Supplemental Materials

Supplemental Methods and Figure Legend

Sex as a biological variable. Our study recruited both male and female participants. For serum protein analysis, healthy controls of both male and female participants were compared to serum samples taken from RIME patients. Serum samples for 8 healthy controls were included from 3 male and 5 female participants ranging from age 4 to 60 years of age. Single-cell RNA sequencing of PBMC was performed on an individual female patient; a sample was obtained after recovery to allow for this patient to serve as their own control.

Demographic information. Demographic information from patients was reported in compliance with NIH guidelines. Information collected was self-identified by patients or their guardians. Multiple designations were permitted.

Study approval. Samples were obtained from RIME patients. All participants, or parents or legal guardians if the patient was a minor, provided written informed consent under protocols approved by the UCSD Institutional Review Board and performed in accordance with the ethical standards set in the 1964 Declaration of Helsinki and its later amendment. Written informed consent was obtained for clinical photographs. UCSD IRB protocol #804364.

Skin biopsy. 3 mm punch biopsies were performed of the lip and thigh lesions and preserved in 10% formalin solution and prepared and stained using standard histopathologic processing for clinical practice. Photomicrographs were obtained.

PBMC and plasma isolation. Whole blood samples were collected using EDTA anticoagulant tubes. The active sample was collected during active RIME, prior to initiation of immunomodulatory therapy. The recovered sample was collected 6 months after hospital discharge and after resolution of all clinical symptoms of RIME. 1 mL of blood was centrifuged at 400 x g for 10 minutes to obtain plasma, which was stored at –80C and later analyzed with NOMIC Bio multiplex protein profiling. 1 ml of blood was taken for PBMC isolation using SepMate™ tubes and STEMCELL Technologies Lymphoprep™ protocol. Cells were then centrifuged at 400 x g for 5 minutes and resuspended in FBS with 10% DMSO for cryopreservation.

BD Rhapsody. Cryopreserved PBMCs from active and recovered RIME samples were processed simultaneously for loading onto a single-lane BD Rhapsody Cartridge and cell capture using Enhanced Cell Capture Beads V1 (BD Biosciences, cat# 664887). Reagent preparation and cartridge priming were conducted as per manufacturer's protocol prior to thawing cells.[+] All centrifugation steps were conducted at 400 x g for 5 minutes at 4°C.

From each thawed sample, $5x10^5$ cells were taken for staining with the BD Sample Tag multiplexing kit for human cells (BD Biosciences, cat# 633781). Cells were incubated for 30 minutes on ice and washed twice with BD Stain Buffer (BSA) (BD Biosciences, cat# 554657). Samples were then resuspended in 620µL of Sample Buffer (BD Biosciences, Cartridge Reagent Kit, cat#633731) and stained using viability dyes Calcein AM at 2mM and Draq7. Each sample was counted with the BD Rhapsody Scanner then pooled together targeting 10,000 live captured cells per PBMC sample and final cartridge loading volume of 380µL. Subsequent processing of cells once loaded onto the cartridge and downstream library preparation was performed in accordance with BD Rhapsody Human TCR/BCR + WTA + Sample Tag Library Preparation Protocol.[+] AMPure® XP magnetic beads were used for PCR cleanups (Beckman Coulter, cat# A63880) and libraries were dual indexed with Illumina TruSeq and Nextera primers. Quality

check of indexed libraries was done using the Invitrogen Qubit™ dsDNA HS Kit and Agilent High Sensitivity D5000 Tapestation kit.

Sequencing. The Whole transcriptome analysis (WTA) and Sample Tag libraries for this cartridge were pooled and sequenced alongside other BD Rhapsody libraries by the IGM Core at UCSD. We targeted 3.4 billion reads per lane plus a 3% PhiX spike-in. All libraries were diluted down to 5mM then pooled to a final volume of 50µL. The volume of each library was calculated by multiplying the captured cells Scanner metric, in this case 31,760, to the target number of reads per cell (50,000 for WTA and 3,000 for sample tag) and dividing by the lane capacity: (captured cells * target reads/cell) / $1.4x10^9$) * 50µL. Once pooled, samples were further quality checked with qPCR and sequenced on the Illumina NovaSeq X 25B flow cell with run configuration paired end 150 bp reads and loading concentration 100pM.

Sequencing Data Preprocessing. Raw sequencing files were uploaded to the cloud platform Seven Bridges (https://www.sevenbridges.com). WTA files were trimmed using the Cutadapt app v1.18 trim function (DOI:10.14806/ej.17.1.200). [+++] Parameters were set as follows: -e 0.1 -n 5 -O 20, --quality-base=33, the adapter file for argument -a was set to a fasta file with 10 repeating base pairs of each nucleotide, R1 and R2 reads were trimmed separately --length 51 and --length 71 respectively in order to mimic the BD recommended run configuration.[+] Sample tag and trimmed WTA files were input to the app BD Rhapsody™ Sequence Analysis Pipeline v2.2.1.[++] The reference input was set to the manufacturer-provided file RhapRef_Human_WTA_2023-02.tar.gz made from hg38 and GENCODE v43. App settings were as follows: Sample Tags Version set to “Single-Cell Multiplex Kit – Human" and Exact Cell Count set to 31,760. The output Seurat object file was used for downstream analysis.

[+] New protocol with 51x71bp BD Rhapsody™ System TCR/BCR Next, Targeted mRNA, and Sample Tag Library Preparation Protocol 23-24505(01)

[++] BD Rhapsody™ Sequence Analysis Pipeline 2.2.1 https://bd-rhapsody-bioinfo-docs.genomics.bd.com

[+++] Cutadapt https://doi.org/10.14806/ej.17.1.200

Single-cell clustering and cell type annotations. Single-cell RNA-seq data was processed using the R package Seurat v5.0.1 (5). To eliminate low quality cells and doublets, cells expressing < 250 or > 3000 unique genes or > 6000 total unique molecular identifiers or with > 20% of reads mapping to mitochondrial transcripts were removed. The transcriptome data was log transformed, normalized by a factor of 10,000 per cell and scaled using Seurat default settings. Principal component analysis was performed using the 2000 variable genes on scaled counts. Samples from active disease and upon recovery were integrated using the *IntegrateLayers* function with Harmony as the integration method. Cells were clustered using the *FindNeighbors* and *FindClusters* functions, with a resolution of 0.1. Clusters were annotated using canonical gene markers for each cell type. Monocyte and T cell clusters were subsetted and re-clustered independently using the same procedure, with clustering resolutions of 0.2 and 0.5, respectively. Monocyte T cell complexes and monocyte B cell complexes were identified based on the expression of both monocyte and T/B cell genes (Supplemental Figure 2B) (6,7). Clusters containing < 50 cells were excluded from downstream analysis.

Differential gene expression analysis of single-cell data. Differential gene expression analysis of pseudo-bulk data was performed using the R package edgeR v4.0.12 (8). Raw counts from single cell RNA-seq data were used to estimate the normalization factor using the *calcNormFactors* function. The cellular detection rate (CDR) was calculated from the feature-by-cell matrix of raw counts as the fraction of measured features with a count > 0 in each cell, yielding values between 0 and 1. The CDR, along with the group (disease stage) information, was included as a covariate in the model matrix. Dispersion

estimates were obtained using the *estimateDisp* function, and differential gene expression analysis was conducted using a generalized linear model (GLM) with quasi-likelihood F-test, implemented in the *glmQLFit* and *glmQLFTest* functions. Genes with a false discovery rate (FDR) < 0.05 and an absolute $\log_2$ fold change > 0.5 were considered significant.

Cell-cell communication analysis. Cell-cell interaction analysis was performed using CellPhoneDB v5 (9). Ligand-receptor (L-R) interactions between different cell populations were predicted using the statistical analysis method with default parameters. Differential L-R interactions were defined as those statistically significant (p-value < 0.05) in one disease stage but not in the other. Dot plots were generated in R v4.3.2 to visualize these differential L-R interactions.

Statistics: Statistics were carried out using specific functions in R as described. No samples were excluded from the analysis.

Study Approval: The University of California, San Diego (UCSD) Institutional Review Board has approved this study (804364).

Data availability: All data relevant to the study is readily available in supplemental documents. Single-cell RNA-seq datasets are available in the NCBI Gene Expression Omnibus (GEO GSE314040). Raw data from NOMIC Bio multiplex protein profiling is provided as an accompanying excel file in the Supporting Data Values file.

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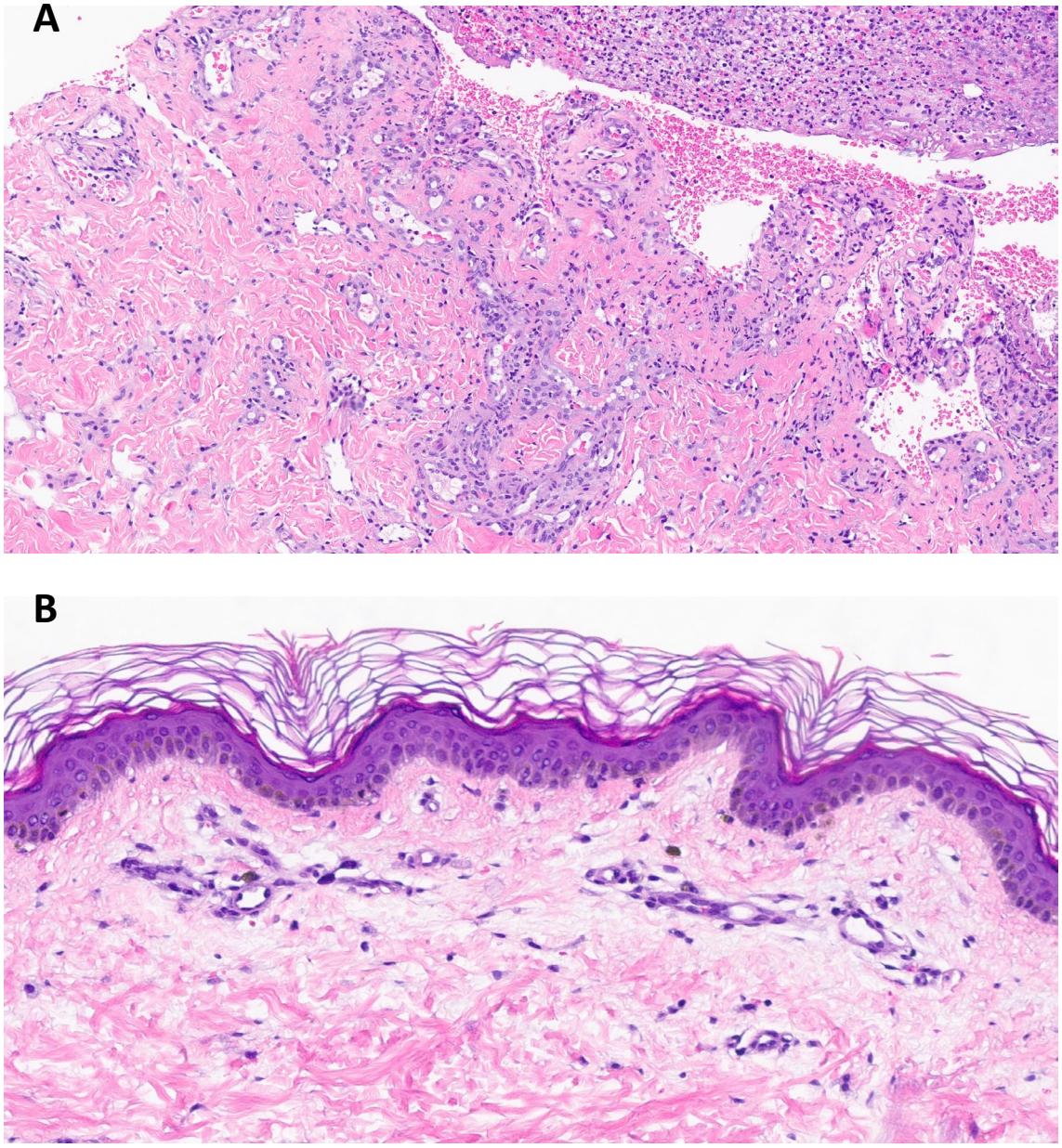
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Conflicts-of-Interest statement: RO received consulting fees from Novartis and Blueprint Medicines. HH is or has been a consultant for Novartis, Sobi, Aclaris, Ventyx and has received research funds from Jecure, Zomagen, Inapill, Takeda. LB has been a speaker for SOBI, and contracted site PI for a clinical trial with Novartis, Inc.DE has served as consultant, speaker, advisory board member, and/or clinical study investigator for Amryt Pharma/ Chiesi Farmaceutici, Apogee Therapeutics, Beiersdorf AG, Dermatology Digest LLC, Dermatology Times, Eli Lilly and Company, Galderma, Incyte, Jassen Pharmaceuticals/ Johnson and Johnson, Med-IQ LLC, Nobelpharma America LLC, Ortho Dermatologics, Pelthos, Regeneron Pharmaceuticals, Sanofi, Solici, Sun Pharmaceutical Industries, Verrica. WLT has served as a clinical trial investigator for Abbvie, Dermira, Incyte, Janssen, Eli Lilly, Pfizer, and Regeneron Pharmaceuticals, and as a Data Safety Committee Member for LEO Pharma.LG received consulting fees from ADMA Biologics.BG has served as either consultant, advisor, and/or received research funding from Sanofi, Regeneron, Pfizer, Abbvie, Leo, Lilly, Amgen, Incyte, Arcutis, Novartis, Genentech, Pharming, Grifols, Takeda, Kedrion, ADMA, Koru, Blueprint, GSK, Biocryst, Kalvista, Astria, Insmed, Kenota, DBV.DS has served as a clinical study investigator for Sanofi. LFE has served as a consultant, speaker advisory board member or investigator for AbbVie, Almirall, Amgen, Apogee, Arcutis, Attovia, Bristol Myers Squibb, Castle Biosciences, CorEvitas, Dermavant, Eli Lilly, Forte, Galderma, Incyte Corporation, Janssen, Johnson & Johnson, Kenvue, LEO Pharma, Novartis, Ortho Dermatologics, Pfizer, Regeneron, Sanofi Genzyme, Target RWE, T-Rex, and UCB.

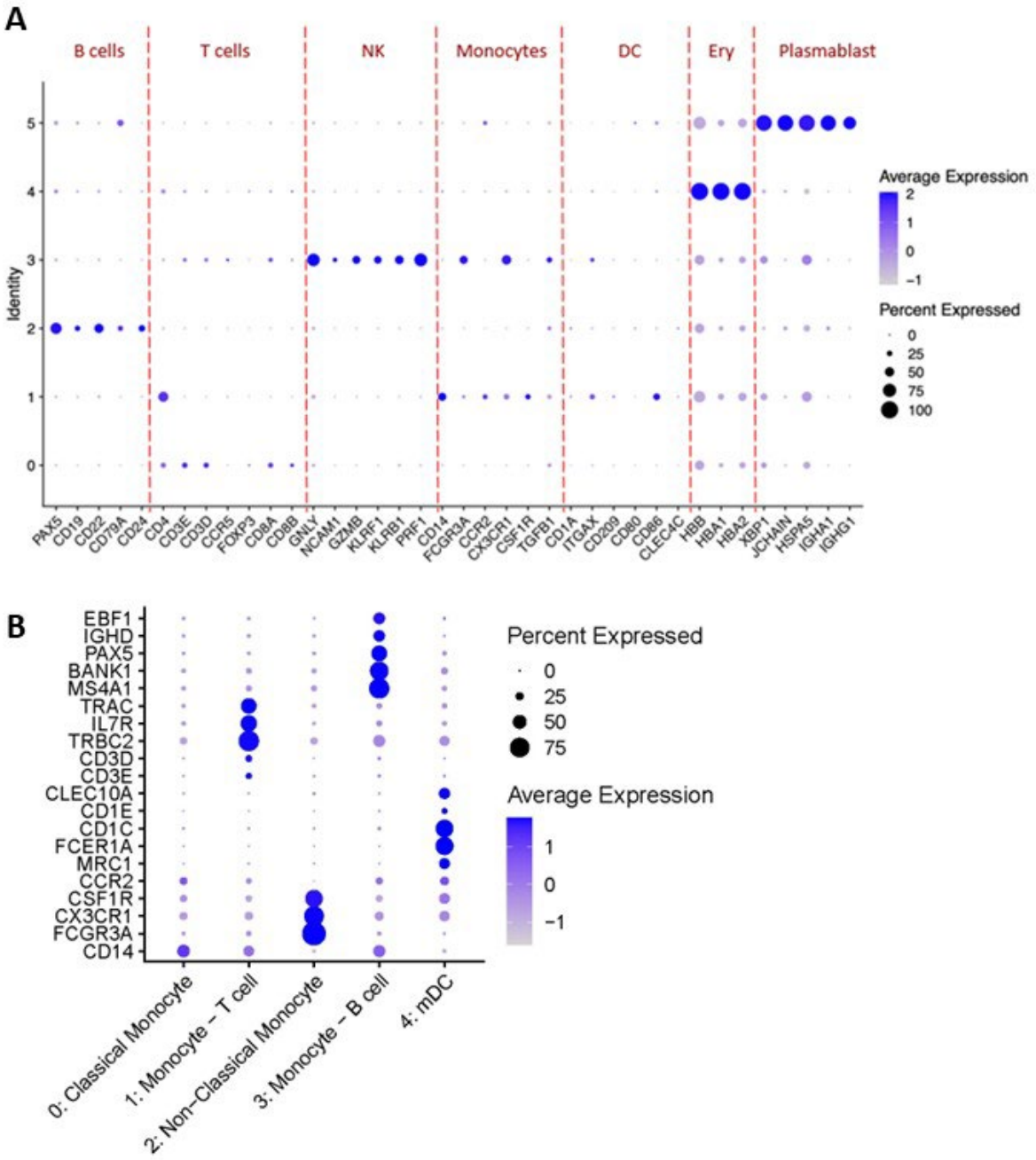
Author contributions: First authorship was assigned to ZT, GW and DF for protocol development, and to DF for development of bioinformatics pipelines and analyses. Final authorship attributed to BC for project oversight, FA for bioinformatics oversight and RO clinical and project oversight and analysis. Data were analyzed by DF, GW, FA, BC, MM, PD, ZJ, BH, RO. All authors wrote the manuscript.

Supplemental References

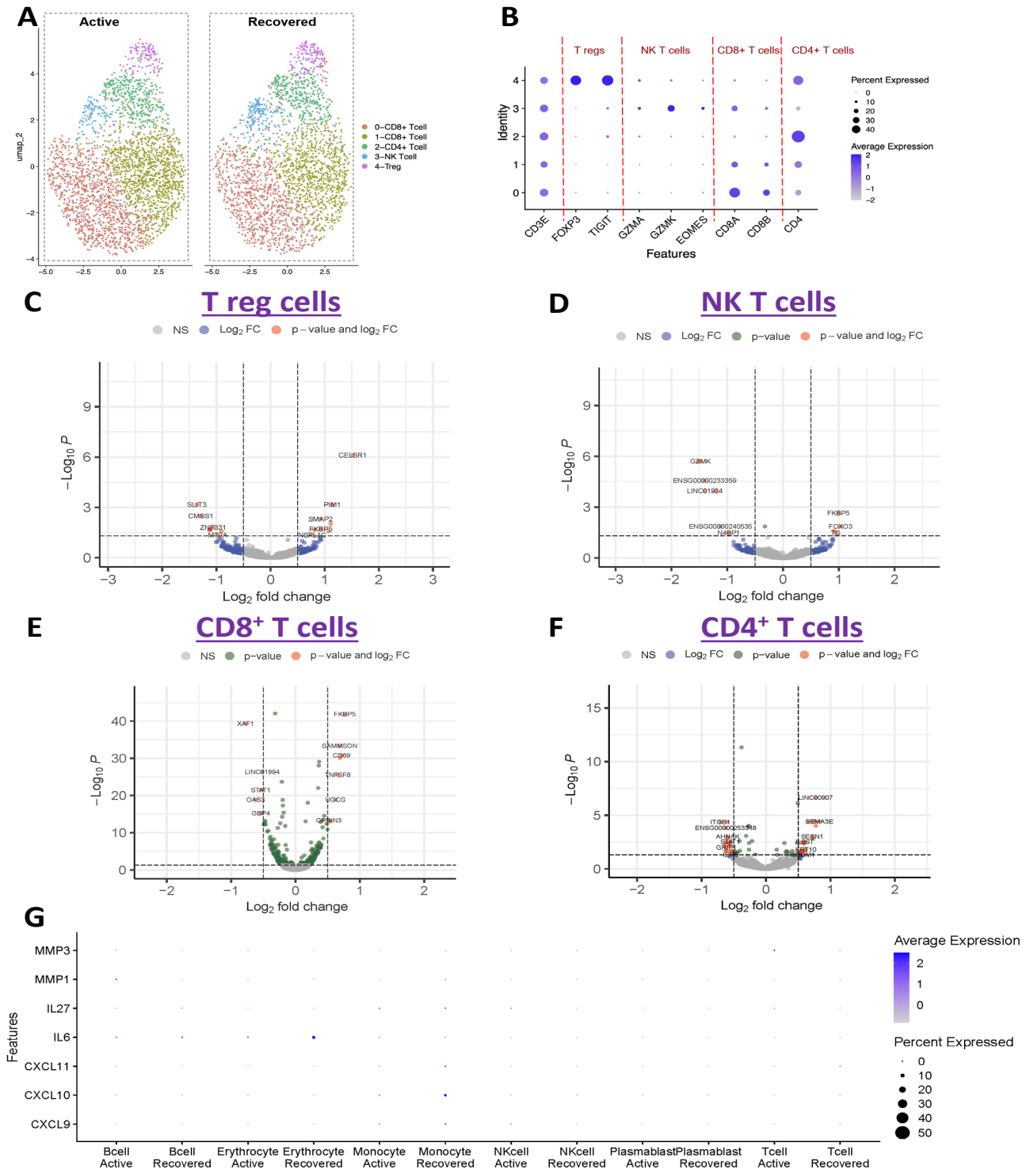
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Supplemental Figure 1. **Histopathologic evaluation of mucocutaneous lesions in RIME demonstrates mononuclear infiltrates and epidermal destruction.** (A) Hematoxylin and eosin (H&E) stain was performed on a punch biopsy from the lip. 100x magnification demonstrated detached full-thickness epidermal necrosis with a thick hemorrhagic and neutrophilic crust. The dermis was notable for a predominantly mononuclear perivascular infiltrate, lacking eosinophils. (B) Biopsy of an erythematous macule on the thigh of the same patient (H&E, 200x) revealed a sparse superficial perivascular lymphocytic infiltrate with subtle vacuolar interface dermatitis.



Supplemental Figure 2. **Canonical gene markers used to identify cell types in scRNA-seq data.** (A-B) Dot plot showing the average expression of canonical gene markers for each major cell type (A) and monocyte subclusters (B). The dot size indicates the percentage of cells expressing each marker and color indicates the normalized expression level.



Supplemental Figure 3. **Single- cell analysis of T cell subsets during active RIME and on recovery.** (A) Separated UMAP visualizations of T cell subclusters in active disease (left) and after recovery (right). (B) Dot plot showing the average expression of canonical gene markers for T cell subclusters (B). The dot size indicates the percentage of cells expressing each marker and color indicates the normalized expression level. (C-F) Volcano plots of differentially expressed genes between active disease and recovered in regulatory T cells (C), natural killer T cells (D), CD8+ T cells (E) and CD4+ T cells (F). Genes with a -log10 adjusted p-value > 1.3 (adjusted p-value < 0.05) and an absolute $\log_2$ fold change > 0.5 are highlighted in red. Statistical significance was determined using the quasi-likelihood F-test (QLF). (G) Dot plot showing the average expression of specific gene markers in all leukocytes. The dot size indicates the percentage of cells expressing each marker and color indicates the normalized gene expression level.

	RIME 1	RIME 2	RIME 3	RIME 4	RIME 5	Controls
Age (years)	18	11	10	14	14	4-60
Sex assigned at birth	M	M	F	M	F	5 Females 3 Males
Ethnicity	Hispanic, non-white	Hispanic, non-white	Filipino, non-hispanic	White, non-Hispanic	White, non-hispanic	
Fever	No	Yes	Yes	No	Yes	
Estimated BSA involvement (%)	<1	6	<1	<1	<1	
Mucosal site involvement	Mouth	Mouth, peri-urethral, peri-anal	Eyes, mouth, vaginal	Mouth, peri-urethral	Mouth	
Hospitalized	No	Yes	Yes	No	Yes	
Length of Stay (days)	n/a	6	19	n/a	5	
ICU admission	No	No	Yes	No	No	
ESR	n/a	26	17	n/a	4	
CRP	n/a	8.8	6.0	n/a	0.10	
Infectious Trigger	COVID	Unknown	Adenovirus	URI symptoms, no trigger identified	Mycoplasma, COVID	
Systemic immunomodulatory therapy	None	Etanercept 0.8mg/kg (2 doses)	Etanercept 0.8mg/kg (2 doses), methylprednisolone 1gm pulse and 7-day taper, IVIg 1gm/kg (2 infusions)	None	Etanercept 0.8mg/kg (1 dose), methylprednisolone/prednisolone taper	
Recurrent episode	Yes	No	No	Yes	Yes	
Associated findings:			Intubated, Hepatitis with resolution over 6 months			

Supplemental Table 1. Clinical characteristics and disease course of RIME patients. A total of 5 patients were recruited, comprising 3 males and 2 females with ages ranging from 10-18 years. Two patients had no identifiable trigger, 1 patient had evidence of adenovirus and rhinovirus infection, 1 patient had evidence of both COVID and *M. pneumoniae* infection, and 1 patient had evidence of COVID infection. Patients displayed a range of clinical severity and received a variety of immunomodulatory therapy. Etanercept 0.8mg/kg and IVIg 1gm/kg/infusion were used. Abbrevations: BSA, body surface area; LOS, length of stay; CXR, chest X ray; RPP, respiratory pathogen panel; BAL, bronchoalveolar lavage; ICU, intensive care unit; HSV, herpes simplex virus; PCR, polymerase chain reaction; RR, reference range.