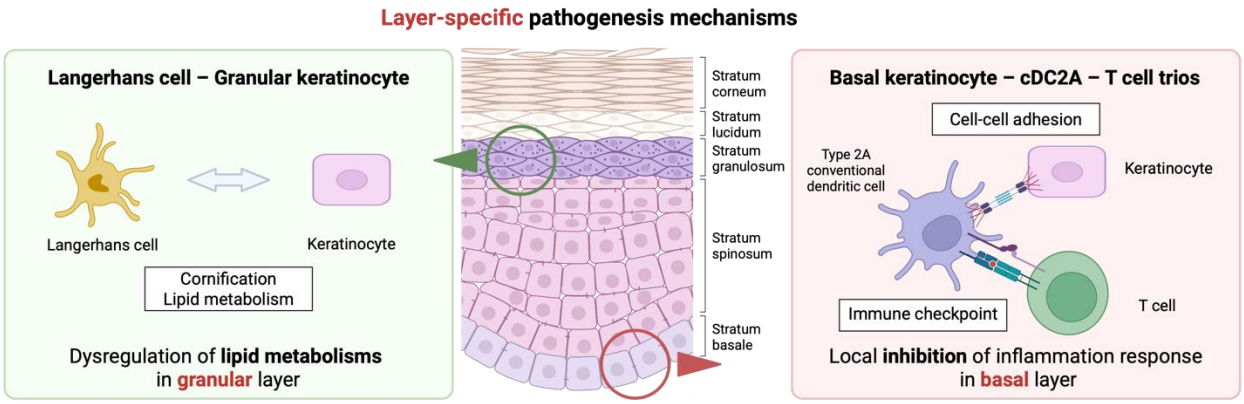
Together, our results demonstrate that, at the single-cell level, lipoxin-related lipid metabolic functions in Langerhans cells are significantly impaired in psoriasis but are restored during IL-23 inhibition therapy (**Fig. 6G, H**). We further propose that these processes are regulated through specific interactions with granular keratinocytes. Based on our analyses, we summarized two previously unreported epidermal layer-specific cross-cell-type interaction mechanisms in psoriasis in a schematic illustration (**Fig. 6J**).

[Added to the Discussion, page 23, lines 453–459]

In summary, we propose two epidermal layer-specific pathogenic mechanisms in psoriasis based on integrative analyses of various IL-23 inhibition-based multimodal datasets (**Fig. 6J**). In the basal epidermal layer, enhanced physical interactions between keratinocytes, cDC2A, and T cells are associated with localized immune suppression, which may contribute to the excessive proliferation of basal keratinocytes. In the granular epidermal layer, disrupted interactions between keratinocytes and Langerhans cells are associated with the dysregulation of lipid metabolism in both cell types.

[Added Figure 6J]

Figure 6J. A proposed schematic illustration of two layer-specific mechanisms of psoriasis pathogenesis.



[Added to the Methods, pages 37–38, lines 762–791]

Cell-cell interaction analysis

Cross-cell-type communication analysis was performed using the *MultiNicheNet* R package, a framework designed to identify ligand-receptor pairs that are differentially active between conditions of interest. In this study, we compared non-lesional, lesional, and treatment conditions using IL-23 inhibition treatment datasets focusing on keratinocytes, myeloid cells, and T cells. Ligand-receptor analyses were restricted to interactions between the predefined module pairs highlighted in **Fig. 6H**, including KER-M3 and MYE-M2, as well as KER-M1, MYE-M5, and T-M1. Data preprocessing and differential tests were conducted using the default parameters, following the recommendations of the tool developers. For each module pair, ligand-receptor interactions were evaluated by matching the ligand genes expressed in the sender cell modules with the receptor genes expressed in the receiver cell modules. Owing to the sparsity of single-cell data, results from differential tests were filtered using a nominal $P < 0.05$ threshold, while adjusted *P* values were also provided in results.

Spatial transcriptome data

Spatial transcriptomic data obtained from the study by Castillo et al. was used to validate the validation of cell-cell communication analysis. We used preprocessed data provided in the original study. Nine non-lesional samples from psoriasis patients were excluded from this analysis, resulting in seven normal skin samples and 14 lesional skin samples included in the analysis. Spatial spots corresponding to basal keratinocytes and cDC2A were defined based on the co-expression of two marker genes for each cell type (basal keratinocytes: *DSG3* and *KRT5*;

cDC2A: *DSC2* and *LAMP3*). For each spot, a composite expression score was calculated using the product of the log-normalized expression values of the corresponding marker genes as a measure of cell-type-specific signal enrichment. To assess the spatial proximity between the two cell types, we calculated the bivariate spatial autocorrelation using the Lee's L statistic. The Lee's L statistic quantifies the degree of spatial colocalization between two spatially distributed variables across neighboring spatial units. The statistic ranges from −1 to 1, where values close to 0 indicate spatial randomness, positive values indicate spatial colocalization, and negative values indicate spatial segregation. Lee's L statistics was calculated for each sample to evaluate spatial associations between defined cell-type-specific spots.

References

Ref. 1. Browaeys R, et al. MultiNicheNet: a flexible framework for differential cell-cell communication analysis from multi-sample multi-condition single-cell transcriptomics data. BioRxiv, 2023.2006. 2013.544751 (2023).

Ref. 2. Harrison OJ, et al. Structural basis of adhesive binding by desmocollins and desmogleins. Proceedings of the National Academy of Sciences 113, 7160-7165 (2016).

Ref. 3. Castillo RL, et al. Spatial transcriptomics stratifies psoriatic disease severity by emergent cellular ecosystems. Science immunology 8, eabq7991 (2023).

Ref. 4. Lee S-I. Developing a bivariate spatial association measure: an integration of Pearson's r and Moran's I. Journal of geographical systems 3, 369-385 (2001).

Ref. 5. Spanbroek R, et al. 5-Lipoxygenase expression in Langerhans cells of normal human epidermis. Proceedings of the National Academy of Sciences 95, 663-668 (1998).

Ref. 6. Fu T, et al. Therapeutic potential of lipoxin A4 in chronic inflammation: focus on cardiometabolic disease. ACS pharmacology & translational science 3, 43-55 (2020).

Additional comments from the authors

We have updated the **Figures**, **Supplementary Figures**, and **Supplementary Tables** accordingly while addressing the reviewers' comments. The updates and rearrangements of the figures and tables are listed below.

Figures

Initially	After revision	Comment on changes in the structure
Figure 1	Figure 1	The text "Downstream analysis" has been modified to "Functional analysis".
Figure 2	Figure 2	The definition of novel loci has been updated.
Figure 3	Figure 3	
Figure 4	Figure 4	
Figure 5	Figure 5	
Figure 6	Figure 6	Panel F has been modified. Panel J has been added to Figure 6.

Supplementary Figures

Initially	After revision	Comment on changes in the structure
	Supplementary Figure 1	UK Biobank samples used in this study.
Supplementary Figure 1	Supplementary Figure 2	
	Supplementary Figure 3	PRS analysis evaluating the effect of Duffy's adjustment.
Supplementary Figure 2	Supplementary Figure 4	
	Supplementary Figure 5	Identified independent loci counts corresponding to effective sample size in psoriasis GWAS.
Supplementary Figure 3	Supplementary Figure 6	
	Supplementary Figure 7	The relationship between Local heritability and genome-wide significance.
	Supplementary Figure 8	Estimating potential novel discovery of LD blocks that have not reached genome-wide significance in current GWAS.
Supplementary Figure 4	Supplementary Figure 9	
Supplementary Figure 5	Supplementary Figure 10	
Supplementary Figure 6	Supplementary Figure 11	

	Supplementary Figure 12	RNA velocity analysis of keratinocyte clusters.
	Supplementary Figure 13	scDRS heterogeneity-based analysis.
	Supplementary Figure 14	MYE-M2 module connectivity analysis.
Supplementary Figure 7	Supplementary Figure 15	
Supplementary Figure 8	Supplementary Figure 16	
	Supplementary Figure 17	Module-level cell-cell interaction analysis among keratinocytes, myeloids, and T cells.
	Supplementary Figure 18	Replication analysis of single-cell analysis using Kim et al. dataset.

Supplementary Tables

Initially	After revision	Comment on changes in the structure
Supplementary Table 1	Supplementary Table 1	
Supplementary Table 2	Supplementary Table 2	
Supplementary Table 3	Supplementary Table 3	
	Supplementary Table 4	Psoriasis summary statistics of sample sizes and independent loci counts
Supplementary Table 4	Supplementary Table 5	
Supplementary Table 5	Supplementary Table 6	
	Supplementary Table 7	Psoriasis susceptibility loci reported in previous GWAS located in LAVA-significant blocks
Supplementary Table 6	Supplementary Table 8	
Supplementary Table 7	Supplementary Table 9	
Supplementary Table 8	Supplementary Table 10	
Supplementary Table 9	Supplementary Table 11	
Supplementary Table 10	Supplementary Table 12	
Supplementary Table 11	Supplementary Table 13	
Supplementary Table 12	Supplementary Table 14	
Supplementary Table 13	Supplementary Table 15	
Supplementary Table 14	Supplementary Table 16	
Supplementary Table 15	Supplementary Table 17	
Supplementary Table 16	Supplementary Table 18	
	Supplementary Table 19	Associated pathways with differential connectivity between normal and lesional samples in MYE-M2 module genes
Supplementary Table 17	Supplementary Table 20	

Supplementary Table 18	Supplementary Table 21	
Supplementary Table 19	Supplementary Table 22	
Supplementary Table 20	Supplementary Table 23	
Supplementary Table 21	Supplementary Table 24	
Supplementary Table 22	Supplementary Table 25	
	Supplementary Table 26	Demographics of transcriptomic samples used in this study

1560

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Manuscript Number: NCOMMS-25-41335B

Manuscript: Genome-wide association study of 1 million people and single-cell analysis for psoriasis identify disease-associated cell subtypes and novel therapeutic targets

Responses from the Authors for Review Comments:

We appreciate the reviewers for the thoughtful and constructive feedback. In response to the reviewers' comments, we have revised and refined our description in the manuscript. We believe that these revisions have enhanced the clarity of our study. The responses to the reviewers' comments are highlighted in blue and the revised text in the manuscript is highlighted in red.

Reviewers' Comments:

Reviewer #1 (Remarks to the Author):

I thank the authors for carefully considering my comments on the initial submission and providing a very thorough response; I appreciate the substantial additional work this has entailed. In many cases my concerns have been satisfactorily resolved. However, there are several places where important issues remain.

Response: We sincerely thank the reviewer for recognizing our efforts and for the further assessment of our work. We have carefully addressed the comments and provided point-by-point responses below.

> The authors have partially taken on board my comment that their manuscript ignores three recent large psoriasis GWAS meta-analyses, e.g. adjusting the number of unreported loci that they claim to find. However, their Results section (lines 132-136) still claims "Previously unreported loci were mapped to genes implicated in immune-related functions (IRF2BP2, CXCR4, NRROS, ULBP3, IL7R, CRTAM, SOCS3, and NFATC2), mitochondrial functions (SDHD, ACOXL, and BCO2), and cell growth and differentiation (CDC7, POU2F3, PIK3R5, and MED15)". Considering the meta-analysis published in Nature Communications last year (PMID: 40021644): not only are some of the listed genes located in loci identified by that study, but some actually appear in the main text of that paper. This raises serious doubts about the authors' appreciation of the existing body of knowledge concerning psoriasis genetics.

Response: We apologize for unintentionally failing to update the gene descriptions and functional categories in the main text. While we had updated the unreported loci and mapped genes in the **Supplementary Table 3** in our previous revision, the corresponding descriptions in the main **Results** were left unchanged. We have now thoroughly cross-referenced our results with the three recently published large-scale psoriasis GWAS meta-analyses (PMID: 38763176, PMID: 39885523, and PMID: 40021644), as well as the latest records in the GWAS Catalog. Following this rigorous re-evaluation, we have confirmed that the newly identified loci do not

reach the genome-wide significance threshold ($p < 5 \times 10^{-8}$) in any of these three publications or prior literature. Accordingly, we have updated the list of genes in the **Results** section.

Response Note 1. Newly identified gene list in this study.

CDC7, *RGS1*, *IRF2BP2*, *CXCR4*, *ULBP3*, *MKLN1*, *NEK6*, *TAF3*, *DGKZ*, *SDHD*, *TEX12*, *BCO2*, *CRTAM*, *KCTD5*, *PIK3R5*, *STXBP4*, *SOCS3*, *ZNF320*, *MLC1*

[Revised in the Results, page 8, lines 133–135]

Previously unreported loci were mapped to genes implicated in immune-related functions (*IRF2BP2*, *CXCR4*, ~~*NRROS,*~~ *ULBP3*, *CRTAM*, and *SOCS3*~~, and *NFATC2*~~), mitochondrial functions (*SDHD*~~*ACOXL,*~~ and *BCO2*), and cell growth and differentiation (*CDC7*~~, *POU2F3,*~~ and *PIK3R5*~~, and *MED15*~~)

> I also feel that the authors have not fully understood or addressed my point regarding the functional enrichment in synovial fluid. They have added (line 171) "This suggests a high genetic overlap between psoriasis and psoriatic arthritis". I still disagree with this conclusion. Psoriasis cases in their study will be very strongly enriched for the presence of psoriatic arthritis compared to controls; their GWAS meta-analysis is to some extent therefore also a psoriatic arthritis GWAS and they cannot make comparisons as if it were a distinct disease. On the same point I still disagree with the statement in Discussion: "which may explain the progression to psoriatic arthritis observed in some patients". And at the end of the Discussion: "Comparative studies on cutaneous psoriasis and psoriatic arthritis may offer additional insight into shared and distinct disease mechanisms" – as pointed out in my review of the initial submission, such genetic studies do already exist.

Response: We thank the reviewer for the comment. We fully agree with the reviewer's perspective and acknowledge the inherent limitation that our study population included patients

with psoriatic arthritis (PsA). We have explicitly stated in both the **Results** and **Discussion** sections that our findings are subject to limitations arising from the clinical definition of psoriasis in this study. Furthermore, as suggested, we have incorporated and discussed the previous study pointed out by the reviewer in the **Discussion** section to provide a more accurate and comprehensive context regarding the genetic relationship between cutaneous psoriasis and psoriatic arthritis.

[Revised in the Results, page 10, lines 173–174]

~~This suggests a high genetic overlap between psoriasis and psoriatic arthritis.~~

This result may be derived from the study cases including individuals with PsA.

[Revised in the Discussion, pages 20–21, lines 387–389]

Our results underscore the pivotal role of T helper 17 (Th17) cells in psoriasis and highlight the significant heritability enrichment in the synovial fluid~~, which may explain the progression to psoriatic arthritis observed in some patients~~ and immune-related cell types.

[Removed from the Discussion, page 24, lines 500–501]

However, their potential as therapeutic targets should be further explored using complementary functional approaches. ~~Finally, Comparative studies on cutaneous psoriasis and psoriatic arthritis may offer additional insight into shared and distinct disease mechanisms. Integrating single-cell data from synovial fluid with skin-based analyses may further clarify immune cell-driven processes across psoriatic diseases.~~

[Added to the Discussion, page 24–25, lines 503–510]

This study has several fundamental limitations. First, the GWAS analysis was conducted exclusively on datasets of European ancestry, and additional investigations are required to

evaluate the applicability of these findings to other populations. Second, a subset of psoriasis patients in our study likely has comorbid PsA. This may account for the observed functional enrichment within tissues such as synovial fluid, rather than reflecting a systemic or cutaneous pathogenesis. As previous studies explored the shared and distinct genetic architectures between cutaneous psoriasis and PsA, our results should be interpreted with caution, particularly in distinguishing cutaneous signals from PsA-driven effects [**Refs. 1, 2**].

References

Ref. 1. Soomro, M. *et al.* Comparative genetic analysis of psoriatic arthritis and psoriasis for the discovery of genetic risk factors and risk prediction modeling. *Arthritis & rheumatology* **74**, 1535-1543 (2022).

Ref. 2. Gupta, Y., Sezin, T. & Thaçi, D. Genome-wide meta-analysis and integrative fine-mapping identify novel susceptibility loci and effector genes in psoriatic arthritis. *medRxiv*, 2025.2008. 2026.25334362 (2025).

Minor comments:

1. Results lines 107-113: "we compared polygenic risk scores (PRS) between ICD-diagnosed psoriasis cases, self-reported cases, and controls […] 73.85% for UK Biobank and 37.5% for 23andMe of cases are likely true positives" – it has been shown that the PRS distribution is higher on average in severe psoriasis cases than in mild psoriasis cases (PMID: 41408349). So the authors' inference about true vs false positive cases in UKB/23andMe is not valid: it may be that there is just lower average psoriasis severity in these groups.

Response: We thank the reviewer for this insightful comment. We agree that the lower average PRS observed in self-reported cases in the UK Biobank and 23andMe cohorts may reflect lower disease severity rather than being false positives.

Our research relies entirely on the binary classification of psoriasis, and we observed that self-reported cases exhibited relatively lower polygenic risk scores and an overall attenuation in the effect sizes of risk alleles compared to clinically-diagnosed GWAS results from a large-scale consortium (**Supplementary Fig. 1B** and **Supplementary Fig. 2**). Because these self-reported cases have weaker genetic signals compared to clinically-diagnosed cases, they can lower the power of our analysis. To account for these differences in genetic signal strength and case definition, we applied the Duffy's method, which resulted in improved PRS performance (**Supplementary Fig. 3**). Notably, this observation is consistent with the study highlighted by the reviewer, which supported the possibility that lower PRS in self-reported cases reflects milder disease severity.

To minimize potential misinterpretation of our statements, we have carefully revised our terminology throughout the manuscript. Furthermore, we have updated the **Results** and **Discussion** sections to incorporate the reviewer's point and to provide a more detailed explanation of how case severity aligns with our observations.

[Revised in the Results, page 7, lines 110–118]

Based on the risk allele frequencies in case samples and control samples, it was estimated that 73.85% for UKB cases and 37.5% for 23andMe cases met the criteria for severe psoriasis (**Supplementary Table 2**). The remaining cases may represent milder disease severity, consistent with previous reports that PRS distribution correlates with clinical severity. After adjusting for the effect size, the effect size distributions of known psoriasis risk variants aligned closely with those reported in previous studies (**Supplementary Fig. 2**). To assess the impact of adjusting for self-reported phenotype definitions, we calculated PRSs in UKB samples using 23andMe psoriasis GWAS summary statistics before and after applying Duffy's adjustment (**Supplementary Fig. 3**).

[Revised in the Discussion, page 19, lines 370–379]

We acknowledge the variation in psoriasis case definition among individual GWAS, which were confirmed by the difference in risk allele frequencies. By applying Duffy's approach to the UKB and 23andMe data, as used in previous studies, the effect size was successfully adjusted, and the proportion of psoriasis cases was appropriately calibrated to approximately 2%. Based on two PRS analyses, we demonstrated the rationale for including self-reported cases and showed that~~,~~ ~~despite their relatively high false-positive rates,~~ these cases can be appropriately incorporated into GWAS analyses using Duffy's adjustment method. A recent study revealed that higher genetic risk was linked to more severe psoriasis [**Ref. 1**]. This suggests potential heterogeneity in disease severity between self-reported and clinically-diagnosed cases, implying that Duffy's approach could be an approach to account for such heterogeneity arising from binary classification.

[Revised in the Methods, page 27–28, lines 556–558]

Following the approach previously used in large-scale psoriasis GWAS, we applied Duffy's correction to account for variations in case definition due to self-reports within the UKB and 23andMe datasets.

Reference

Ref. 1. Saklatvala, J. R. *et al*. Genetic liability to psoriasis predicts severe disease outcomes. *Genome medicine* **18**, 14 (2026).

2. Results line 121: "identified a total of 198 independent SNPs from conditional and joint (COJO) association analysis across 125 independent loci" – I still feel the use of "independent" twice here is confusing and unclear. The meaning should be made more explicit, or preferably use another word (e.g. distinct loci).

Response: We thank the reviewer's constructive suggestion to clarify the terminology. As the reviewer recommended, we have replaced "independent loci" with "distinct loci" to avoid confusion and clearly distinguish between the independent SNPs and their respective genomic regions.

[Revised in the Results, page 8, lines 122–124]

our meta-analysis identified a total of 198 independent SNPs from conditional and joint (COJO) association analysis across 125 distinct loci, including 17 previously unreported loci.

[Revised in the Results, page 8, lines 126–129]

This GWAS included the largest sample size for psoriasis among the published studies, and a comparison of our GWAS with previous psoriasis GWASs based on the effective sample size (**Supplementary Table 4**) revealed an approximately linear increase in the number of significant distinct loci (**Supplementary Fig. 5**).

3. Results lines 150-52: "We compared 106 independent SNPs located in 72 LD blocks that showed statistically significant heritability with 42 independent SNPs within the 152 non-

significant LAVA regions identified in the GWAS." - The authors have not stated any findings here. In the next sentence they say that SNPs were categorised into four classes; I think this classification is sufficiently bespoke that they should direct the reader to the Methods here.

Response: We thank the reviewer for pointing out that the specific findings were not stated in this section. Following the reviewer's suggestion, we have revised the **Results** section to explicitly describe the results. Additionally, we have updated the terminology for clarification in line with Comment #19, revising "Significant" and "NS" into "LAVA-significant (LAVA-S)" and "LAVA-not significant (LAVA-NS)" blocks, respectively. The figure legends have been updated accordingly to reflect this change. We noted that LAVA-NS independent SNPs generally exhibited smaller effect sizes and lower statistical significance compared to those in LAVA-S regions (**Supplementary Fig. 7B, C**).

Furthermore, to facilitate a better understanding of the classification methodology, we have added the statement "(see **Methods**)" regarding the SNP categorization.

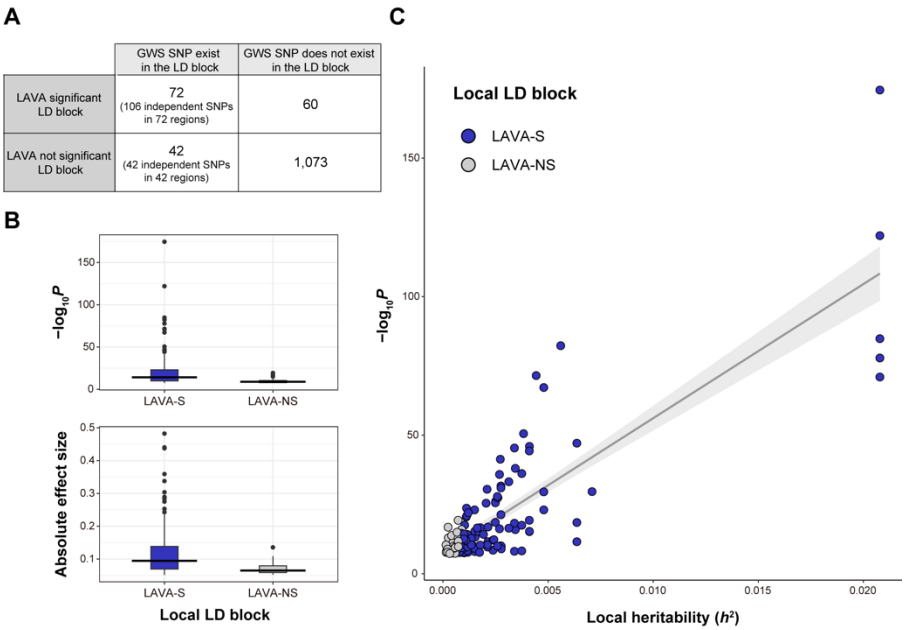
[Revised in the Results, page 9, lines 149–156]

We first examined 42 regions in which LD local heritability was not significant but contained significant SNPs (referred to as LAVA-NS independent SNPs). When we compared these with 106 independent SNPs located in 72 LD blocks that showed statistically significant heritability (referred to as LAVA-S independent SNPs), we identified that LAVA-NS independent SNPs exhibited smaller effect sizes and lower statistical significance (**Supplementary Fig. 7B, C)**. Next, 60 significant LAVA regions where no genome-wide significant SNPs were identified were categorized into four classes for each LD block to explore the potential for novel discoveries (**Supplementary Fig. 8A**, see **Methods**).

[Revised the Supplementary Fig. 7]

Supplementary Figure 7. The relationship between local heritability and genome-wide significance.

(A) Contingency table of LD blocks categorized by the significance of local heritability (LAVA) and the presence of genome-wide significant (GWS) SNPs. **(B)** Comparison of GWAS p-values and effect sizes between 106 independent SNPs located in LAVA-significant (LAVA-S) LD blocks and 42 independent SNPs in LAVA-not significant (LAVA-NS) LD blocks. This analysis was restricted to independent SNPs within LD blocks having at least one GWS SNP. **(C)** Scatter plot showing the relationship between local heritability and psoriasis association *p*-value of LAVA-S and LAVA-NS independent SNPs described in **(B)**.



4. Results line "These patterns suggests that the genetic architecture of psoriasis may diverge from that of allergic diseases" – I disagree that the revised p-value of 0.0005 for atopic dermatitis supports this conclusion.

Response: We thank the reviewer for the comment. We agree that the strong magnitude of genetic correlation and *p*-value for atopic dermatitis contradict our previous conclusion. Accordingly, we have removed the relevant statement to ensure accurate interpretation of our results.

[Revised in the results, page 11, lines 192–194]

Allergic traits, including allergies (r_g = 0.103; p = 0.034) and atopic dermatitis (r_g = 0.272; p = 0.0005), showed positive genetic correlations with nominal significance ($p < 0.05$). ~~These patterns suggests that the genetic architecture of psoriasis may diverge from that of allergic diseases, pointing to distinct underlying disease mechanisms from a global genetic architecture perspective.~~

5. Results line 270-73: "Notably, RGS14, NEK6, PRSS53, and PRDX5 exhibited opposite directions of effects on psoriasis risk between the blood and skin expression levels. For example, RGS14, NEK6, and PRDX5 exhibited negative effects in the skin eQTL datasets, whereas their effect directions were positive in the blood eQTL datasets." – I still feel the wording here needs to be more carefully considered, as it remains imprecise. What I believe the authors are actually describing is that the same alleles that increase psoriasis risk are associated with lower expression of these genes in skin eQTL datasets, but higher expression in blood eQTL datasets.

Response: We thank the reviewer for pointing out the imprecise wording and providing a clearer expression. We have revised the **Results** accordingly based on the reviewer's suggestion.

[Revised in the Results, page 14, lines 271–276]

Notably, some prioritized genes exhibited tissue-specific regulatory patterns. For *RGS14*, *NEK6*, *PRDX5*, and *FKBPL*, the same alleles associated with increased psoriasis risk were linked to decreased expression levels in skin eQTL datasets, but increased expression in blood eQTL datasets. In contrast, for *PRSS53*, the risk allele was associated with increased expression in the skin and decreased expression in the blood. These results highlight the importance of tissue-specific gene regulation (**Supplementary Table 17**).

6. In the same part of the results, I also feel that the authors should treat with more caution the genes where HEIDI p-values are found to be <0.05 and therefore the analysis is less convincing that the causal variants are shared.

Response: We thank the reviewer for highlighting the importance of the HEIDI test threshold. We acknowledge that genes with HEIDI *p*-values between 0.01 and 0.05 require interpretation with more caution, as they may suggest potential heterogeneity or linkage rather than a single causal variant. Accordingly, we have retained these results but have updated statements in **Methods** section and revised the **Supplementary Table**. Additionally, we have corrected the gene arrangement in the SMR results for GTEx whole blood where it was previously inconsistent.

[Revised in the Methods, page 35, lines 726–730]

Genes were considered to have a causal relationship with psoriasis if they met the Bonferroni-adjusted *p*-value threshold (0.05/110) and passed the HEIDI test (*p*-value < 0.01), which evaluates the likelihood of pleiotropic effects due to linkage disequilibrium. Results with HEIDI *p*-values between 0.01 and 0.05 require careful interpretation due to potential heterogeneity, with further details provided in **Supplementary Table 17**.

[Modified the Supplementary Table 17]

Table S17. SMR and HEIDI analysis in four eQTL datasets

Statistical significance of SMR *p*-value in independent eQTL dataset is 0.05/110 = 4.55E-4.

For the HEIDI test, results are color-coded based on the strength of evidence.

HEIDI *p*-value between 0.01 and 0.05 indicates the suggestive evidence of a shared causal variant (highlighted in yellow). HEIDI *p*-value > 0.05 indicates robust evidence of no heterogeneity (highlighted in green).

Gene	SMR (GTEx Skin – Sun exposed)	SMR (GTEx Skin – Sun not exposed)

	b_SMR	se_SMR	p_SMR	p_HEIDI	b_SMR	se_SMR	p_SMR	p_HEIDI
RGS14	-0.21151	0.031999	**3.85E-11**	**0.112518**	-0.19877	0.03119	**1.85E-10**	**0.061814**
IRF5	0.15145	0.030466	**6.66E-07**	**0.41538**	0.144837	0.029715	**1.09E-06**	**0.240585**
NEK6	-0.35646	0.086277	**3.60E-05**	**0.541294**	-0.16706	0.078307	0.032894	0.007117
CCDC88B	0.258976	0.04581	**1.57E-08**	0.040854	0.274524	0.051967	**1.27E-07**	0.003396
CNPY2	-1.02024	0.198577	**2.78E-07**	0.020103	NA	NA	NA	NA
UBE2L3	0.572916	0.107774	**1.06E-07**	**0.203646**	NA	NA	NA	NA
MFN2	-0.36576	0.080599	**5.68E-06**	0.010977	-0.31124	0.065177	**1.80E-06**	**0.206256**
DENND1B	0.252637	0.083094	0.002363	0.002583	NA	NA	NA	NA
BLOC1S2	-0.0344	0.031996	0.282322	0.045858	-0.02788	0.025913	0.282047	0.044283
RPS6KA4	0.522146	0.119482	**1.24E-05**	**0.255473**	NA	NA	NA	NA
ZC3H12C	-0.01899	0.048915	0.697904	6.39E-05	-0.38357	0.066933	**1.00E-08**	3.13E-06
IL23A	NA	NA	NA	NA	NA	NA	NA	NA
RHOF	-0.23203	0.04973	**3.07E-06**	**0.08396**	-0.24631	0.05257	**2.79E-06**	**0.317337**
SOCS1	NA	NA	NA	NA	NA	NA	NA	NA
HSD3B7	0.158776	0.02221	**8.76E-13**	0.022735	0.176214	0.025796	**8.43E-12**	0.029457
VKORC1	0.374989	0.059801	**3.60E-10**	0.001115	0.403387	0.071246	**1.50E-08**	0.000459
SSBP4	-0.23739	0.046519	**3.34E-07**	**0.326476**	-0.1593	0.030349	**1.53E-07**	**0.40133**
ELL	NA	NA	NA	NA	0.229454	0.086301	0.007843	0.049867

(The rows and columns are truncated due to space constraints.)

7. Results lines 306-07: "Pathway analysis of genes with decreased connectivity in lesional samples revealed the enrichment of lipoxin (LX) biosynthesis pathway" – needs further interpretation. What does lower connectivity actually mean?

Response: We thank the reviewer for the comment. In hdWGCNA analysis, module connectivity (referred to as kME) represents the strength of the correlation between each gene's expression and the overall module score (module eigengene). Genes with high connectivity are considered

hub genes, which are central to the module's biological functions (**Supplementary Fig. 14A**). By calculating and comparing connectivity separately in lesional and non-lesional samples, we identified genes whose connectivity significantly decreased in the diseased state (**Supplementary Fig. 14C**). We interpreted the decreased connectivity in lesional samples as a disruption of the co-expression network, indicating a loss of normally coordinated biological functions. We specifically identified that genes with decreased connectivity in lesional samples were significantly associated with lipoxin biosynthesis pathway (adjusted *p*-value = 0.020). This suggests that this anti-inflammatory pathway is dysregulated in psoriatic lesions, which may contribute to the persistent inflammation and impaired wound healing characteristic of the disease.

Accordingly, we have added the explanations in the **Results** and **Methods** sections.

[Revised in the Results, page 16, lines 308–314]

Pathway analysis of genes with decreased connectivity in lesional samples revealed the enrichment of lipoxin (LX) biosynthesis pathway (adjusted *p*-value = 0.020) (**Supplementary Table 20**). This lower connectivity reflects a disruption of coordinated biological functions within the network. Given the established role of the lipoxin pathway in immunoregulation and wound healing, its disruption suggests that this anti-inflammatory mechanism may be impaired in psoriatic lesions. This dysregulation might contribute to the persistent inflammation and defective tissue repair characteristic of the disease.

[Added to the Methods, page 36, lines 751–754]

This analysis was independently applied to keratinocyte, myeloid, and T-cell populations, which were previously identified as significant in the scDRS analysis. All other parameters were set to defaults following the recommendations of the tool developers, including the calculation of kME using the Pearson correlation.

8. Line 331-32: “were differentially active between non-lesional and lesional skin, as well as between lesional and treatment conditions” – for clarity should the latter comparison be between *baseline* lesional and treatment conditions?

Response: We thank the reviewer for the suggestion. We have added “baseline” accordingly as the reviewer suggested.

[Revised in the Results, page 17, lines 338–341]

Using the MultiNicheNet framework, we performed differential analyses of ligand-receptor pairs to identify interactions that were differentially active between non-lesional and lesional skin, as well as between baseline lesional and treatment conditions (**Supplementary Fig. 17A**).

9. PASI = Psoriasis Area and Severity index – acronym is stated incorrectly in line 343 and Supp Fig. 17 legend.

Response: We thank the reviewer for correcting the acronym. We have revised it accordingly as the reviewer suggested.

[Revised in the Results, page 17–18, lines 350–352]

cDC2A-associated spots were detected in most samples, and spatial co-localization between basal keratinocytes and cDC2A showed a significant positive association with Psoriasis Area and Severity Index (PASI) scores (**Supplementary Fig. 17C**).

[Revised the legend of Supplementary Fig. 17]

(C) Evaluating associations between Psoriasis Area and Severity Index (PASI) and Lee’s L index.

10. Discussion line 370 – for clarity, consider “lacked *genome-wide* significant signals”.

Response: We thank the reviewer for the comment. We have revised the statement accordingly.

[Added to the Discussion, page 19, lines 382–383]

Furthermore, a subset of LD blocks showed high local heritability but lacked genome-wide significant signals.

11. Discussion lines 382-84: “With the increased statistical power of our GWAS, we detected stronger genetic associations with conditions such as myocardial infarction, asthma, and anxiety disorders.” – are they actually stronger (larger magnitude)? Or similar magnitude to earlier estimates but now with lower p-values?

Response: We thank the reviewer for this clarification. We agree that the term “stronger” was imprecise in this context. Our results represent more significant genetic associations (lower *p*-values) rather than a larger magnitude of genetic correlation. We have revised the statement accordingly.

[Revised in the Discussion, page 20, lines 395–397]

With the increased statistical power of our GWAS, we detected statistically more robust genetic associations with conditions such as myocardial infarction, asthma, and anxiety disorders.

12. Discussion lines 389-91: “The observed positive genetic correlation between sedentary behavior and increased cardiovascular disease risk in individuals with psoriasis may therefore reflect the shared genetic factors underlying these phenotypes” – I don’t believe the authors

have looked at CVD risk in individuals with psoriasis; I think the authors intend a different meaning and this sentence needs to be reworded to be clearer.

Response: We thank the reviewer for identifying this ambiguity. We agree that the original wording could be misinterpreted as clinical observation. We have rephrased the sentence to reflect the shared genetic factors underlying these phenotypes.

[Revised in the Discussion, page 20, lines 400–403]

Time spent watching television is a form of leisure-time sedentary behavior that has been previously shown to be causally associated with cardiovascular disease. The observed positive genetic correlations between sedentary behavior, cardiovascular disease, and psoriasis may reflect shared genetic factors underlying these phenotypes.

13. Discussion lines 403-05: "Cells in the outermost nucleated epidermal layer, which is characterized by pronounced cornification features, showed the strongest association with psoriasis" – if I understand the proposed novelty of this correctly, it is about the potentially causal role of the later-stage epidermal cells (and that these cells are not just 'passengers' in the process – they are to some extent causal and not just consequential). Perhaps this point can be made more clearly.

Response: We thank the reviewer for the constructive comment. We agree that highlighting the fundamental role of these cells helps the interpretation of our findings. We have revised the Discussion to clarify that the genetic risk is specifically enriched in the genes related to terminal differentiation, suggesting that these later-stage epidermal cells may play a causal role in psoriasis pathogenesis.

[Revised in the Results, page 21, lines 414–419]

Notably, keratinocytes exhibited a continuous expression pattern from the basal to granular layers, reflecting the spatial organization of the epidermis. Specifically, cells in the outermost nucleated epidermal layer, which is characterized by pronounced cornification features, showed the strongest association with psoriasis. This implies that genetic susceptibility to psoriasis is fundamentally linked to the dysregulation of biological functions within the later-stage epidermal layer, reflecting its potential causal role in the pathogenesis of the disease.

14. Discussion lines 472-73: "One possible hypothesis is that immune cells, keratinocytes and endothelial cells may already possess a genetically determined susceptibility to psoriasis." – since cells cannot have psoriasis, I think this should be reworded.

Response: We thank the reviewer for the comment. In response to the reviewer's comment, we have revised the statement to be more precise.

[Rephrased in the Results, page 24, lines 485–486]

One possible hypothesis is that the genetic risk for psoriasis is closely linked to the inherent functional programs of immune cells, keratinocytes, and endothelial cells.

15. Methods: the stated date of accession of GWAS Catalog remains October 22, 2023. Does this remain correct following the revisions, where the authors have included newer studies?

Response: We thank the reviewer for pointing out our mistake. We have updated the GWAS catalog accession date to "October 2, 2025" in the revised manuscript.

[Revised in the Results, page 29, lines 580–582]

Loci that had already reached genome-wide significance within a 1-Mb region in previous studies (GWAS Catalog on October 2, 2025) or any of the four independent GWAS included in this study were classified as previously known loci.

16. Methods line 571: "We investigated the two cases in the contingency table of the local heritability" – do the authors mean 'two classes of LD block' here?

Response: We thank the reviewer for this helpful suggestion. We agree that "classes of LD blocks" is a clearer and more precise term. We have revised the sentence accordingly.

[Rephrased in the Methods, page 29, lines 599–600]

We investigated the two classes in the contingency table of the local heritability significance and genome-wide significance in LD blocks (**Supplementary Fig. 7A**).

17. Since I feel it is a limitation of the analysis approach, I feel that "original effect estimates" (line 681) should have added the word "marginal" as the authors disclosed in their rebuttal. I think it would also be prudent to disclose that one of the assumptions of the coloc method is of a single causal variant underlying the tested association signals, and in the authors' use case this may not always hold.

Response: We thank the reviewer for the constructive feedback. Following the suggestion, we have replaced "original effect estimates" with "marginal effect estimates" to align with the terminology used in the paper introducing GCTA-COJO algorithm. Additionally, we have updated the **Methods** section to disclose that the coloc method assumes a single causal variant per locus, acknowledging that this assumption may not always hold in complex loci with multiple independent signals.

[Rephrased in the Methods, pages 34, lines 711–716]

Fourth, we conducted a Bayesian colocalization analysis[1] to evaluate whether the independent COJO SNPs for psoriasis colocalized with eQTL signals based on marginal effects from GWAS summary statistics. This colocalization framework assumes a single causal variant underlying the tested association signals at each locus. Genes with a posterior probability (PP.H4) > 0.8 were considered colocalized, based on eQTL data from GTEx (two skin tissue types and whole blood) and eQTLGen (whole blood).

18. Methods lines 765-66: "In this study, we compared non-lesional, lesional, and treatment conditions using IL-23 inhibition treatment datasets focusing on keratinocytes, myeloid cells, and T cells." – I feel more detail is needed on the analysis approach. For example, are these pairwise or multivariable comparisons?

Response: We thank the reviewer for the comment. Following the reviewer's suggestion, we have updated the **Methods** section to specify that we utilized pairwise contrasts (lesion versus non-lesion, and treatment versus lesion) and statistical framework to identify differential cell-cell communication (as implemented in the MultiNicheNet package).

[Revised in the Methods, page 39, lines 807–811]

In this study, we estimated condition-specific cell-cell communications by performing pairwise contrasts using IL-23 inhibition treatment datasets, focusing on keratinocytes, myeloid cells, and T cells. Specifically, two contrasts were tested: lesion versus non-lesion to identify disease-associated interactions, and treatment versus lesion to identify treatment-induced changes.

[Added to the Methods, page 39, lines 814–818]

For each module pair, ligand-receptor interactions were evaluated by matching the ligand genes expressed in the sender cell modules with the receptor genes expressed in the receiver cell

391 modules. Differential tests were conducted using the default MultiNicheNet workflow, which
392 employs a generalized linear model based on sample-level pseudobulk analysis to determine log2
393 fold changes and *p*-values.

394

19. In the newly added Supplementary Figure 7 – part C, suggest clarifying the legend ("Significant", "NS") to indicate what is meant by significant here.

395

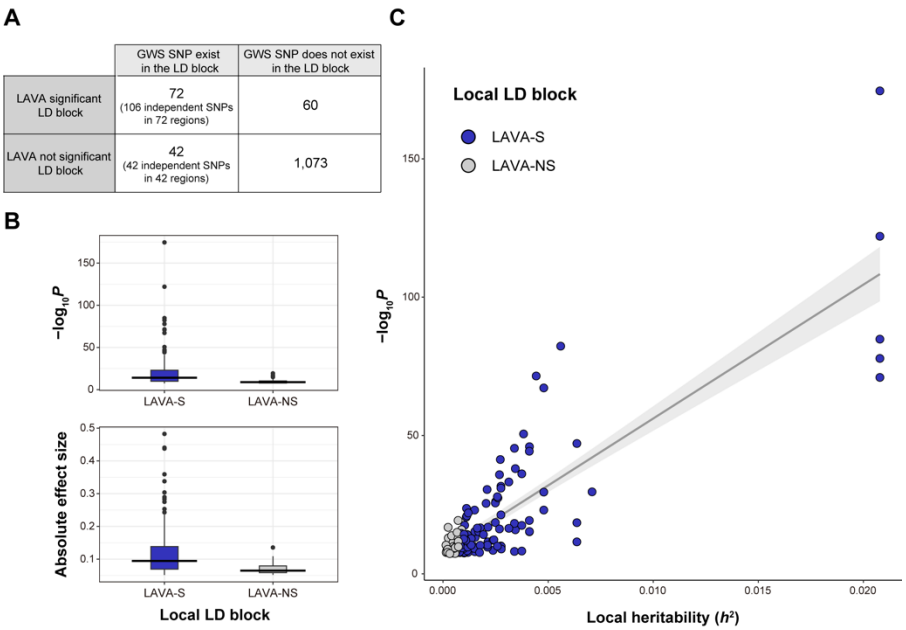
396 **Response**: We thank the reviewer for the comment. Consistent with our response to the
397 reviewer's Comment #3, we have refined notations of LD blocks having genome-wide
398 significant (GWS) SNPs based on the significance of their local heritability (LAVA).
399 Specifically, we have updated the previous notations of "Significant" and "NS" to "LAVA-S"
400 (LAVA-significant) and "LAVA-NS" (LAVA-not significant).

401

402 **[Revised Supplementary Fig. 7]**

403 **Supplementary Figure 7.** The relationship between local heritability and genome-wide
404 significance.

405 **(A)** Contingency table of LD blocks categorized by the significance of local heritability (LAVA)
406 and the presence of genome-wide significant (GWS) SNPs. **(B)** Comparison of GWAS p-values
407 and effect sizes between 106 independent SNPs located in LAVA-significant (LAVA-S) LD
408 blocks and 42 independent SNPs in LAVA-not significant (LAVA-NS) LD blocks. This analysis
409 was restricted to independent SNPs within LD blocks having at least one GWS SNP. **(C)** Scatter
410 plot showing the relationship between local heritability and psoriasis association *p*-value of
411 LAVA-S and LAVA-NS independent SNPs described in **(B)**.



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413

20. In the newly added Supplementary Figure 13 – part A, it's not clear to me which are the high heterogeneity cell types or where the difference between lesional and non-lesional tissues is significant. Perhaps these features could be added to the plot? In part F, what do the line and point colours mean?

414

415 **Response**: We thank the reviewer for the constructive comment. In the revised **Supplementary**
416 **Figure 13A**, we have performed the Wilcoxon rank-sum test for each cell type to compare
417 scDRS scores between lesional and non-lesional tissues, with asterisks denoting *p*-values < 0.05.
418 Additionally, we have added panel B to compare scDRS heterogeneity *p*-values (hetero_mcp)
419 with Wilcoxon test p-values and provided the detailed statistical result in **Supplementary Table**
420 **15**. For visualization purpose, Wilcoxon $-\log_{10}$(*p*-values) were clipped at a
421 maximum of 4. The fitted line demonstrated the association between scDRS heterogeneity and
422 between-tissue differences. We identified EC_c1_CDKN1A as the only endothelial cell subset
423 exhibiting both significant scDRS heterogeneity and between-tissue differences. We have used
424 this cluster as a representative case to further investigate the biological pathways associated with
425 this heterogeneity (**Supplementary Figure 13C–G**).

Regarding the panel **G** (formerly panel **F**), which illustrates the association between scDRS decile groups and AGE-RAGE signaling pathway scores, we have removed the line and point colors to avoid confusion. The lines are retained to represent the continuous patterns across deciles. Accordingly, we have revised **Supplementary Figure 13** panels and legends.

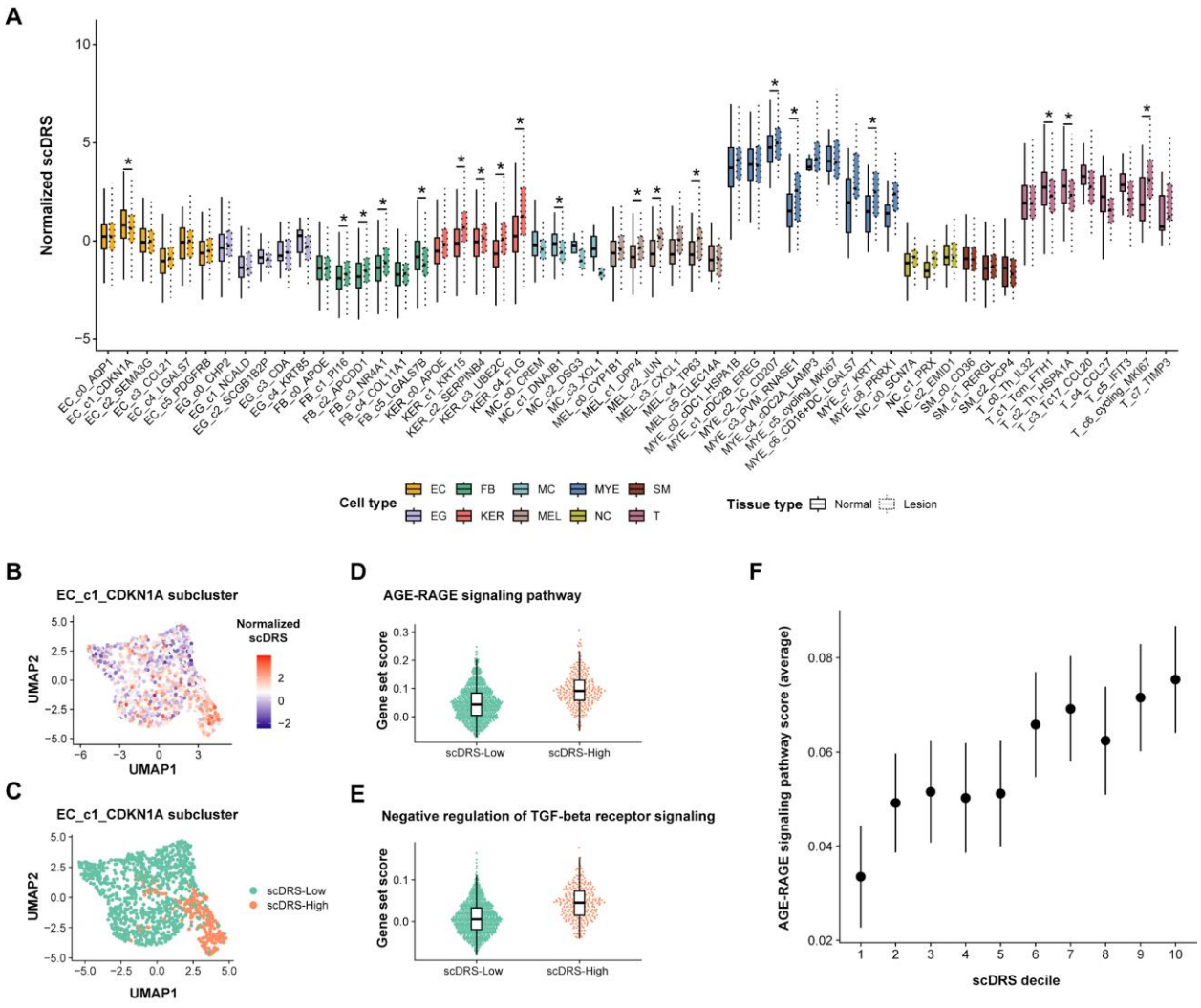
[Revised in the Results, page 13, lines 247–249]

Cell types with significant scDRS heterogeneity showed larger differences in scDRS score distribution between normal and lesional skin (**Supplementary Fig. 13A** and **Supplementary Table 15**).

[Revised in the Supplementary Fig. 13]

Supplementary Figure 13. scDRS heterogeneity-based analysis.

(A) Distributions of scDRS scores for each cell type stratified by tissue type (normal and lesion). Asterisks denote cell types satisfying both scDRS heterogeneity false discovery rate (FDR) < 0.10 and Wilcoxon rank-sum test FDR < 0.10. **(B)** scDRS score distribution of the endothelial cluster EC_c1_CDKN1A in the UMAP space. **(C)** Subclustering of the endothelial cluster EC_c1_CDKN1A. Distributions of gene set scores of two EC_c1_CDKN1A subclusters representing **(D)** the AGE-RAGE signaling pathway and **(E)** negative regulation of the TGF-beta receptor signaling pathway. **(F)** Average gene set scores per scDRS decile group of cells in EC_c1_CDKN1A. Error bars represent 95% confidence intervals.



446

447

448

449 **[Added the Supplementary Table 15]**

450 **Table S15. Comparison of scDRS heterogeneity and scDRS scores between lesional and**
451 **non-lesional cells.**

452 scDRS heterogeneity p-values (hetero_mcp) are identical to those reported in Supplementary
453 Table 14.

454 Statistical differences in scDRS scores between lesional and non-lesional cells were evaluated
455 using the Wilcoxon rank-sum test.

Cell type	hetero_mcp	hetero_mcp FDR	Wilcoxon p-value	Wilcoxon FDR
EC_c0_AQP1	0.035	0.078	0.621	0.759
EC_c1_CDKN1A	0.001	0.009	0.001	0.002
EC_c2_SEMA3G	0.148	0.204	0.759	0.888
EC_c3_CCL21	0.004	0.016	0.0638	0.113
EC_c4_LGALS7	0.725	0.753	0.826	0.909
EC_c5_PDGFRB	0.307	0.359	0.308	0.413
EG_c0_CHP2	0.0173	0.048	0.059	0.112
EG_c1_NCALD	0.006	0.024	0.738	0.883
EG_c2_SCGB1B2P	0.166	0.218	0.137	0.193
EG_c3_CDA	0.949	0.949	0.801	0.899
EG_c4_KRT85	0.0541	0.093	0.090	0.134
FB_c0_APOE	0.0835	0.131	0.892	0.925
FB_c1_PI16	0.0357	0.078	4.66E-08	2.56E-07
FB_c2_APCDD1	0.002	0.014	2.56E-11	2.81E-10
FB_c3_NR4A1	0.009	0.032	5.46E-06	2.50E-05

(The remaining rows are omitted.)

21. The authors state in their response (response document line 411) that local heritability block classifications have been added to Supplementary Table 6 but I do not see these in the submitted version of this table.

Response: We thank the reviewer for identifying this oversight. We apologize for the omission in the previous submission. We have now updated the **Supplementary Table 6** to include the column for discovery potential category of LD blocks.

[Revised the Supplementary Table 6]

Table S6. LAVA local genetic correlation

Bonferroni-adjusted p-values are calculated based on multiple testing correction for a total of 2495 blocks.

Only LD regions where latent heritability was estimated are shown.

The LD block categorization for potential novel discoveries (as shown in the 'Discovery potential category' column) is described in the Methods and Supplementary Fig. 8A.

CHR	START	END	…	LABEL	Discovery potential category
5	158484776	159663733	…	chr5:158484776-159663733	
6	26396201	27261035		chr6:26396201-27261035	Near MHC region
6	25684630	26396200		chr6:25684630-26396200	
1	66778016	67761890		chr1:66778016-67761890	
5	149850204	150981934		chr5:149850204-150981934	
19	10028841	11681978		chr19:10028841-11681978	
6	24950380	25684629		chr6:24950380-25684629	Near MHC region
6	111363444	112342873		chr6:111363444-112342873	
5	130573818	132554690		chr5:130573818-132554690	
17	25872111	27344401		chr17:25872111-27344401	
2	60775067	62429910		chr2:60775067-62429910	
14	35379989	36422077		chr14:35379989-36422077	
1	23907046	25885138		chr1:23907046-25885138	
6	137553213	138825721		chr6:137553213-138825721	
12	55416803	56987105		chr12:55416803-56987105	
5	157191082	158484775		chr5:157191082-158484775	Likely residual LD
6	33864263	34979270		chr6:33864263-34979270	Near MHC region
6	106053916	107309327		chr6:106053916-107309327	

(The remaining rows are omitted.)

22. I thank the authors for correcting my misunderstanding in relation to minor comment 11 on the original submission (response document line 681). The comment related to the consideration of gene modules enriched for IL23 pathway mechanisms, when the data came from patients treated with IL23 inhibitors. If I now understand correctly, the modules were already defined in the Ma dataset, and not dervied directly from the Francis data. Perhaps this point should be made more explicitly.

Response: We thank the reviewer for the comment. As noted by the reviewer, the gene modules were defined using the Ma et al. dataset, in which the MYE-M2 module was identified with

IL23A as a hub gene. We have revised the **Results** and **Discussion** sections to make this point more explicit.

[Revised in the Results, page 16, lines 316–324]

Changes in module activity during IL-23 inhibitor treatment were investigated by analyzing temporal scRNA-seq data from five patients undergoing risankizumab treatment, as reported by Francis et al. (**Fig. 6F** and **Supplementary Fig. 15**). The dataset comprised non-lesional and lesional skin collected at baseline (day 0), as well as lesional skin collected at 3 and 14 days after treatment (days 3 and 14), with each patient contributing samples across all time points. To evaluate their therapeutic dynamics, we projected this independent treatment cohort onto pre-defined gene modules defined in the Ma et al. dataset (see **Methods**). Following the projection, module activity was compared between lesional and non-lesional samples collected at the initial visit (**Fig. 6G** and **Supplementary Fig. 16**).

[Revised in the Discussion, page 22, lines 442–446]

Biological therapies targeting the IL-17/IL-23 axis have recently gained prominence in psoriasis treatment. Remarkably, risankizumab, an IL-23 inhibitor, has shown significant efficacy in clinical trials. In this study, we utilized additional longitudinal scRNA-seq data from patients undergoing risankizumab treatment. By projecting the data onto gene modules defined from untreated psoriatic skin, we captured dynamic changes in disease-relevant transcriptomic programs.

Reviewer #2 (Remarks to the Author):

We thank the authors for their thoughtful responses to the initial comments and the substantial additions to the manuscript. However, several critical concerns remain that must be addressed before this work can be considered for publication.

Response: We appreciate the reviewer for the opportunity to further improve our manuscript with the invaluable suggestions. We have carefully considered the remaining concerns and have revised the manuscript to address them. Detailed point-by-point responses are provided below.

1: We appreciate the detailed explanation of the Duffy approach and agree that Supplementary Figure 3 substantially strengthens the manuscript. # 2: Supplementary Figure 1 is an excellent addition that enhances the paper's clarity.

Response: We thank the reviewer for the positive feedback.

3 (Critical): While we acknowledge the inherent limitations of using publicly available datasets, we remain deeply concerned about the lack of demographic information, particularly regarding sex and age. These variables are standard confounders routinely adjusted in any type of transcriptomic analyses. Furthermore, the datasets employed appear heavily skewed toward male participants, raising serious questions about the generalizability of your findings to female populations and across age groups. Please address this limitation explicitly in the main text and discuss how sex and age differences might impact your result.

Response: We thank the reviewer for pointing out this critical limitation. We fully agree that sex and age are fundamental biological variables; however, many publicly available psoriasis single-cell datasets lack granular, individual-level metadata for these factors. Furthermore, we acknowledge the concern regarding the male-skewed nature of currently available datasets. Previous studies have reported sex-related differences in disease severity (PMID: 28342016), which are potentially linked to the regulation of immune-mediated inflammation by various sex

hormones (PMID: 24459670, 25728622). These findings underscore the need for sex-specific investigations in psoriasis. We have explicitly addressed this limitation in the revised manuscript and highlighted the necessity for future sex-stratified analyses based on large-scale single-cell cohorts.

[Added to the Discussion, page 25, lines 510–520]

Third, many publicly available single-cell datasets did not provide comprehensive demographic information, including age and sex, which precluded us from performing a sex-stratified analysis. Furthermore, the datasets used in this study were heavily skewed toward male participants. Consequently, there are inherent limitations in the generalizability of our findings, as the analysis could not fully account for sex-specific effects. Previous research has indicated that male patients often exhibit greater disease severity compared to females [**Ref. 1**]. Moreover, studies have reported a significant link between sex hormones and immune regulation, noting that high estrogen levels correlate with clinical improvement, whereas low testosterone in males is associated with increased disease severity [**Refs. 2, 3**]. Therefore, future investigations using large-scale and well-annotated single-cell cohorts are needed to elucidate the effects of demographic variables on psoriasis pathogenesis.

References

Ref. 1. Hägg, D., Sundström, A., Eriksson, M. & Schmitt-Egenolf, M. Severity of psoriasis differs between men and women: a study of the clinical outcome measure psoriasis area and severity index (PASI) in 5438 Swedish register patients. *American journal of clinical dermatology* **18**, 583-590 (2017).

Ref. 2. Ceovic, R. *et al.* Psoriasis: female skin changes in various hormonal stages throughout life—puberty, pregnancy, and menopause. *BioMed research international* **2013**, 571912 (2013).

Ref. 3. Cemil, B. C., Cengiz, F. P., Atas, H., Ozturk, G. & Canpolat, F. Sex hormones in male psoriasis patients and their correlation with the P soriasis A rea and S everity I ndex. *The Journal of dermatology* **42**, 500-503 (2015).

4: Lines 1092-1094 remain unclear. When stating that "scDRS analysis robustly and consistently revealed significant enrichment in dendritic cell and T cell clusters," what is the specific comparison being made? Are these cells enriched when comparing pre- to post-treatment samples, or when comparing diseased versus healthy tissue? Additionally, Supplementary Figure 18's heatmap is difficult to interpret due to the confusing notation of ligands (L), receptors (R), and left/right (L or R) comparisons. Please explicitly define all terms in the figure legend and clarify what each comparison represents.

Response: We thank the reviewer for the comment. We would like to clarify that the scDRS enrichment analysis was not performed as a direct comparison between disease and healthy samples. Instead, both disease and healthy control samples were included in the analysis, and sample type (lesion vs. non-lesion) was used as a covariate in the model. The primary goal of this analysis was to assess whether the cell type-specific enrichment patterns observed in the Ma et al. dataset are consistently recapitulated in an independent dataset. Accordingly, we performed scDRS analysis using the Kim et al. dataset with the same tissue types (pre-treatment disease and healthy control samples) and covariates (mitochondrial gene percentages and sample types). As a result, we observed significant enrichment in dendritic cell and T cell clusters, consistent with the findings from the Ma et al. dataset. We have revised the **Results** and **Methods** sections to describe the analytical details more clearly.

Regarding figures of cell-cell interaction results, we agree that previous notations were confusing. We have revised the legends of **Supplementary Figures 17 and 18**. Specifically, we have removed the “Left (L) / Right (R)” in the legends and explicitly defined “L” as ligand and “R” as receptor in both figure panels and legends.

[Revised in the Results, page 18, lines 352–360]

To assess the consistency across multiple datasets and the generalizability of the findings, we analyzed the Kim et al. dataset, which included samples of healthy controls and patients with psoriasis collected at week 28 after risankizumab treatment (**Supplementary Fig. 18** and

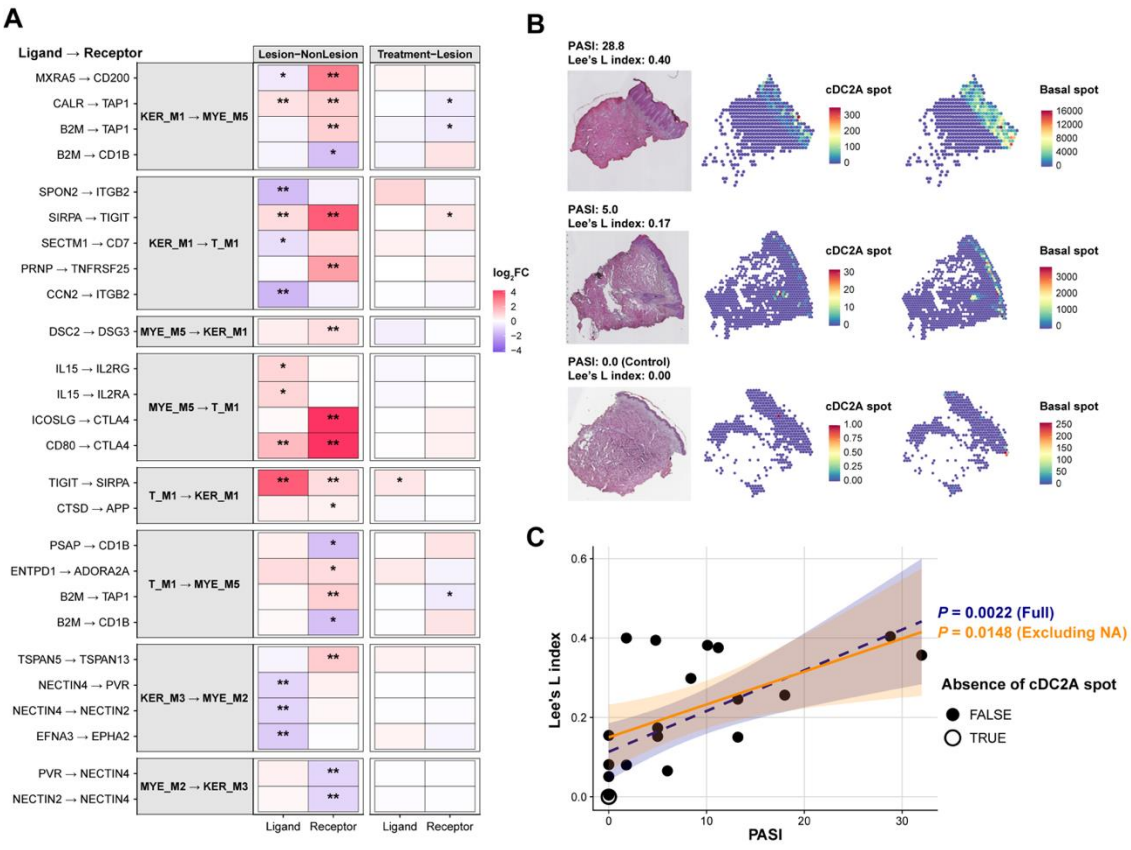
Supplementary Table 27). This dataset exhibited distinct cellular expression profiles compared to other single-cell datasets, including clearer separation of dendritic and other myeloid cells and the clustering of endothelial and fibroblast populations (**Supplementary Fig. 18A–C**). Using scDRS analysis, we revealed significant enrichment of genes associated with psoriasis in dendritic cell and T cell clusters, consistent with the patterns observed in the Ma et al. dataset (**Supplementary Fig. 18D**).

[Added to the Methods, page 37–38, lines 781–793]

Additionally, an independent scRNA-seq dataset from Kim et al. was included as the replication dataset. This dataset included skin scRNA-seq data from healthy controls and patients with psoriasis, as well as samples collected at a long-term post-treatment time point (week 28 after risankizumab treatment). All the preprocessing steps were performed using the same pipeline as described above. Cell type annotation for this dataset was conducted independently using canonical marker genes reported in the original study rather than reference mapping because substantial heterogeneity in data characteristics driven by distinct expression profiles resulted in reference-based mapping being disproportionately assigned to a single cluster. We performed scDRS analysis on the Kim et al. dataset for external validation. To ensure consistency with our primary analysis of the Ma et al. data, we included pre-treatment (baseline) psoriasis samples and healthy control samples. The analysis utilized the same MAGMA-derived gene weights and parameters that we applied to the Ma et al. data. Mitochondrial percentages and sample types (lesional or healthy) were used as covariates.

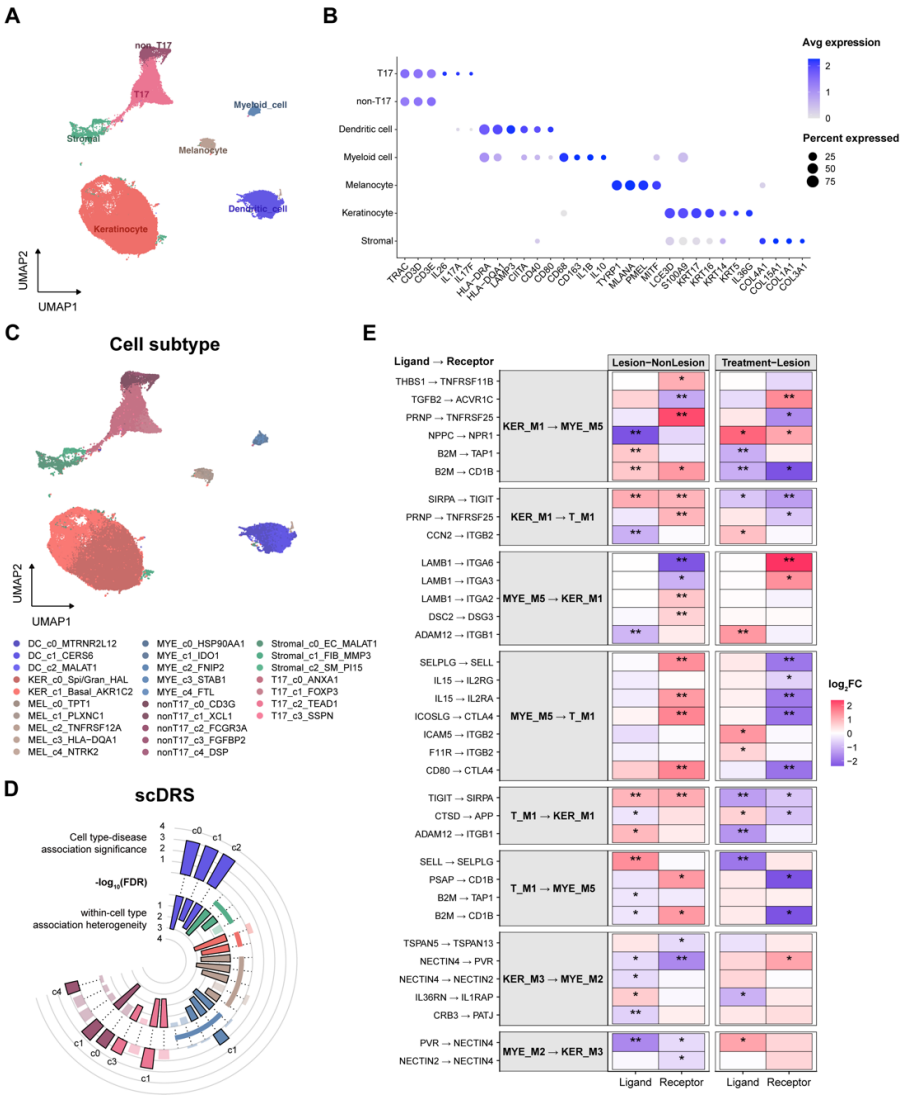
[Revised the Supplementary Fig. 17]

Supplementary Figure 17. Module-level cell-cell interaction analysis among keratinocytes, myeloids, and T cells. (A) Ligand-receptor interactions that exhibit pairwise differential expression between lesion and non-lesion samples ~~(Left)~~ and treatment and lesion samples ~~(Right)~~. The heatmap shows the log2-transformed fold changes of the gene expression between two conditions.



[Revised the Supplementary Fig. 18]

Supplementary Figure 18. Replication analysis of single-cell analysis using Kim et al. dataset. (E) Ligand-receptor interactions that exhibit pairwise differential expression between lesion and non-lesion samples ~~(Left)~~ and treatment and lesion samples ~~(Right)~~. The heatmap shows the log2-transformed fold changes of the gene expression between two conditions.



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600

5: We appreciate the added clarity. We recommend incorporating Supplementary Figure 12b into a main figure, as it provides important context for understanding the results.

601

602 **Response:** We thank the reviewer for the constructive suggestion. We agree that incorporating
603 the RNA velocity-based keratinocyte differentiation results into the main figure provides
604 valuable context. In our previous analysis, we utilized scVelo to estimate velocity streams.
605 During the revision process, we re-performed the RNA velocity analysis using veloVI to provide
606 more accurate and robust results, as recent benchmarking studies have shown that veloVI
607 achieves the highest performance (PMID: 41916302, PMID: 41509202) [**Refs. 1, 2**]. We have

updated **Figure 4F** by overlaying the RNA velocity stream to enhance the visualization of differentiation trajectories. Accordingly, we have revised the **Results** and **Methods** sections.

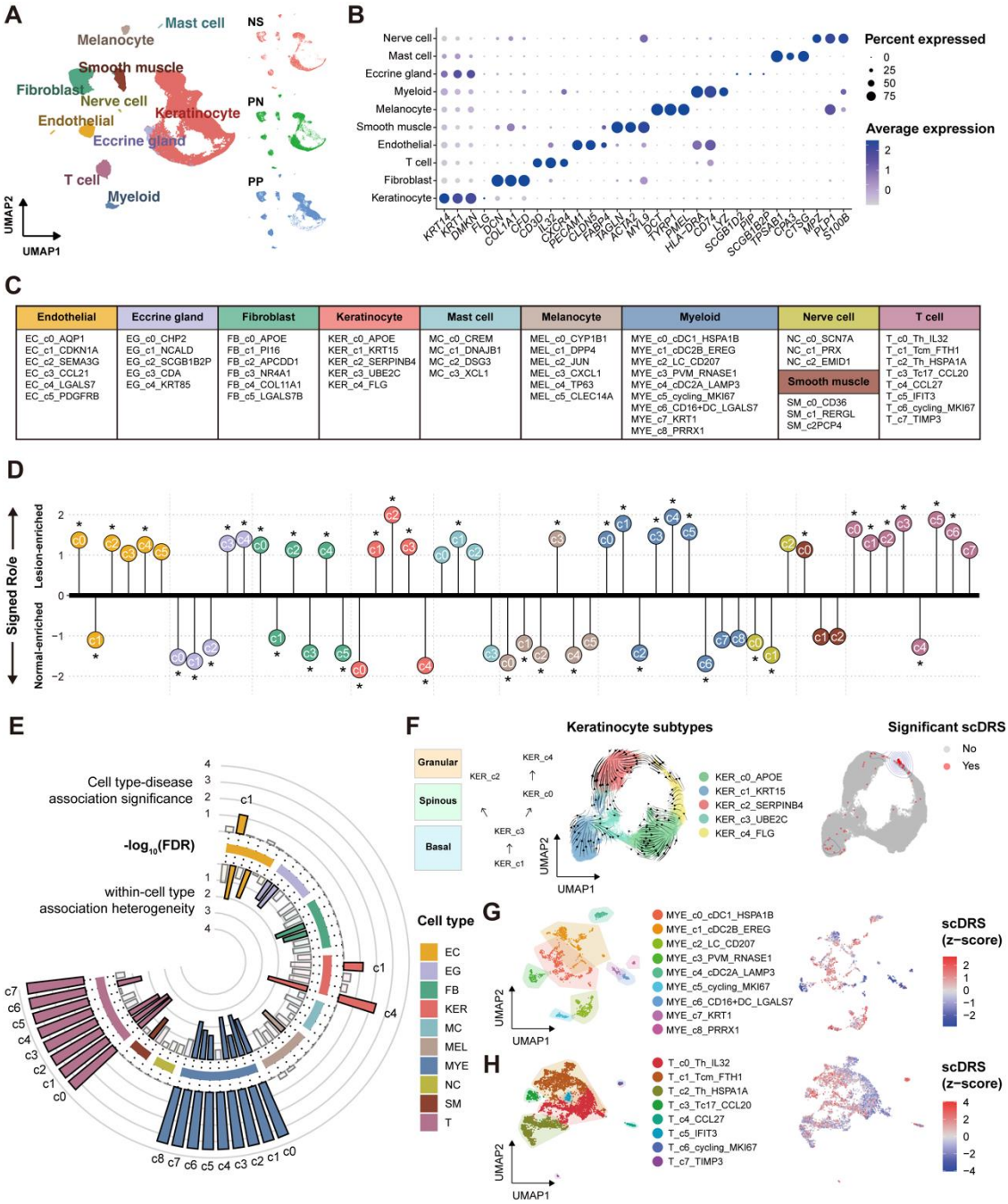
References

Ref. 1. Luo, Y. *et al.* Benchmarking RNA velocity methods across 17 independent studies. *Cell Reports Methods* (2025).

Ref. 2. Huang, K. *et al.* Benchmarking algorithms for RNA velocity inference. *bioRxiv*, 2026.2001. 2003.697314 (2026).

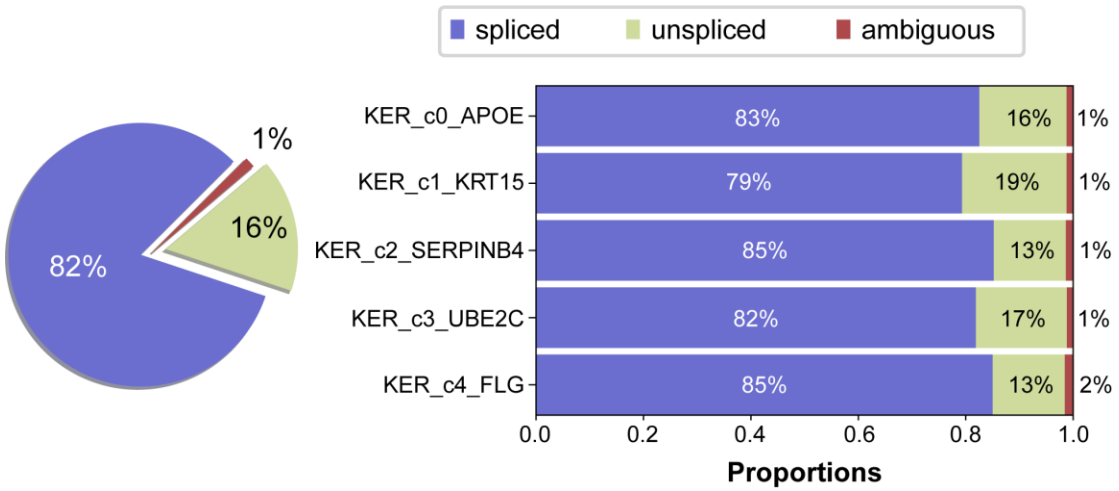
[Revised the Fig. 4F]

Fig. 4: Joint analysis of single-cell transcriptomics and GWAS data. (**F**) Two keratinocyte lineages (left), UMAP with RNA velocity (center), and FDR-adjusted Monte Carlo *p*-values of cells (right) are shown.



[Removed the Supplementary Fig. 12B]

Supplementary Figure 12. The proportion of unspliced and spliced reads per cluster in RNA velocity analysis of keratinocyte clusters.



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628

629 **[Revised in the Methods, pages 33–34, lines 691–698]**

630 To further validate keratinocyte differentiation, RNA velocity analysis was performed using
631 scVelo and veloVI for the functional validation. RNA velocity estimates future transcriptional
632 states of cells by modeling the relative abundance of unspliced and spliced transcripts in the
633 single cell expression profiles. Higher proportions of unspliced reads indicated active
634 transcription and were associated with less differentiated cellular states. For veloVI analysis, the
635 model was implemented using default hyperparameters as specified in the documentation, with
636 the training process conducted for a maximum 500 epochs.

637

6-8: Thank you for the additional descriptions.

638

639 **Response:** We thank the reviewer for the acknowledgement.

640

9: We appreciate the clarification. Please also add the p-value threshold to the Methods section, as currently only the expected value calculation is described.

641

Response: We thank the reviewer for the comment. Following the reviewer's comment, we have implemented a permutation-based statistical test to evaluate the significance of Ro/e results and FDR < 0.05 was considered statistically significant. We have updated **Fig. 4D** to include asterisks denoting significant enrichment and revised the **Methods** section accordingly to describe this statistical approach.

[Revised in the Results, page 12, lines 218–220]

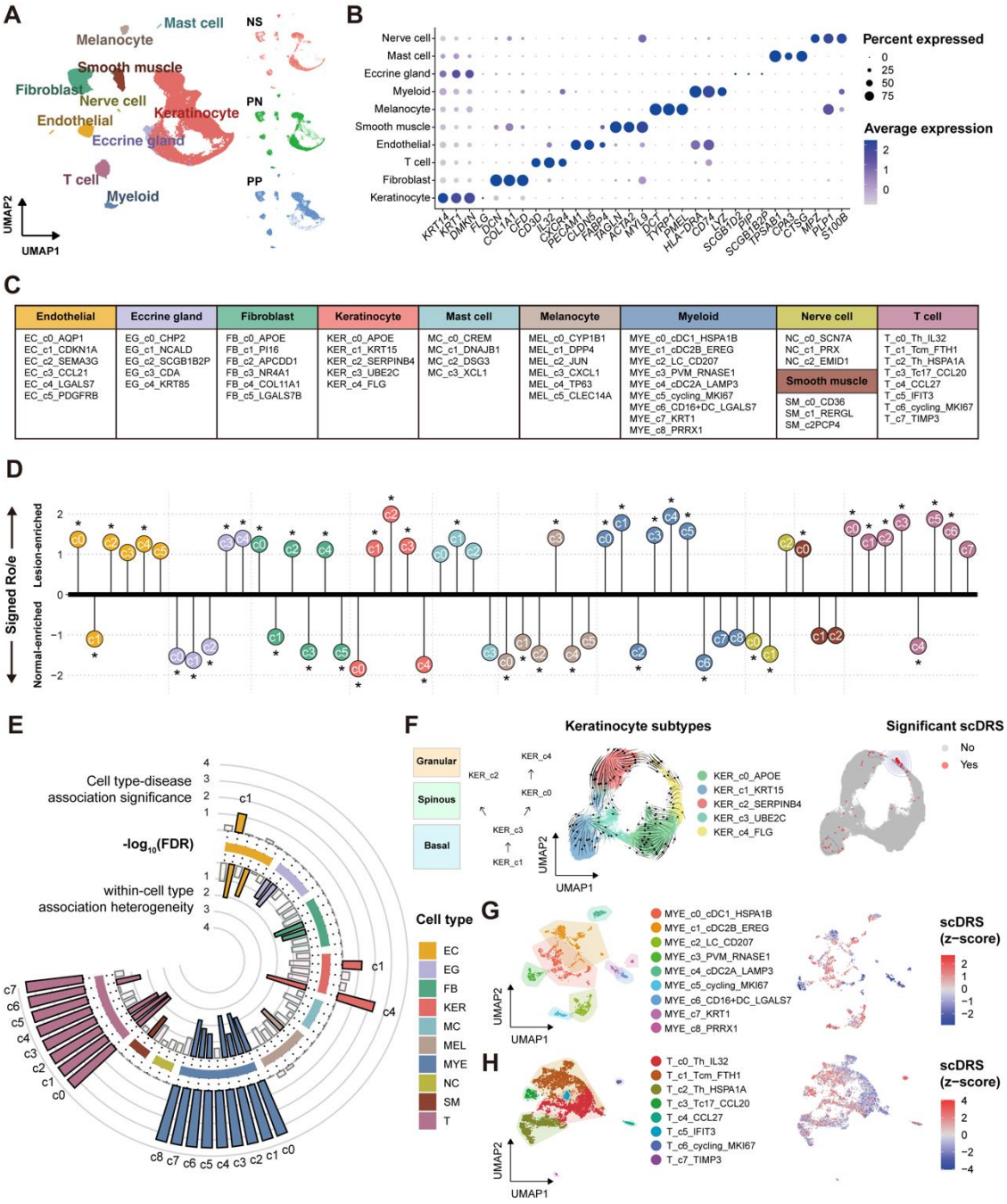
Additionally, KER_c2 was enriched with cells derived from lesion tissues, whereas KER_c4 was enriched with cells from normal tissues (**Fig. 4D** and **Supplementary Table 14**).

[Revised in the Methods, pages 32–33, lines 668–672]

If the Ro/e value for the non-lesion was greater than 1, the cell subtype was defined as normal-enriched. Otherwise, the subtype was defined as lesion-enriched if the Ro/e value for lesional tissue was greater than 1. Statistical significance of Ro/e values was assessed by 10,000 random permutations of tissue labels with two-sided empirical p-values. After multiple testing correction, FDR < 0.05 was considered statistically significant.

[Revised the Figure 4D]

(D) Lollipop plot displaying relative tissue enrichment. We compared the number of non-lesion cells (NS+PN) and lesion cells (PP) in each cell subtype and calculated the ratio of observed and expected (Ro/e). If the Ro/e value for the non-lesion was higher than 1, the Ro/e value for the cell subtype was defined as −1×(Non-lesion Ro/e). Otherwise, Ro/e was defined as (Lesion Ro/e). Asterisks denote the statistical significance with false discovery rate (FDR) < 0.05.



[Revised the Supplementary Table 14]

Abbreviation: Ro/e, Ratio of observed to expected.

group	Enriched tissue	Ro/e	Ro/e permutation p-value	Ro/e permutation FDR	n_cell	n_ctrl	…	n_fdr_0.2
EC_c0_AQP1	Disease	1.37	1.00E-04	1.62E-04	1896	10000		46
EC_c1_CDKN1A	Normal	1.10	5.00E-04	7.64E-04	1150	10000		101
EC_c2_SEMA3G	Disease	1.30	1.00E-04	1.62E-04	866	10000		7
EC_c3_CCL21	Disease	1.04	4.22E-01	4.94E-01	486	10000		0
EC_c4_LGALS7	Disease	1.26	1.60E-03	2.32E-03	178	10000	…	5
EC_c5_PDGFRB	Disease	1.08	4.59E-01	5.26E-01	116	10000		1
EG_c0_CHP2	Normal	1.53	1.00E-04	1.62E-04	908	10000		10
EG_c1_NCALD	Normal	1.63	1.00E-04	1.62E-04	134	10000		0
EG_c2_SCGB1B2P	Normal	1.30	1.10E-03	1.63E-03	114	10000		0

(The remaining rows are omitted.)

References

Ref. 1. Yang, Y. *et al.* Pan-cancer single-cell dissection reveals phenotypically distinct B cell subtypes. *Cell* **187**, 4790-4811. e4722 (2024).

Ref. 2. Shi, Q. *et al.* Cross-tissue multicellular coordination and its rewiring in cancer. *Nature* **643**, 529-538 (2025).

10-12 (Critical): While we appreciate the new computational analyses, they do not provide the mechanistic validation necessary to support the biological insights currently being claimed. The identification of cell-cell communication represents hypothesis generation rather than hypothesis testing. To substantiate your claims, functional validation experiments are essential. Specific examples from the manuscript:

CD80-CTLA4 interaction

Basal keratinocyte-cDC2A-T cell trio

Please provide experimental validation demonstrating the functional relevance of these pathways in your disease context. What is the mechanistic basis for these interactions? Does disrupting one component alter the disease phenotype or treatment response? Computational

prediction alone is insufficient to establish biological significance. Without such validation, the manuscript remains primarily descriptive.

Response: We thank the reviewer for highlighting the importance of mechanistic validation and for the thoughtful comments regarding the interpretation of our cell-cell communication results. We fully agree that mechanistic validation is the gold standard for establishing the mechanisms of cell-cell interaction.

However, we sincerely apologize that we are unable to provide the requested experimental validation. As our research group is a purely computational dry lab, we unfortunately do not have the necessary facilities and resources to conduct experiments. Nevertheless, to compensate for this limitation and ensure that our findings are robust and biologically plausible, we have focused on strengthening the biological evidence through a comprehensive and multi-dimensional approach. Specifically, the inferred cell-cell interaction patterns were consistently observed in the independent single-cell transcriptome dataset (Kim et al.), confirming their reproducibility. Furthermore, spatial transcriptomic analysis using the Castillo et al. dataset demonstrated physical proximity between the implicated cell types, providing crucial spatial context for these functional interactions.

This strategy aligns with the established methodological scope of large-scale genomic studies in psoriasis, which utilize computational and statistical frameworks to establish mechanistically informed hypotheses in the absence of direct experimental validation [**Refs. 1–5**]. For example, an early psoriasis GWAS first identified the *TYK2* gene as a novel psoriasis-associated gene [**Ref. 6**], and the TYK2 inhibitor Deucravacitinib was later developed through clinical studies and FDA-approved [**Ref. 7**]. In another example, a recent psoriasis GWAS identified *AHR* as a novel risk gene [**Ref. 1**], and a subsequently published study further suggested that treatment with a plant mixture to activate the *AHR* gene in a 2D psoriasis model significantly restored psoriasis-related pathological features [**Ref. 8**]. We believe that the prioritized genes and proposed mechanisms in our study will likewise serve as a valuable foundation for developing novel therapeutic strategies in psoriasis.

While these complementary analyses support the reliability of the proposed interactions, we acknowledge that the findings warrant experimental evidence. Accordingly, in response to the

reviewer's feedback, we have carefully toned down our claims throughout the **Results** and **Discussion** sections. In addition, the schematic illustration of the proposed mechanisms (previously added to **Figure 6J**) has been moved to the **Supplementary Figure 19** to avoid potential over-interpretation by readers. We hope that our revised manuscript and responses properly address the reviewer's concerns, and that other experimental research groups find our results informative.

References

Ref. 1. Dand, N. *et al.* GWAS meta-analysis of psoriasis identifies new susceptibility alleles impacting disease mechanisms and therapeutic targets. *Nature communications* **16**, 2051 (2025).

Ref. 2. Zhang, M. *et al.* Multi-ancestry genome-wide meta-analysis with 472,819 individuals identifies 32 novel risk loci for psoriasis. *Journal of Translational Medicine* **23**, 133 (2025).

Ref. 3. Heikkilä, A. *et al.* Genetic Study of Psoriasis Highlights its Close Link with Socioeconomic Status and Affective Symptoms. *Journal of Investigative Dermatology* **144**, 2719-2729 (2024).

Ref. 4. Liu, S. *et al.* Novel genetic insight for psoriasis: integrative genome-wide analyses in 863 080 individuals and proteome-wide Mendelian randomization. *Briefings in Bioinformatics* **26**, bbaf032 (2025).

Ref. 5. Tsoi, L. C. *et al.* Large scale meta-analysis characterizes genetic architecture for common psoriasis associated variants. *Nature communications* **8**, 15382 (2017).

Ref. 6. Strange A, *et al.* Genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1. *Nature genetics* **42**, 985 (2010).

Ref. 7. Papp K, *et al.* Phase 2 trial of selective tyrosine kinase 2 inhibition in psoriasis. *New England Journal of Medicine* **379**, 1313-1321 (2018).

Ref. 8. Heinemann N, Rademacher F, Vollert H, Gläser R, Harder J. Plant extract mixture shows anti-inflammatory and barrier-strengthening effects and activates aryl hydrocarbon receptor in a 2D psoriasis model. *Scientific Reports* **16**, 13638 (2026).

[Revised in the Results, page 17, lines 343–349]

Notably, the DSC2-DSG3 interaction showed increased activity in lesional samples, ~~indicating physical adhesion~~ suggesting potential cellular proximity or interaction between cDC2A and basal keratinocytes. Interactions between cDC2A and T cells were also upregulated in lesional skin, including CD80-CTLA4 interactions, ~~representing~~ reflecting a proposed cDC2A-mediated ~~inhibition~~ modulation of immune activity in the basal epidermal layer. Spatial transcriptomic validation using an independent psoriasis dataset ~~further supported these findings, revealing~~ provided additional context consistent with these predictions, showing close spatial proximity between basal keratinocytes and cDC2A specifically in lesional tissue (**Supplementary Fig. 17B**).

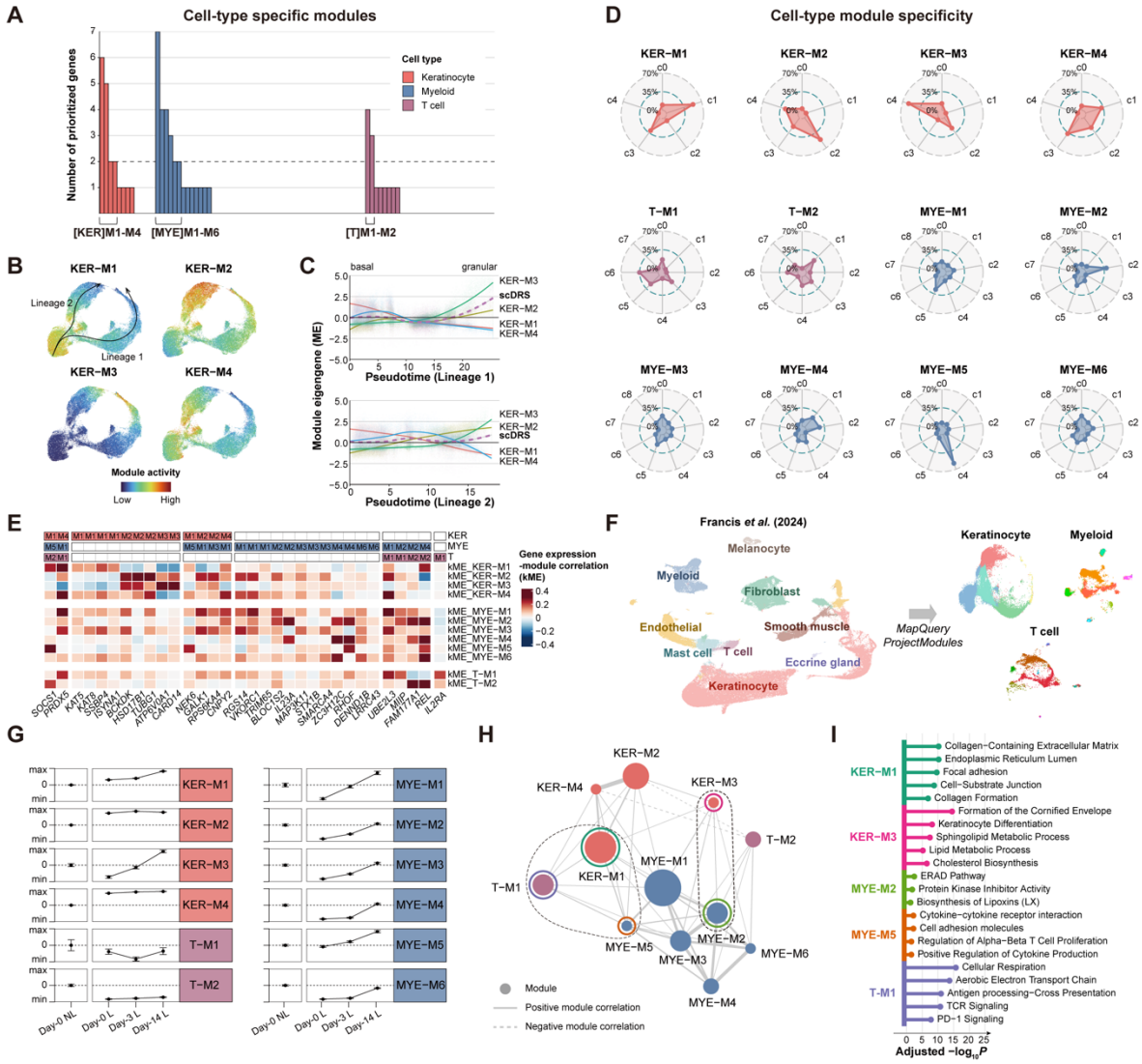
[Revised in the Discussion, pages 25–26, lines 520–533]

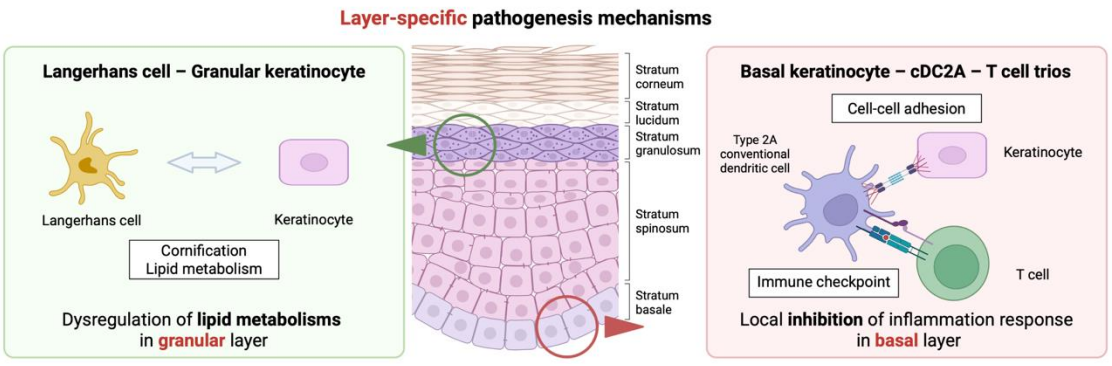
Fourth, although our multi-omics framework identified key psoriasis-associated genes and putative cell-cell communication interactions, these findings remain computationally derived and lack direct mechanistic validation. Specifically, the functional relevance of the CD80-CTLA4 interaction and the basal keratinocyte-cDC2A-T cell trio in psoriasis pathogenesis requires further experimental confirmation. Despite our use of spatial transcriptomics to demonstrate the proximity of these cell types, this does not establish causality. As our findings were derived mainly from computational analyses, they should be interpreted with caution and not as definitive evidence of underlying mechanisms. For instance, future studies utilizing skin organoids or CRISPR-based functional screens are necessary to determine whether disrupting these specific interactions alters the inflammatory phenotype.

Taken together, our multi-omics framework provides a systematic approach identifying cell type-specific molecular features and prioritizing biologically relevant pathways in psoriasis. This framework may help guide future experimental studies, facilitate the identification of potential therapeutic targets, and could be extended to other complex diseases.

[Moved Fig. 6J to Supplementary Fig. 19]

Fig. 6: Cell type-specific gene co-expression modules associated with risankizumab treatment. (A) Cell type specific co-expression gene network identification using hdWGCNA, which identified 13, 49, 30 modules for keratinocyte, myeloid, and T cell, respectively. Each expressed gene was assigned to single module. The bar plot represents the number of assigned genes that were defined as therapeutic targets. **(B)** Module eigengenes (MEs) of keratinocyte module 1 to module 4 (KER-M1 to KER-M4). **(C)** MEs of keratinocyte modules change over the pseudotime of two keratinocyte trajectories. Dots represent the ME score of single cells. Smoothed curves with 95% confidence interval were fitted by the LOESS algorithm. scDRS scores of keratinocyte cells were plotted together (Dashed purple curve). **(D)** Cell type enrichment of 12 modules having two or more prioritized genes. The enrichments represent the proportion of average MEs for all cell subtypes in the major cell type**. (E)** Module memberships and connectivities of prioritized genes. Genes assigned to at least one of the 12 modules are shown. **(F)** Overview of procedure about risankizumab treatment data for psoriasis from Francis et al. **(G)** Temporal enrichment changes of MEs for non-lesions and lesion skins over treatment. NL, Non-lesion; L, Lesion. **(H)** A correlation network for 12 modules from 20 samples of Francis et al. **(I)** Gene-set pathway analysis for five modules of interest. Representative pathways with adjusted p-values < 0.10 were illustrated.





Supplementary Figure 19. Schematic illustration of proposed two layer-specific mechanisms of psoriasis pathogenesis.

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Reviewer #3 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Response: We appreciate the reviewer's valuable time and effort in co-reviewing our manuscript.

Additional revision

In addition to addressing the reviewers' comments, we have further corrected some typos and revised statements in the manuscript, which are listed below.

[Revised in the Results, page 33, lines 689–690]

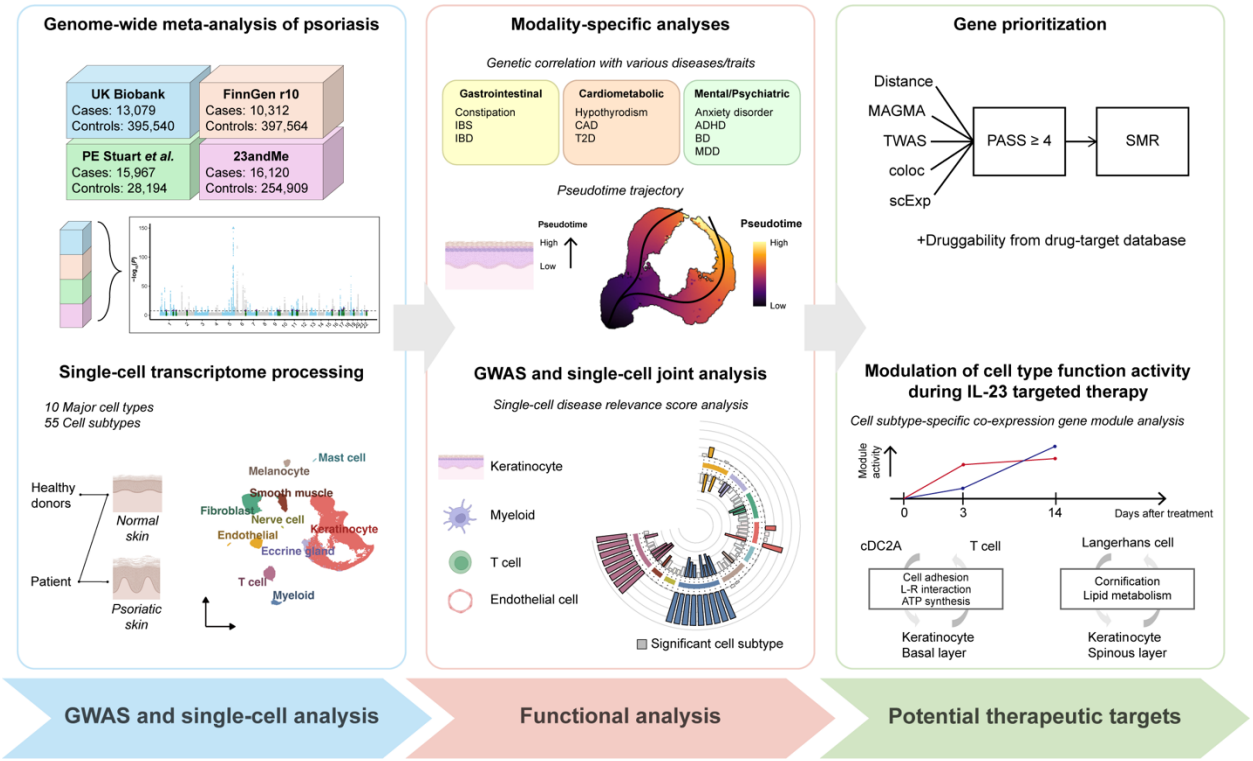
This pattern suggests a gradual differentiation process, and we applied trajectory inference using a Slingshot library with parameter start.clus = "~~KER_c3_UBE2C~~KER_c1_KRT15".

[Revised in the Results, page 39, lines 823–824]

Spatial transcriptomic data obtained from the study by Castillo et al. was used to validate the ~~validation~~ results of cell-cell communication analysis.

[Revised Manhattan plot in the Figure 1]

The Manhattan plot has been revised to reflect the updated unreported loci during the revision.



[Revised in the Supplementary Figure 11]

This plot represents the distribution of pseudotime of cells assigned to the lineages for each cluster by Slingshot analysis.

Additional comments from the authors

We have updated the **Figures**, **Supplementary Figures**, and **Supplementary Tables** accordingly while addressing the reviewers' comments. The updates and rearrangements of the figures and tables are listed below.

Figures

Initially	After revision	Comment on changes in the structure
Figure 1	Figure 1	Manhattan plot has been revised to match Supplementary Figure 4.
Figure 2	Figure 2	
Figure 3	Figure 3	
Figure 4	Figure 4	Panel D has been modified to represent statistical significance in response to the reviewer #2's comment #9. Panel F has been modified to overlay RNA velocity onto UMAP.
Figure 5	Figure 5	
Figure 6	Figure 6	Figure 6J panel has been moved to Supplementary Figure 19.

Supplementary Figures

Initially	After revision	Comment on changes in the structure
Supplementary Figure 1	Supplementary Figure 1	
Supplementary Figure 2	Supplementary Figure 2	
Supplementary Figure 3	Supplementary Figure 3	
Supplementary Figure 4	Supplementary Figure 4	
Supplementary Figure 5	Supplementary Figure 5	
Supplementary Figure 6	Supplementary Figure 6	
Supplementary Figure 7	Supplementary Figure 7	Figure panels and legends have been revised in response to the reviewer #1's comments #3 and #19.
Supplementary Figure 8	Supplementary Figure 8	
Supplementary Figure 9	Supplementary Figure 9	
Supplementary Figure 10	Supplementary Figure 10	
Supplementary Figure 11	Supplementary Figure 11	

Supplementary Figure 12	Supplementary Figure 12	Previous RNA velocity plot has been integrated to Figure 4F.
Supplementary Figure 13	Supplementary Figure 13	Figure panels and legends have been revised in response to the reviewer #1's comment #20.
Supplementary Figure 14	Supplementary Figure 14	
Supplementary Figure 15	Supplementary Figure 15	
Supplementary Figure 16	Supplementary Figure 16	
Supplementary Figure 17	Supplementary Figure 17	Figure panels and legends have been revised in response to the reviewer #1's comment #9 and the reviewer #2's comment #4.
Supplementary Figure 18	Supplementary Figure 18	Figure panels and legends have been revised in response to the reviewer #2's comment #4.
-	Supplementary Figure 19	Previous Figure 6J panel has been moved to Supplementary Figure 19.

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820 Supplementary Tables

Initially	After revision	Comment on changes in the structure
Supplementary Table 1	Supplementary Table 1	
Supplementary Table 2	Supplementary Table 2	
Supplementary Table 3	Supplementary Table 3	
Supplementary Table 4	Supplementary Table 4	
Supplementary Table 5	Supplementary Table 5	
Supplementary Table 6	Supplementary Table 6	The column "Discovery potential category" has been added in response to the reviewer #1's comment 19.
Supplementary Table 7	Supplementary Table 7	
Supplementary Table 8	Supplementary Table 8	
Supplementary Table 9	Supplementary Table 9	
Supplementary Table 10	Supplementary Table 10	
Supplementary Table 11	Supplementary Table 11	
Supplementary Table 12	Supplementary Table 12	
Supplementary Table 13	Supplementary Table 13	
Supplementary Table 14	Supplementary Table 14	A permutation test for Ro/e has been added in response to the reviewer #2's comment #9.
-	Supplementary Table 15	Added new Supplementary Table 15. Comparison of scDRS heterogeneity and scDRS scores between lesional and non-lesional cells.

Supplementary Table 15	Supplementary Table 16	
Supplementary Table 16	Supplementary Table 17	Visualization for p_HEIDI and related notes have been revised in response to the reviewer #1's comment #6.
Supplementary Table 17	Supplementary Table 18	
Supplementary Table 18	Supplementary Table 19	
Supplementary Table 19	Supplementary Table 20	
Supplementary Table 20	Supplementary Table 21	
Supplementary Table 21	Supplementary Table 22	
Supplementary Table 22	Supplementary Table 23	
Supplementary Table 23	Supplementary Table 24	
Supplementary Table 24	Supplementary Table 25	
Supplementary Table 25	Supplementary Table 26	
Supplementary Table 26	Supplementary Table 27	

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Manuscript Number: NCOMMS-25-41335B

Manuscript: Integration of GWAS and single-cell analysis for psoriasis identifies disease-associated cell subtypes and therapeutic targets

Responses from the Authors for Review Comments:

We appreciate the reviewers for the continued interest in our work and for providing further constructive feedback. We have addressed each of the comments point-by-point, which has allowed us to further improve the quality of our manuscript. The responses to the reviewers' comments are highlighted in blue and the revised text in the manuscript is highlighted in red.

Reviewers' Comments:

Reviewer #1 (Remarks to the Author):

The authors have done an excellent job of responding to my comments.

Response: We sincerely thank the reviewer for the encouraging comment and for recognizing our efforts in addressing the previous comments.

However, I think there is one missing figure panel: the proposed new Supp Fig 13 panel B sounds like it should fully address my concern in minor comment 20, but is missing in the resubmission.

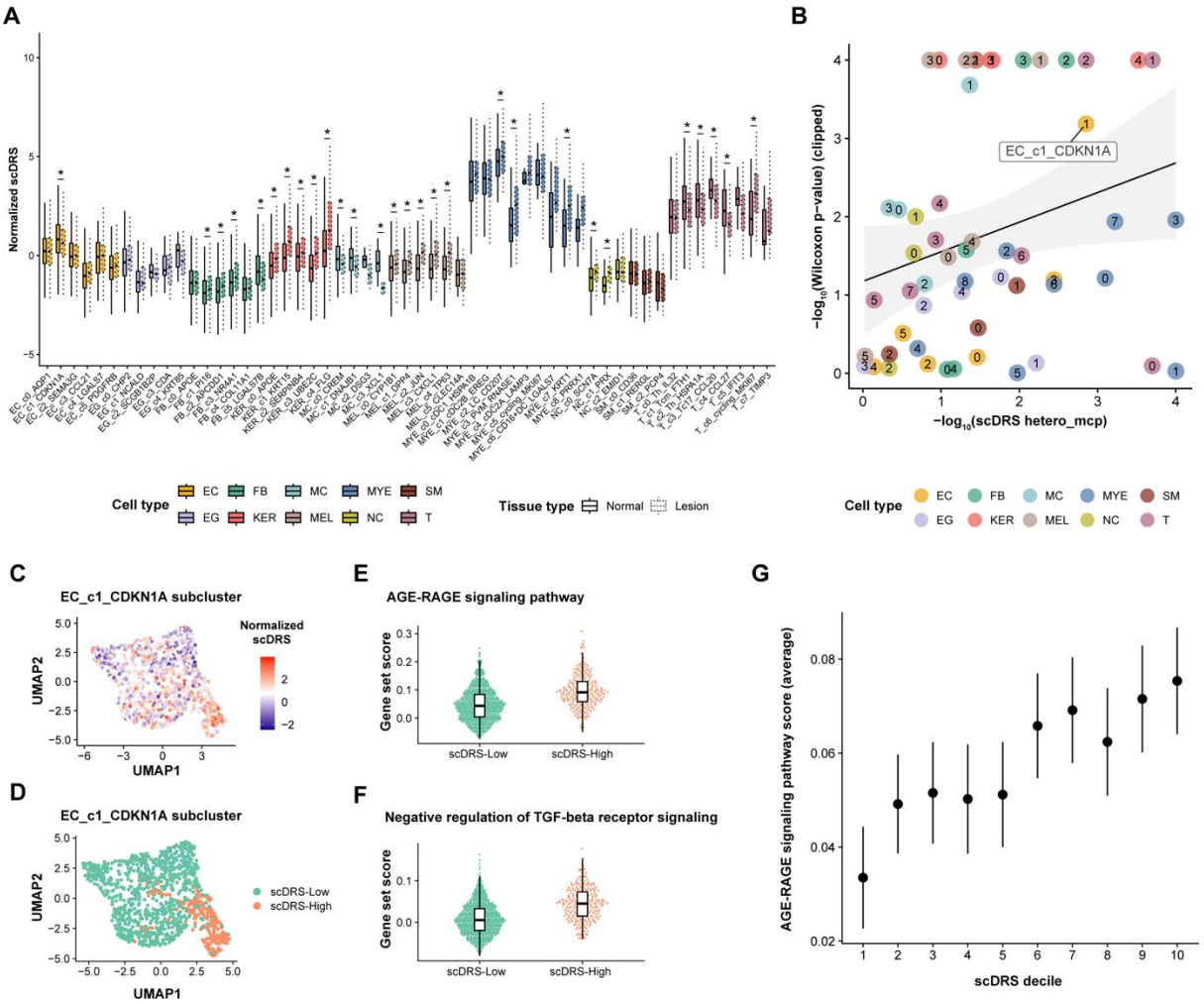
Response: We thank the reviewer for pointing out the missing panel. We have now inserted panel B in the Supplementary Figure 13, accordingly. In this panel, the fitted line illustrates the association between scDRS heterogeneity and the Wilcoxon rank-sum test of scDRS scores between tissues.

[Revised the Supplementary Figure 13]

Supplementary Figure 13. scDRS heterogeneity-based analysis.

(A) Distributions of scDRS scores for each cell type stratified by tissue type (normal and lesion). Asterisks denote cell types satisfying both scDRS heterogeneity false discovery rate (FDR) < 0.10 and Wilcoxon rank-sum test FDR < 0.10. P-values for scDRS heterogeneity were calculated using two-sided permutation tests (10,000 permutations). **(B)** Comparison between scDRS heterogeneity p-values and tissue-specific two-sided Wilcoxon test p-values. For visualization, Wilcoxon test p-values are clipped at a maximum of 4. A solid black line represents a linear fit between two variables and the surrounding shaded band denotes the 95% confidence intervals. Detailed statistical results are provided in Supplementary Data 15. **(C)** scDRS score distribution of the endothelial cluster EC_c1_CDKN1A in the UMAP space. **(D)** Subclustering of the endothelial cluster EC_c1_CDKN1A. Distributions of gene set scores of two EC_c1_CDKN1A

subclusters representing **(E)** the AGE-RAGE signaling pathway and **(F)** negative regulation of the TGF-beta receptor signaling pathway. **(G)** Average gene set scores per scDRS decile group of cells in EC_c1_CDKN1A. Error bars represent 95% confidence intervals.



If I were being very picky, I would also note that:

- In response to minor comment 1, I don't think it is quite correct to say that 73.85% of the UK Biobank cases "met the criteria for severe psoriasis" while the remainder may "represent milder disease severity" (and similar for 23andMe). If this were the case, I believe the estimate would come out higher than 73.85%. Isn't the point that the total genetic liability across cases is *consistent with* 73.85% severe psoriasis and the remainder *unaffected* (misclassified)? But it doesn't mean that the distribution actually looks like this; in reality it could be that a

much higher proportion are true cases but with lower average genetic liability (perhaps due to lower severity).

Response: We fully agree with the reviewer's insightful point. The proportion *q* is an estimate used to correct for the reduced genetic signal of self-reported cases, and it cannot be used to infer the actual number of true cases. As the reviewer highlighted, if milder cases contribute to a value such as 73.85%, the true proportion of cases could be higher, since these self-reported cases still showed a genetic signal that is attenuated relative to clinically diagnosed cases but distinct from controls (Supplementary Fig. 1B and Supplementary Fig. 2). Accordingly, q should be interpreted as reflecting the total genetic signal across cases rather than the proportion of severe cases. To avoid this misinterpretation, we have revised the term to "estimated true-positive proportion" and removed the previous statement regarding milder cases from the Results.

[Revised in the Results, page 7, lines 113–117]

Based on the risk allele frequencies in case samples and control samples, the total genetic liability across cases was consistent with estimated true-positive proportions of 73.85% for UKB cases and 37.5% for 23andMe cases, reflecting the weaker genetic signal of self-reported cases (Supplementary Data 2). ~~The remaining cases may represent milder disease severity, consistent with previous reports that PRS distribution correlates with clinical severity.~~

[Revised the Supplementary Data 2]

Supplementary Data 2. Estimated parameters of the Duffy's adjusting method for UK Biobank and 23andMe.

Parameter	Description	UK Biobank	23andMe
q	Estimated true-positive proportion	0.7385	0.375

- In response to minor comment 16, I still find the revised phrasing “We investigated the two classes in the contingency table” to be unclear.

Response: We thank the reviewer for the comment. We agree that the phrase "two classes" was unclear. It was intended to refer to two sequential analyses of LD blocks: we first examined the relationship between local heritability and the presence of genome-wide significant SNPs (Supplementary Fig. 7), and then categorized LAVA-significant blocks lacking genome-wide significant SNPs to explore the potential for identifying additional loci (Supplementary Fig. 8). As these analyses are already described in detail in the Results, we have revised the Methods to state clearly what the contingency table compares, and to avoid unclear wording.

[Revised in the Methods, page 30, lines 610–611]

We examined the association between local heritability significance and the presence of genome-wide significant SNPs across LD blocks using a contingency table (Supplementary Fig. 7A).

Beyond these minor points, I have no remaining major concerns. Congratulations on a through, insightful and well-written exploration of psoriasis genetics and mechanisms.

Response: We thank the reviewer for the valuable feedback that has helped strengthen our manuscript.

Reviewer #2 (Remarks to the Author):

The authors address my comments. No more comments.

Response: We sincerely appreciate the valuable time and effort you have dedicated to reviewing our manuscript.