



Molecular differentiation trajectories of peripheral helper T cells in the chronically inflamed tissue of patients with ANA positive juvenile idiopathic arthritis



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Introduction

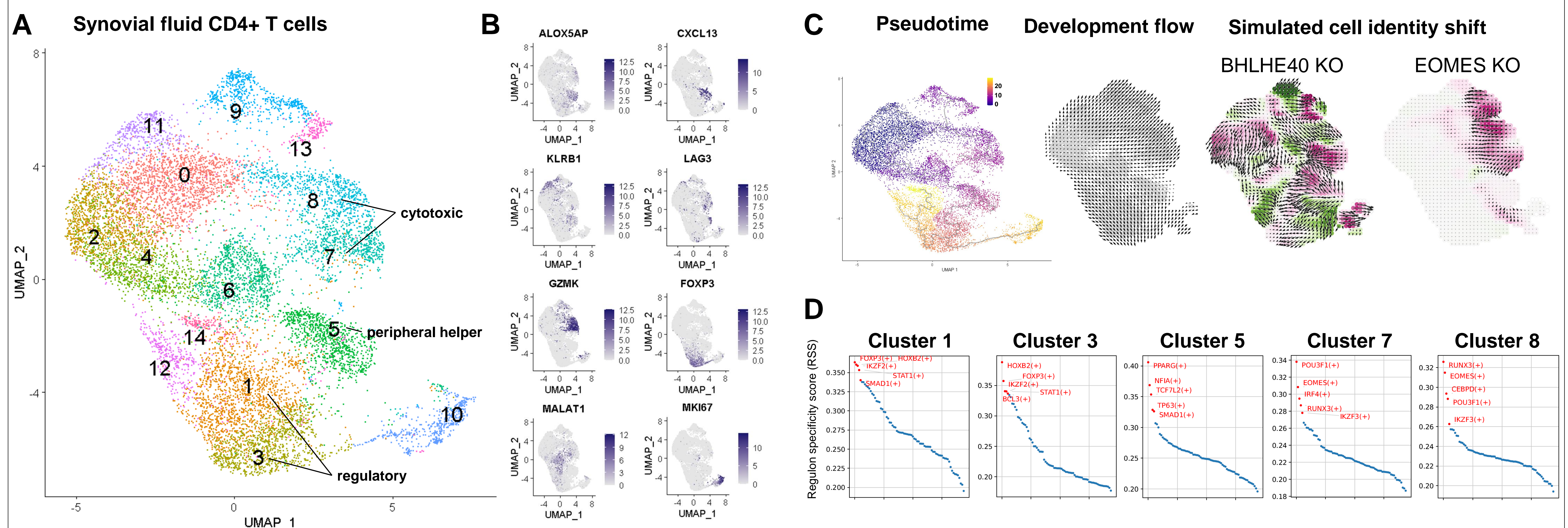
Previous research has demonstrated that antinuclear antibody (ANA)-positive juvenile idiopathic arthritis (JIA) is characterized by a synovial expansion of PD1^{high}CXCR5⁺ peripheral T helper cells (T_{PH}) that co-express interleukin (IL)-21 and interferon (IFN)- γ and can provide efficient B cell help.(1) Similar cell populations are implicated in the pathogenesis of several autoimmune diseases.(2) However, the molecular mechanisms underlying the differentiation of this T cell population remain poorly understood.

Objectives and Methods

To elucidate the differentiation trajectories and identify key transcription factors that induce a TPH-like effector program in juvenile idiopathic arthritis, we conducted a comprehensive analysis of T helper cells in the synovial fluid from 4 JIA patients using single-cell RNA sequencing. *In silico* perturbation simulations were performed to investigate potential key molecules driving TPH differentiation. Naïve T helper cells from peripheral blood were stimulated in the presence of recombinant cytokines (TGF β , IL12, IFN α , IL1). Cell phenotype, cytokine profiles and transcriptome signature were assessed using flow cytometry, ELISA and bulk RNA sequencing. The CRISPR-Cas9 system was employed via electroporation to knockout potential regulators of TPH differentiation.

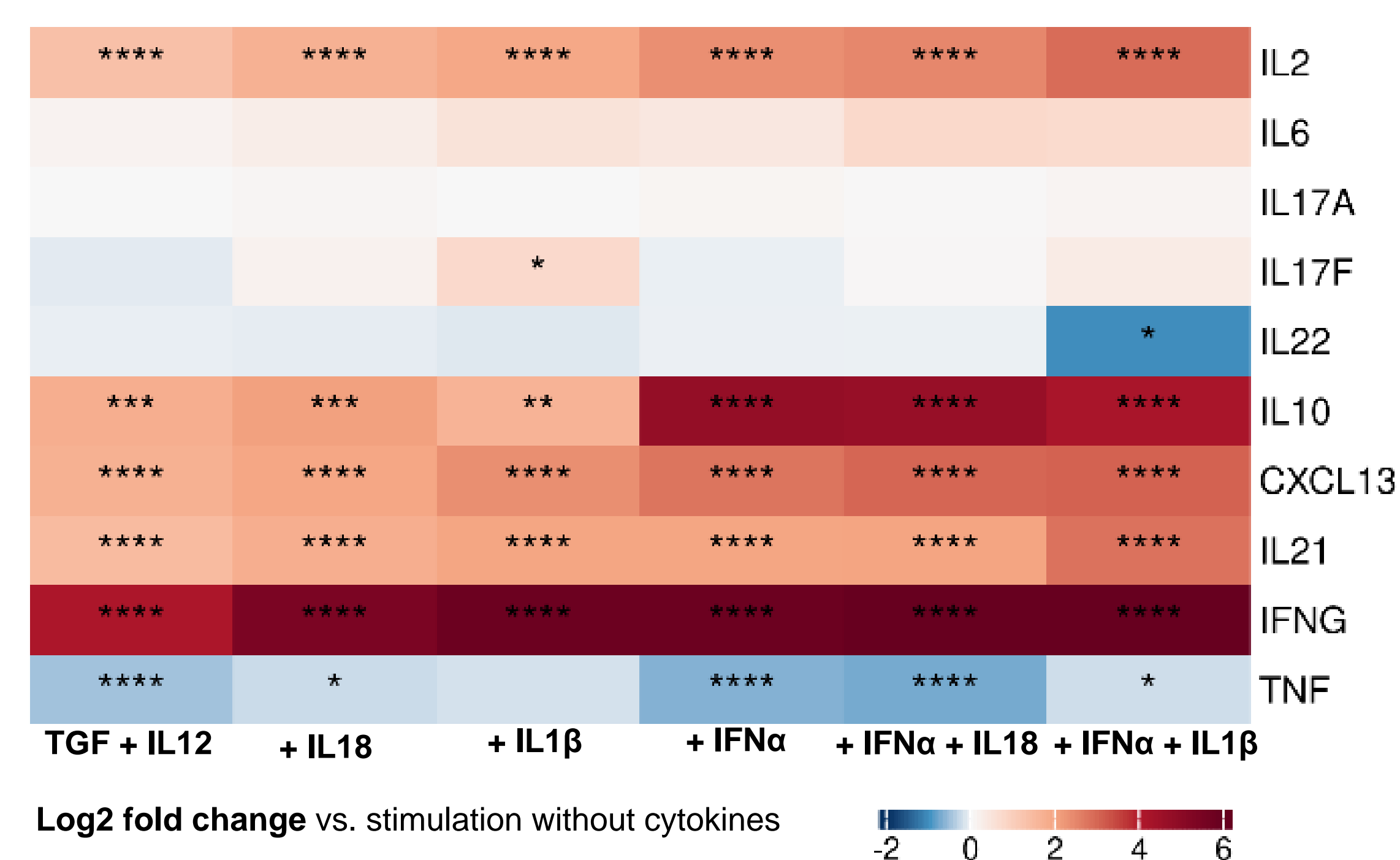
Results

1. Single cell RNA sequencing reveals potential drivers of TPH differentiation



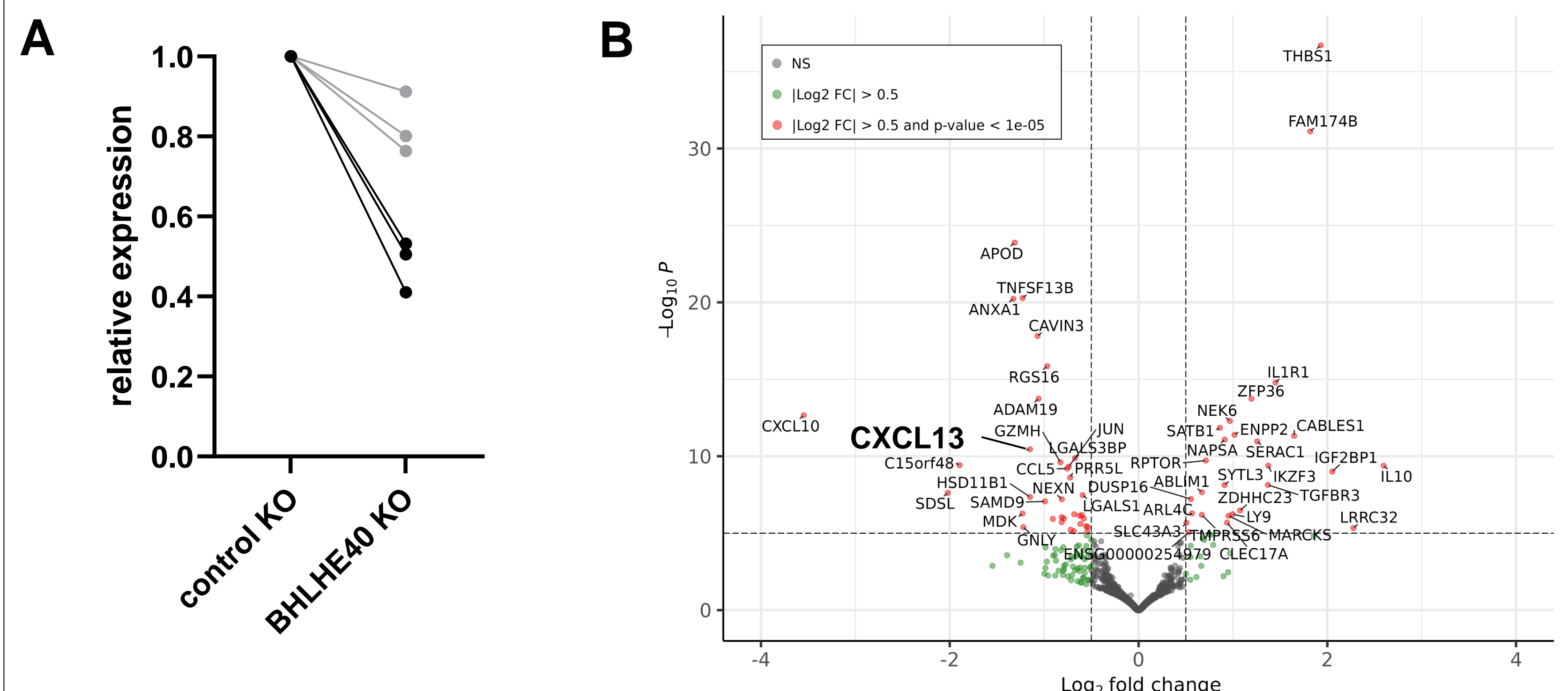
A Umap representation of transcriptomic clusters after integration of scRNA seq data of SF CD4⁺ T cells of 4 oligoJIA patients. **B** Marker gene expression in the clusters from A. **C** *in silico* perturbation analysis using CellOracle. Arrows indicate predicted identity shift in response to knockout, red color indicates shift direction against development flow. **D** most specific regulons for selected clusters from A. Regulon specificity scores were calculated using SCENIC.

2. TGF β , IL12 and IFN α induce TPH effector cytokines



Naive T helper cells were stimulated with plate-bound CD3 and CD28 antibody in presence of TGF β and IL12 with and without IFN α , IL18 or IL1 β for 5 days. Heatmap showing cytokine mRNA expression as log2 fold change compared to „neutral“ stimulation without addition of recombinant cytokines

3. BHLHE40 Knockout leads to decreased expression of CXCL13 in CD4⁺ cells



Naive T helper cells were stimulated for 24h with plate-bound CD3 and CD28 antibody before CRISPR-Cas9 mediated knockdown of BHLHE40 via electroporation. **A** relative expression of BHLHE40 5 days after CRISPR-Cas9-editing in BHLHE40 knockout vs. control knockout measured by qPCR. Black data points indicate decrease by > 40% as quality cutoff for RNA sequencing **B** Volcano plot showing differentially up- (right) or downregulated (left) genes in BHLHE40 knockout compared to negative control knockout by bulk RNA sequencing.

Conclusion

- Single cell RNA sequencing revealed BHLHE40 among others as potential key regulator of TPH differentiation.
- TGF β , IL12 and IFN α in combination induce TPH effector cytokines CXCL13, IL21 and IFN γ
- Knockout experiments suggest that BHLHE40 positively regulates CXCL13 expression.