Identification of psoriasis-protective *IL17D* variant associated with increased *IL17D* and *FAM19A5* expression in psoriatic skin

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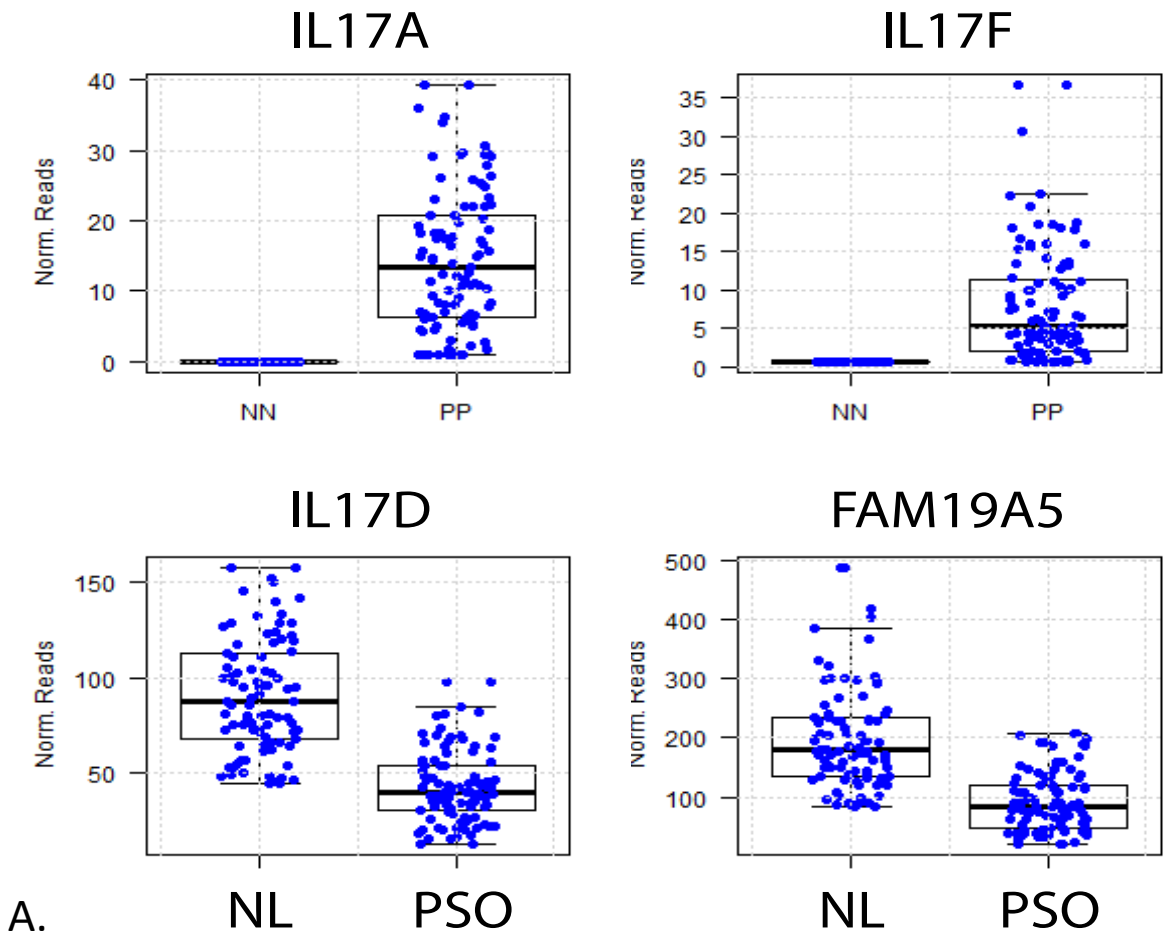
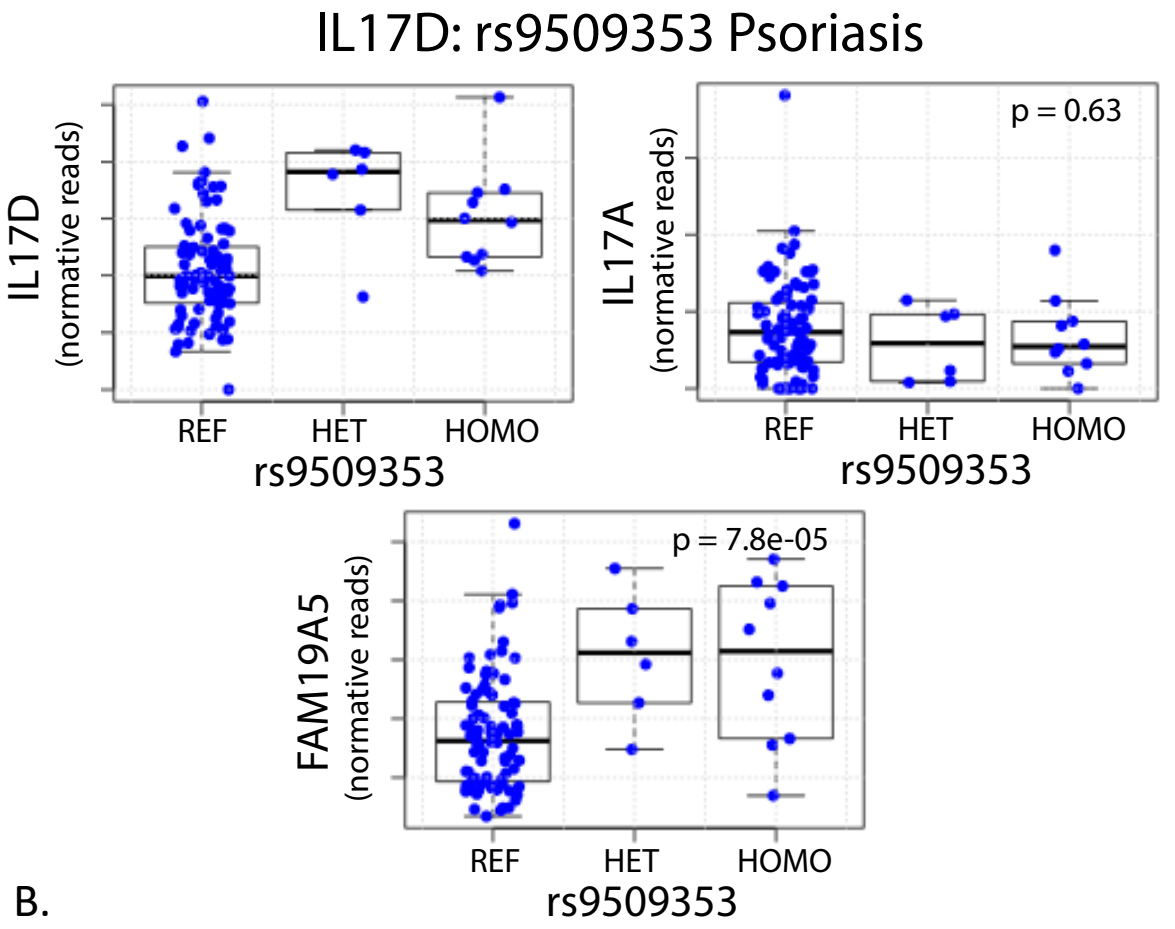
IL17D expression and variant-associated changes



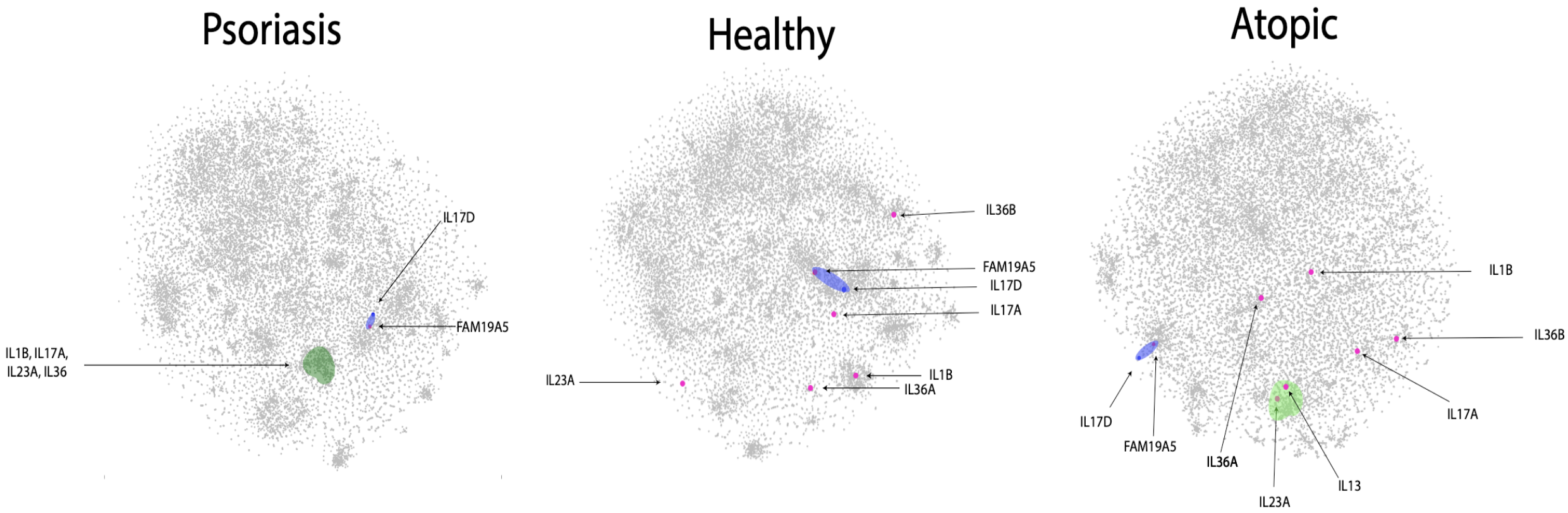


Psoriasis is a chronic inflammatory skin disease with an estimated heritability of 80% (Rahman P, Elder JT 2005). The IL-17 family plays a central role in the mediation of inflammatory pathways, including psoriasis. The most well-studied of these, IL-17A, is a major pro-inflammatory cytokine (Monin L, Gaffen SL 2018; Blauvelt A, Chiricozzi A 2018).

Methods: RNA-Seq FASTQ files of human normal and psoriasis lesional skin were downloaded from the NCBI Sequence Read Archive (http://www.ncbi.nlm.nih.gov/Traces/sra). The RNA-Seq dataset used for this analysis consisted of 99 psoriasis vulgaris skin biopsy samples obtained from patients washed out of all systemic and topical therapies and 90 healthy controls.

Results: A, *IL17A* is highly upregulated in psoriatic skin and appears to be a driver of disease (Rembilla NC, Senra L, Boehncke, 2018). *IL17F* shares the greatest homology to *IL17A*[3] and is also highly upregulated in psoriasis. In contrast, RNA-Seq datasets identified that *IL17D* is highly expressed in normal skin but downregulated (Fold Change = 0.33; p = 2.5×10^{-14})[5] in psoriatic plaques.[6] B, an *IL17D* regulatory variant, rs9509353, was found to be protective against psoriasis (OR = 0.20, p-value = 5.9e-07), increasing expression of *IL17D* and *FAM19A5*, a chemokine-like molecule that is also normally downregulated in psoriasis.

2D correlation & distribution: tSNE



Methods: Gene expression clusters were mapped using the t-Distributed Stochastic Neighbor Embedding (t-SNE) method. Distance was calculated as (d) = $1\text{-}r^2$, where r equals Pearson's correlation coefficient, calculated with Rtsne package (Le et al. 2019).

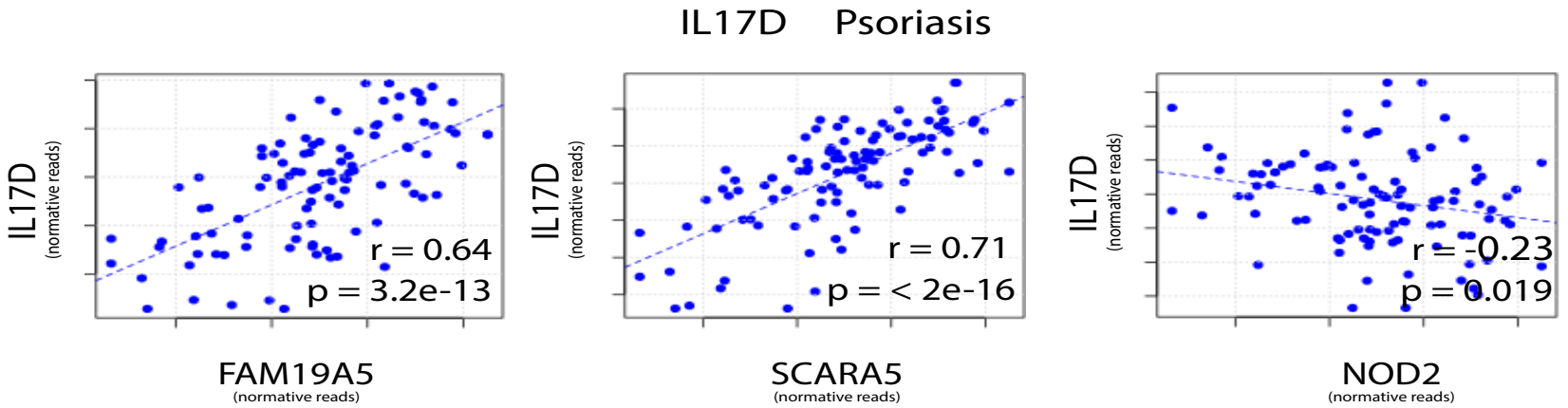
Results: 2D images of the psoriasis and healthy transcriptome illustrate the close spatial relationship between *IL17D, FAM19A5, and SCARA5,* which (1) map together and (2) away from the proinflammatory cytokine cluster comprised of *IL17A*, *IL23A*, *IL36,* and *IL1B.* Notably, these proinflammatory cytokines do not cluster together in healthy skin. Correlation studies found these cytokine clusters to be independent of *IL17D and FAM19A5*. Combined, these results highlight a putative regulatory role for IL-17D and FAM19A5 in psoriasis.

IL17D meta-analysis and correlations

A. **Methods:** Correlation analyses of gene expressions were performed on read counts of each identified gene normalized with DESeq2 package. Values were subsequently log transformed and winsorized when necessary. Spearman's correlation coefficients were calculated (r_s) using the cor.test function in R. P values were estimated by algorithm AS 89 (Le, et al. 2019). **Results:** Correlative studies demonstrate a close relationship between *IL17D* and *FAM19A5, IL17D* and *SCARA5,* and an inverse relationship between *IL17D* and *NOD2* in both the healthy and psoriasis transcriptomes. Other examples of highly correlated genes include *TGF-B2*, *TGF-R3*, *BTRC*, *TP63*, and *TIMP3*.

B. **Methods:** Meta-analysis was completed using the R package "metafor." A weighted random-effects model was used to estimate a summary effect size. To estimate between-study variance, a restricted maximum-likelihood estimator was applied. A weighted estimation with inverse-variance weights was used to fit the model (Le al. 2019). **Results:** Meta-analysis across multiple psoriasis datasets confirmed close relationships between *IL17D*, *FAM19A5*, *SCARA5*, and *NOD2*, all depicted here.

A.



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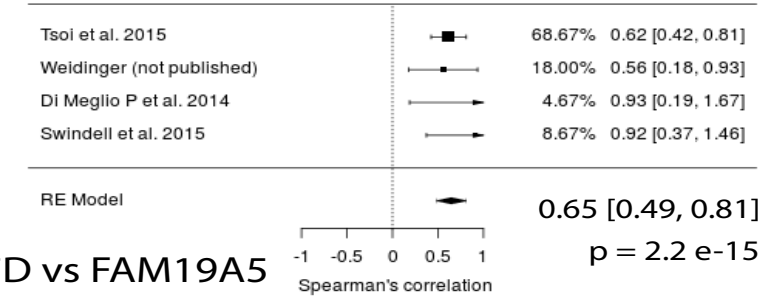
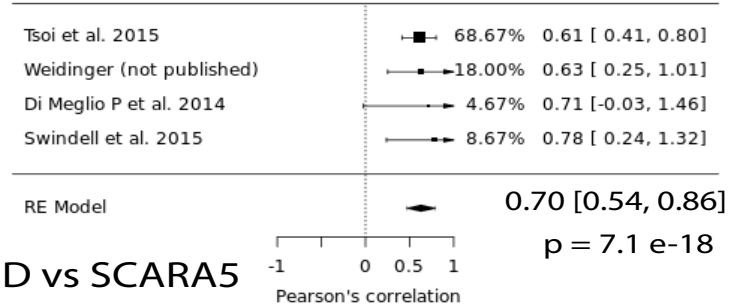
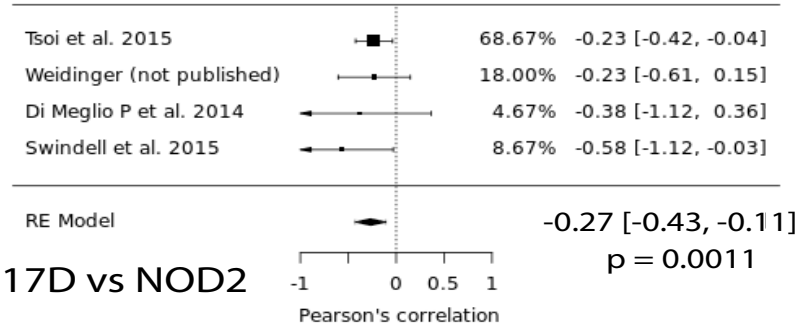


IL17D vs FAM19A5

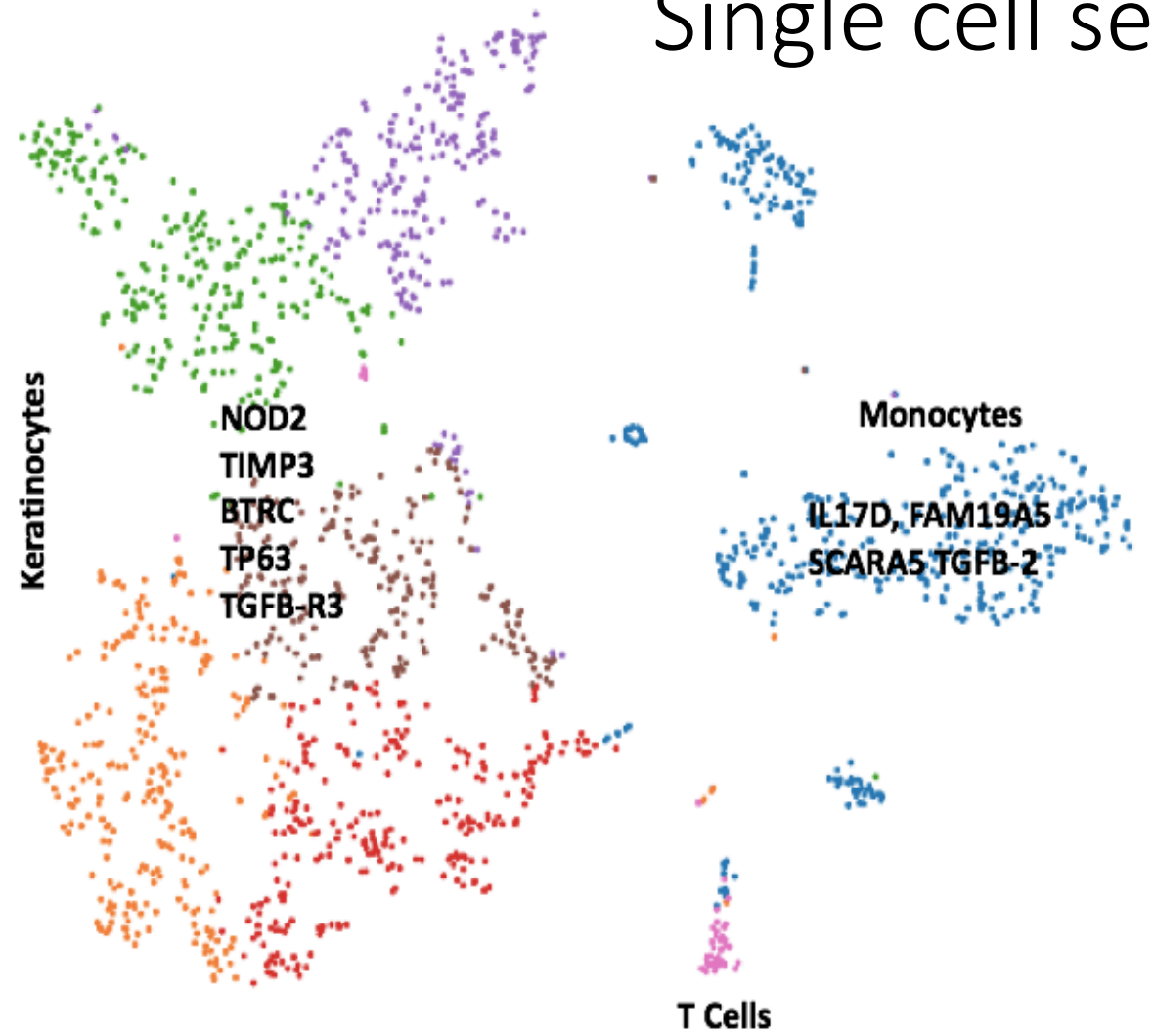


IL17D vs SCARA5

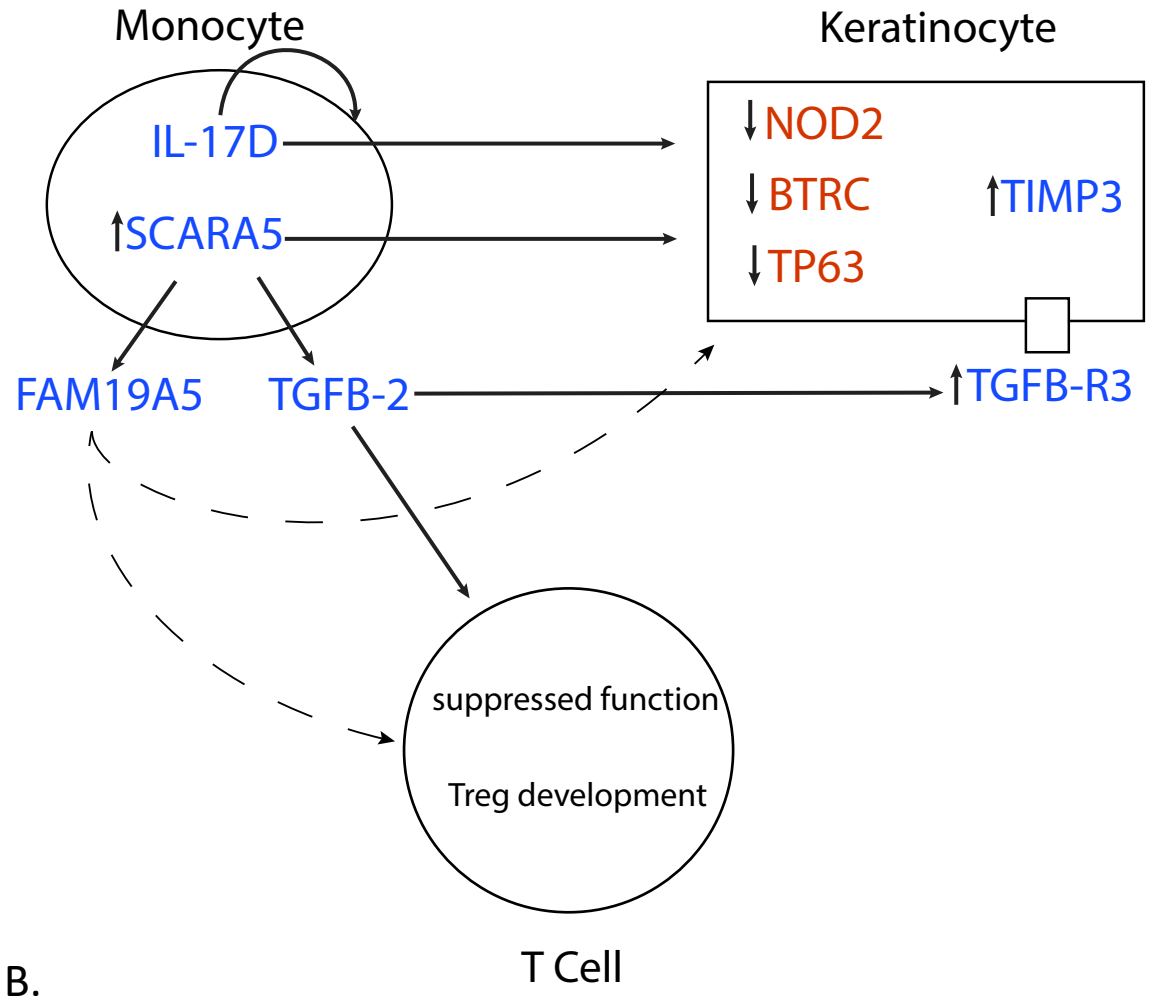


IL17D vs NOD2

Single cell sequencing data



A.



B.

Methods: Raw read data was processed using the 10x Genomics Cell Ranger and results were analyzed and visualized with 10x Genomics Loupe cell Browser.

Results: A, single cell data demonstrating clusters of genes from different cell populations. B, putative mechanism of IL-17D regulation in psoriasis. *IL17D* is expressed by monocytes and acts in an autocrine-like fashion to increase *SCARA5* expression intracellularly, and to increase FAM19A5 and TGFB-2 secretion. IL-17D then acts on keratinocytes to reduce expression of pro-inflammatory genes, *NOD2*, *BTRC*, and *TP63,* and to increase the expression of anti-inflammatory genes, *TIMP3* and *TGFB-R3*, which encodes a TGFB receptor. TGFB-2 from monocytes then interacts with its upregulated receptor on keratinocytes and separately acts on T cells to increase T regulatory cell production and to suppress T cell function, thereby reducing inflammation in psoriasis.