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1 **Title:** Single cell transcriptomics reveals distinct effector profiles of infiltrating T cells in lupus
2 skin and kidney
3
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5 **Running Title:** Single-cell analysis of T cells in cutaneous lupus
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7
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33 **Abstract**
34
35 Cutaneous lupus is commonly present in patients with systemic lupus erythematosus (SLE) but
36 can also exist as an isolated manifestation without further systemic involvement. T cells have
37 been strongly suspected to contribute to the pathology of cutaneous lupus, yet our understanding
38 of the T cell phenotypes and functions in the skin in lupus remains incomplete, and the extent to
39 which lupus T cell infiltrates in skin resemble those in other tissue beds is unknown. Here, we
40 present a detailed single-cell RNA sequencing profile of T and NK cell populations present
41 within lesional and non-lesional skin biopsies of patients with cutaneous lupus. We identified
42 multiple lymphocyte clusters, including both CD4 and CD8 T cells, NK cells, regulatory T cells,
43 and a population of strongly interferon-responding cells that was present in patients with
44 cutaneous lupus but absent in healthy donors. T cells across clusters from both lesional and non-
45 lesional skin biopsies expressed elevated levels of interferon simulated genes (ISGs); however,
46 compared to T cells from control skin, T cells from cutaneous lupus lesions did not show
47 elevated expression profiles of activation, cytotoxicity, or exhaustion. Integrated analyses
48 comparing skin T/NK cells to lupus nephritis kidney T/NK cells indicated that skin lymphocytes
49 appeared less activated and lacked the expanded cytotoxic populations prominent in lupus
50 nephritis. An integrated comparison of skin T cells from lupus and systemic sclerosis revealed
51 similar activation profiles but an elevated ISG signature specific to cells from lupus skin
52 biopsies. Overall, these data represent the first detailed transcriptomic analysis of the of T and
53 NK cells in cutaneous lupus at the single cell level and have enabled a cross-tissue comparison
54 that highlighted the stark differences in composition and activation of T/NK cells in distinct
55 tissues in lupus.

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56 **Introduction**

57 Systemic lupus erythematosus (SLE) is a highly heterogenous disease, with the potential
58 to manifest an array of pathologies across the body (1). Around 70% of individuals affected by
59 SLE have cutaneous involvement, known as cutaneous lupus erythematosus, though such
60 manifestations can also occur without further systemic symptoms (2). To date, no therapies
61 specifically aimed at cutaneous lupus have been approved, and the current standard of care
62 generally relies on topical corticosteroids and calcineurin inhibitors to attempt to address
63 symptoms (3). A deeper understanding of the cells implicated in cutaneous lupus, both with and
64 without broader systemic involvement, will be beneficial to the development more efficacious
65 and targeted therapies.

66 T cells are suspected to play a major role in lupus pathology. Expanded populations of
67 CD4 T follicular helper (Tfh) and T peripheral helper (Tph) cells promote B cell activation and
68 autoantibody production (4–7). Work analyzing the role of regulatory T (Treg) cells in lupus has,
69 on the other hand, been more contentious with conflicting results suggesting both increased and
70 decreased presence in the disease (8–12). Single cell transcriptomic analyses of lupus nephritis
71 (LN) kidney biopsies have suggested a role for cytotoxic T cell subsets in the kidneys of affected
72 patients, with populations of NK cells, cytotoxic T cells, and Granzyme K+ CD8 T cells all
73 being highly represented among lymphocytes in kidney biopsies (13). Further, histologic
74 analyses of cutaneous lupus biopsies identified heterogenous staining of granzyme B+ T cells in
75 cutaneous lesions (14). The extent to which T cell infiltrates within different target tissues in
76 lupus, for example skin and kidney, demonstrate similar effector phenotypes, remains unclear.

77 Advances in single-cell RNA sequencing (scRNA-seq) technologies have allowed for
78 the high-throughput generation and analysis of cellular states in disease and homeostasis (15,

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79 16). To date, these tools have been applied to multiple rheumatic diseases, including lupus (17).
80 Previous studies in lupus have produced single-cell catalogs of the cell states present in kidney
81 biopsies of patients with LN and in PBMCs of patients with SLE (13, 18, 19). These scRNA-seq
82 datasets have served as a foundation for a better understanding of the cell types relevant to lupus
83 pathology in these tissues. A single-cell transcriptomic analysis of cutaneous lupus could
84 likewise help to reveal insights into the pathogenesis of the disease and serve as a means to
85 compare the role of a particular cell type, such as T cells, across multiple tissues of lupus
86 pathology.

87 Here, we report the first detailed transcriptomic evaluation of the T and NK cells in
88 cutaneous lupus lesions at the single-cell level. With paired lesional and non-lesional skin
89 biopsies from patients with cutaneous lupus, as well as skin biopsies from healthy donors, we
90 define and compare the T cell populations present across these samples. Further, we employ a
91 previously published dataset of kidney biopsies in LN patients (13) to perform an integrated
92 analysis of the T cell states present across both pathologic kidney and skin. Lastly, we perform a
93 comparison of skin T cells between cutaneous lupus and systemic sclerosis (SSc) patients,
94 providing a cross-disease examination of the role of T cells across different rheumatic skin
95 pathologies (21). Combined, these analyses reveal both parallels and distinct differences between
96 the T cells in skin and kidney in lupus, suggesting that the effects of therapeutic targeting of T
97 cells may differ in different target tissues.

98 **Results**

99 **Single cell transcriptomic identities of skin-localized T cells in cutaneous lupus**

100 Skin biopsies were obtained from both lesional and non-lesional locations on 6 SLE
101 patients and an additional patient with isolated cutaneous lupus, as well as 14 healthy controls

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102 (Table S1). Biopsies were dissociated and droplet-based scRNA-seq was used to obtain the
103 transcriptomes of T cells. Following the application of quality control filters, we obtained a total
104 of 3,499 T and NK cells for further analysis (Fig. 1A). Among these, we broadly identified
105 populations of CD4+ T cells, CD8+ T cells, and NK cells (marked by *TYROBP* expression) (Fig.
106 1B). Further analysis identified 13 subclusters, which largely appear independent of any batch
107 effects (Fig. 1C and S1).

108 We next set out to define the unique transcriptional programs of each subcluster (Table
109 S2). Across subclusters containing CD4+ cells, conventional memory T cells accounted for 3 of
110 these clusters (#0, 1, and 5). FOXP3+ Treg cells were found across 2 clusters, both sharing
111 expression of *CTLA4*, *ICOS*, and *CD27* (#4 and 10). Notably, the Tregs associated with cluster
112 #4 were found to have stronger expression of *CTLA4*, *TIGIT*, and *ICOS* than those in cluster #10,
113 while those in cluster #10 were marked by higher expression of *CD27*. The differing expression
114 profiles of these Treg clusters suggest that those cells contained in cluster #4 belong to a more
115 activated and suppressive subset (22). In addition, we noted a population of Tfh and/or Tph cells,
116 defined by the absence of *FOXP3* and the expression of markers such as *CXCL13*, *PDCD1*,
117 *ICOS*, and *MAF* (Fig. 1D).

118 Across the NK and CD8+ T cell clusters identified in these biopsies, tissue-resident
119 memory CD8+ T (Trm) cells, identified by expression of markers such as *ZNF683* and *XCL1*,
120 formed 2 clusters (#2 and 8). A population of CD8+ T cells marked by the strong expression of
121 *GZMK* but relative absence of *GZMB* was identified (#3), consistent with a phenotype previously
122 recognized in LN kidneys and rheumatoid arthritis synovium (13, 23). In contrast, cluster #7
123 expressed higher levels of *GZMB* compared to cluster #3, suggesting a cytotoxic T lymphocyte
124 (CTL) phenotype. Clusters #3 and #7, though, were both found to similarly express the cytokine

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125 *IFNG* and chemokines *CCL4* and *CCL5*. Aside from these CD8+ clusters, 2 subclusters of NK
126 cells were identified in the dataset (#9 and 11). While both share a similar core transcriptional
127 program composed of markers such as *KLRB1*, *TYROBP*, and *NKG7*, the NK cells of cluster #9
128 are largely differentiated by stronger expression of *XCL1*, suggestive of a CD56^{bright} NK cell
129 population, while those of cluster #11 overexpress *PRF1*, multiple granzyme genes, and *CCL5*,
130 likely representing a CD56^{dim} population (24).

131 In addition, a cluster marked by the expression of interferon-stimulated genes (ISGs)
132 such as *IFIT1*, *IFIT3*, *IFI44*, *OAS1*, and *LY6E*, among others, was identified. This population of
133 cells appears to be a mixture of CD4+, CD8+, and CD4-CD8- cells, likely comprising cells from
134 the other major subclusters (Fig. 1D). This ISG-enriched cluster is consistent with previously
135 published scRNA-seq studies of the T cells in nephritic kidney, blood, and skin of SLE patients,
136 further highlighting a conserved interferon signature across cell types and tissues in SLE (13, 19,
137 20).

138 **Similarly elevated interferon-response signature in T cells at both lesional and non-lesional**
139 **sites in cutaneous lupus**

140 We next sought to determine if certain cell subsets were differentially represented among
141 the lesional, non-lesional, and control samples obtained by this study. Of the 3,499 T and NK
142 cells sequenced, 2,116 and 1,383 were collected from cutaneous lupus patients and healthy
143 controls, respectively. In total, 687 cells were obtained from the lesional biopsies of these lupus
144 patients, and 1,429 were sequenced from their paired non-lesional samples. Accounting for
145 differences in cell numbers for each sample type, we identified the ISG-high cluster to have
146 greater representation in the lupus patient samples compared to healthy controls (Fig. 2A and B).

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147 The cluster of ISG-high T cells was nearly exclusively represented by cells from the
148 cutaneous lupus samples, including cells from both lesional and non-lesional samples (Fig. S2).
149 In addition, an elevated ISG transcriptional signature was seen across clusters in both the lesional
150 and non-lesional samples compared to controls (Fig. 2C). This is consistent with previous studies
151 that have identified a conserved elevation in type I interferon-regulated genes across patients
152 with cutaneous lupus erythematosus and SLE (25–27).

153 We next sought to use the transcriptomic data to compare the functional status of T cells
154 in cutaneous lupus and healthy skin biopsies. For this effort, we generated activation-,
155 cytotoxicity-, and exhaustion-relevant gene lists and calculated signature scores for lesional, non-
156 lesional, and healthy controls (Table S3). Surprisingly, T cells from cutaneous lupus lesions and
157 non-lesional sites appeared quite similar to T cells from control skin across these measures,
158 suggesting a lack of wide-scale activation in T cells within cutaneous lupus skin lesions (Fig.
159 2C).

160 A focused analysis of the expression of key cytotoxic genes specifically within the CTL
161 cluster similarly revealed no expression differences (Fig. 2D). In contrast, a focused analysis on
162 the Tph/Tfh cell cluster, a population of cells strongly implicated in pathologic T/B cell
163 interactions in SLE, revealed some differences. Notably, we found an upregulation of
164 costimulatory genes such as *ICOS* and *TIGIT* in Tph/Tfh cells from cutaneous lupus patients
165 compared to their healthy control counterparts, and further noted an upregulation of HLA-DRA
166 and the transcription factor *MAF*, which has been demonstrated to promote Tph/Tfh cell function
167 (Fig. 2E) (13).

168 Altogether, these data highlight the systemic nature of detection of interferon response
169 genes in T cells of the skin and suggest a potential increase in activity of the B cell-helping

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170 Tph/Tfh cells, but otherwise indicate limited features of activation or cytotoxicity in skin-
171 localized T cells in cutaneous lupus.

172 **Low cytotoxicity and effector signatures in T cells from cutaneous lupus compared to lupus**
173 **nephritis**

174 To better understand our results in the broader context of lupus, we sought to define
175 conservation and potential differences across T cells found at different tissue sites in SLE. To
176 accomplish this, we performed an integration of our dataset with T cells from a previously
177 published scRNA-seq dataset of immune cells obtained from kidney biopsies of patients with LN
178 (25). Within this dataset, we isolated 1719 T and NK cells from 24 patients with LN. Following
179 integration of these datasets using canonical correlation analysis (CCA), we produced a single
180 unified visualization of data from both tissues (Fig. 3A). Cells of the same subset largely
181 clustered together irrespective of tissue origin, and all of the T cell types were found in both
182 tissues, including Trm CD8+, Tregs, cytotoxic CD8+, Tph/Tfh cells, and NK cells (Fig. 3B,C).

183 When comparing representation of these cell states across tissues, we noted an increased
184 proportion of memory T cells in the skin of cutaneous lupus patients, including CD4 memory
185 and CD8 Trm subsets, along with a larger percentage of cells in the ISG elevated cluster, when
186 compared to kidney samples of LN patients. Conversely, we observed a strongly increased
187 representation of cells with cytotoxic function, including CD8+ and NK clusters, in samples
188 obtained from the kidneys of LN patients (Fig. 3D).

189 Gene-level examination of cytotoxic marker expression between cutaneous lupus and LN
190 further highlighted this difference between T cells from the different tissues, with LN T cells
191 having increased expression of genes associated with cytotoxicity, including *GZMB, GZMH,*
192 *PRF1,* and *GNLY* (Fig. 3E). In comparison, ISGs showed similar expression levels between

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193 cutaneous lupus and LN T cells, indicative of the systemic interferon response in lupus. Along
194 with an increase in cytotoxicity genes, T cells from LN kidneys also showed an increased
195 activation signature score across clusters compared to T cells from skin (Fig. 3F).

196 **Elevated IFN signatures in skin-localized T cells from lupus compared to systemic sclerosis**

197 To further extend our exploration of the T cells present in cutaneous lupus, we sought to
198 compare our data to T cells from the skin of another rheumatic disease. A recent study focused
199 on systemic sclerosis (SSc) obtained skin biopsies from 27 patients with SSc and an additional
200 10 healthy controls (21, 28). After isolating T cell transcripts from this data, we integrated the
201 dataset with our dataset of cutaneous lupus T cells, providing a unified visualization of both sets
202 in the same UMAP space (Fig. 4A). Similar to our results upon integrating data from skin T cells
203 with data from kidney T cells in lupus, we observed the presence and co-clustering of all major
204 cell types described above in T cells from both systemic sclerosis skin and lupus skin samples
205 (Fig. 4B).

206 Comparing the distribution of cells from each disease for each cluster, we noted a
207 deficiency of cells from SSc samples in the cluster associated with the strongest ISG signature
208 (Fig. 4C). Examination of the sample groups and healthy controls revealed that while there is a
209 significant increase in ISGs comparing T cells from control skin and T cells from cutaneous
210 lupus lesional and non-lesional sites, no such difference exists between SSc samples and their
211 respective healthy controls (Fig. 4D). A heatmap analysis of the expression patterns of multiple
212 ISGs in cutaneous lupus and SSc T cells further corroborated this finding, suggesting that IFNs
213 more strongly influence the cutaneous T cell response in lupus than in SSc (Fig. 4E).

214 We them aimed to profile differences in the activation and effector function of T cells
215 between cutaneous lupus and SSc. Through an examination of signature scores, our analysis

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216 noted no differences in these scores between the cutaneous lupus and SSc components of each
217 identified cluster (Fig. 4F). Lastly, we sought to compare the Tph/Tfh cluster, which is marked
218 by *CXCL13+* CD4 T cells in cutaneous lupus and SSc. While ISGs represented many of the most
219 upregulated genes in cutaneous lupus, we also noticed an upregulation of multiple activation and
220 exhaustion genes. Notably, we found higher levels of *PDCD1*, *TOX*, *LAG3*, *TNFRSF18*, and
221 *MAF* on average in T cells from cutaneous lupus samples compared to SSc samples (Fig. 4G).
222 Together, these results emphasize the strength of interferon-response in lupus, even in
223 comparison with another rheumatic disease, and suggest an increased activity of B cell-helping T
224 cell subsets in cutaneous lupus compared to SSc.

225 **Discussion**

226 This study represents the first in-depth examination of skin-localized T cells in cutaneous
227 lupus using single-cell transcriptomics. Our analysis revealed deep transcriptional heterogeneity
228 within the T cells collected from the skin of these patients, including subsets of CD4 and CD8
229 memory T cells, Tregs, cytotoxic T cells, helper T cells, and others. One of the defining
230 characteristics in our comparison of T cells from lupus patients and healthy donors is the
231 significant upregulation of ISGs. Though the role of IFN is increasingly well-understood in the
232 pathogenesis of lupus (28), this study furthers the notion of systemic effects of this signaling that
233 can be detected across tissues. Our results demonstrate robust upregulation of ISGs in T cells of
234 both the kidney and skin of lupus patients, far exceeding that in T cells from both healthy skin
235 and skin from SSc patients.

236 Aside from strong differences related to interferon response, we noted surprisingly little
237 transcriptional evidence of increased activation or effector function engagement when comparing
238 T cells from cutaneous lupus skin to those from healthy skin. In contrast, when comparing T

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239 cells from the kidneys of lupus patients with T cells from the skin of lupus patients, our analysis
240 suggests that the T cell infiltrates differ substantially at these two tissue sites. We noted a marked
241 increase in T cells associated with cytotoxic function, and likewise observed an increase in
242 expression of effector function-related genes, in T cells from LN kidneys. These findings suggest
243 a different role of T cells in the pathology of lupus in disparate tissues, whereby T cells in organs
244 such as the kidney may mediate cytotoxic effects that contribute to tissue injury, while T cells in
245 the skin may contribute alternative functions, including B cell help, or may be primarily
246 bystanders.

247 There are several limitations that raise caution about this interpretation of the data. First,
248 the total number of lesional T cells analyzed is limited. Second, it is possible that T cell
249 activation signatures are downregulated during the longer processing time required for isolation
250 of T cells from skin. However, the robust detection of ISGs suggests that at least some of the
251 disease-associated transcriptomic signatures are retained during processing. Third, it is possible
252 that activated T cells are inefficiently collected or preferentially depleted during tissue
253 processing, or that the activated cells represent a small minority of the total cells that is not well
254 visualized in our analyses. Further analyses by complementary approaches will be helpful to
255 address these considerations. Yet taken together, the striking differences between transcriptomes
256 of T cells from kidney and skin appear to suggest substantial differences in their functions.

257 Notably, our analysis of the Tph/Tfh cells of cutaneous lupus patients suggests an
258 increased activation of Tph/Tfh cells obtained from lesional and non-lesional lupus skin
259 compared to control skin or SSc skin. These cells may have a role in directing B cell responses
260 within the skin. However, the frequency of Tph/Tfh cells in cutaneous lupus lesions is low and
261 not clearly different from controls; thus, it is also possible that these cells in skin are a reflection

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262 of the activated circulating Tph/Tfh cell populations in SLE patients (29). The findings related to
263 this cell subset and their potential role in the skin of lupus patients warrant further examination
264 with larger populations of isolated cells.

265 The search for effective and targeted therapies for lupus remains difficult, and certain
266 gaps in our knowledge of the diseases pathogenesis across tissues remain. Here, we delineate the
267 phenotypes of T cells in cutaneous lupus, both in relation to healthy donor T cells as well as T
268 cells from other affected organs. These results suggest a limited activation of T cells in the skin
269 of lupus patients, especially in comparison to those of the kidney, and suggest that the pathologic
270 roles of T cells in lupus differ depending on the target tissue. These findings may help to explain
271 the differences in the efficacy of antimetabolite or T cell-directed therapies in lupus
272 manifestations in skin and kidney and may influence the search for the most efficacious
273 pathways to target to treat cutaneous lupus.

274 **Methods**

275 **Subjects and Sample Collection**

276 Skin punch biopsies were obtained from lesional (primarily back, chest, arms) and non-lesional
277 (sun-protected hip) locations from 7 patients with active cutaneous lupus (Supplementary Table
278 1). A diagnosis of SLE was confirmed for 6 of 7 patients via the 2019 EULAR/ACR criteria, and
279 the remaining patient was noted to have cutaneous involvement only. In addition, 14 healthy
280 donors were recruited to obtain control skin punch biopsies from sun-protected skin. Sample
281 collection for this study was approved by the University of Michigan Institutional Review Board
282 (IRB).

283 **Single-cell cDNA and Library Preparation**

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284 The collected biopsies were incubated overnight in 0.4% dispase (Life Technologies) in Hank's
285 Balanced Saline Solution (Gibco) at 4°C as previously detailed (29). Single-cell libraries for all
286 samples were generated using the 10x Genomics Chromium Single Cell 3' (v3 Chemistry)
287 protocol through the University of Michigan Genomics Core. Finally, libraries were sequenced
288 on an Illumina NovaSeq 6000 sequencer to generate 150 bp paired end reads.

289 **Data Analysis**

290 *Processing FASTQ reads-*

291 Initial processing of the sequencing output, including quality control, read alignment to a
292 reference genome (GRCh38), and gene quantification was performed using the 10x Genomics
293 Cell Ranger pipeline (v3.1.0). Following the generation of barcode and UMI counts, samples
294 were merged into a single expression matrix using the cellranger aggr pipeline.

295 *Data subsetting and quality control filtering-*

296 T and NK cell clusters were subset from the above dataset of skin biopsies from cutaneous lupus
297 patients and healthy donors in Seurat (v4.0.3). The remaining cells were then filtered to only
298 include cells with between 200 and 3500 detected features, and those with less than 15% of reads
299 associated with mitochondrial genes.

300 *Dimensionality reduction and clustering-*

301 Cells that passed all subset and filter steps then underwent another round of principal component
302 analysis, where the first 20 PCAs were subsequently used for downstream analysis. To ensure
303 integration of our samples, we corrected batch effects at the level of the sample using Harmony
304 (30). We employed Harmony using our PCA reduction with theta = 2, max.iter.cluster = 20, and
305 max.iter.harmony = 10. After integration, cells were clustered using Louvain clustering in Seurat
306 with a resolution of 0.6, determined through an iterative analysis of clustering results. Resulting

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307 clusters were then visualized using Uniform Manifold Approximation and Projection (UMAP)
308 plots. Differential gene expression between clusters was determined using a Wilcoxian Rank
309 Sum Test, and the resultant list was filtered to only include genes with a log fold-change greater
310 than 0.25 and those which were detected in at least 50% of the population being tested.

311 *Dataset integration-*

312 Inter-dataset integration was performed using canonical correlation analysis (CCA) in Seurat
313 (31). First, the lupus nephritis and systemic sclerosis datasets were downloaded and filtered on
314 the above quality control metrics, before being clustered. The T and NK cells were then subset
315 out from the respective datasets. Following this, CCA integration was performed by first finding
316 anchors between the dataset pairs, using the first 20 dimensions. Following, the *IntegrateData*
317 function in Seurat was used to display the datasets on the same UMAP projection.

318 **Data Availability**

319 The cutaneous lupus single-cell transcriptomics data analyzed in this publication is available in
320 the Gene Expression Omnibus (GEO) database under the accession number XXXXXXXXX.
321 The lupus nephritis analyzed during this study can be accessed within the Single Cell Portal
322 hosted by the Broad Institute using the study ID SCP279.
323 The systemic sclerosis data analyzed during this study is available in the Gene Expression
324 Omnibus database under the accession numbers GSE138669 and GSE181957.

325 **Author Contributions**

326 Conceived and designed the analysis: GSD, JMK, DAR.
327 Collected the data: ACB, MPM, RW, LCT, JEG.
328 Performed the analysis: GSD, FM.
329 Wrote the manuscript: GSD, DAR.
330 Supervised the work: JMK, DAR.
331

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341 and National Psoriasis Foundation.

342 **<u>Declaration of Interests</u>**

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352 **References**

353 1. Kaul A et al. Systemic lupus erythematosus. *Nat Rev Dis Primers* 2016;2:16039.

354 2. Stannard JN, Kahlenberg JM. Cutaneous lupus erythematosus: updates on pathogenesis and
355 associations with systemic lupus. *Curr. Opin. Rheumatol.* 2016;28(5):453–459.

356 3. Shi H, Gudjonsson JE, Kahlenberg JM. Treatment of cutaneous lupus erythematosus: current
357 approaches and future strategies. *Curr. Opin. Rheumatol.* 2020;32(3):208–214.

358 4. Bocharnikov AV et al. PD-1hiCXCR5- T peripheral helper cells promote B cell responses in
359 lupus via MAF and IL-21 [Internet]. *JCI Insight* 2019;4(20). doi:10.1172/jci.insight.130062

360 5. Liarski VM et al. Cell distance mapping identifies functional T follicular helper cells in
361 inflamed human renal tissue. *Sci. Transl. Med.* 2014;6(230):230ra46.

362 6. Makiyama A et al. Expanded circulating peripheral helper T cells in systemic lupus
363 erythematosus: association with disease activity and B cell differentiation. *Rheumatology*
364 2019;58(10):1861–1869.

365 7. Lin J, Yu Y, Ma J, Ren C, Chen W. PD-1+CXCR5-CD4+T cells are correlated with the
366 severity of systemic lupus erythematosus. *Rheumatology* 2019;58(12):2188–2192.

367 8. Franz B et al. Low number of regulatory T cells in skin lesions of patients with cutaneous
368 lupus erythematosus. *Arthritis Rheum.* 2007;56(6):1910–1920.

369 9. Maczynska I et al. CD4+CD25high regulatory T cells and CD4+CD69+ T cells in peripheral
370 blood of patients with cutaneous lupus erythematosus. *Cent Eur J Immunol* 2009;34(4):213–217.

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371 10. Gambichler T, Pätzholz J, Schmitz L, Lahner N, Kreuter A. FOXP3+ and CD39+ regulatory
372 T cells in subtypes of cutaneous lupus erythematosus. *J. Eur. Acad. Dermatol. Venereol.*
373 2015;29(10):1972–1977.

374 11. Lin S-C et al. The quantitative analysis of peripheral blood FOXP3-expressing T cells in
375 systemic lupus erythematosus and rheumatoid arthritis patients. *Eur. J. Clin. Invest.*
376 2007;37(12):987–996.

377 12. Valencia X, Yarboro C, Illei G, Lipsky PE. Deficient CD4+CD25high T regulatory cell
378 function in patients with active systemic lupus erythematosus. *J. Immunol.* 2007;178(4):2579–
379 2588.

380 13. Arazi A et al. The immune cell landscape in kidneys of patients with lupus nephritis. *Nat.*
381 *Immunol.* 2019;20(7):902–914.

382 14. Grassi M, Capello F, Bertolino L, Seia Z, Pippione M. Identification of granzyme B-
383 expressing CD-8-positive T cells in lymphocytic inflammatory infiltrate in cutaneous lupus
384 erythematosus and in dermatomyositis. *Clin. Exp. Dermatol.* 2009;34(8):910–914.

385 15. Stubbington MJT, Rozenblatt-Rosen O, Regev A, Teichmann SA. Single-cell transcriptomics
386 to explore the immune system in health and disease. *Science* 2017;358(6359):58–63.

387 16. Efremova M, Vento-Tormo R, Park J-E, Teichmann SA, James KR. Immunology in the Era
388 of Single-Cell Technologies. *Annu. Rev. Immunol.* 2020;38:727–757.

389 17. Gao Y, Dunlap G, Elahee M, Rao DA. Patterns of T-Cell Phenotypes in Rheumatic Diseases
390 From Single-Cell Studies of Tissue. *ACR Open Rheumatol* 2021;3(9):601–613.

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391 18. Der E et al. Tubular cell and keratinocyte single-cell transcriptomics applied to lupus
392 nephritis reveal type I IFN and fibrosis relevant pathways. *Nat. Immunol.* 2019;20(7):915–927.

393 19. Nehar-Belaid D et al. Mapping systemic lupus erythematosus heterogeneity at the single-cell
394 level. *Nat. Immunol.* 2020;21(9):1094–1106.

395 20. Billi AC et al. Non-lesional and Lesional Lupus Skin Share Inflammatory Phenotypes that
396 Drive Activation of CD16+ Dendritic Cells [Internet]. *bioRxiv* 2021;
397 doi:10.1101/2021.09.17.460124

398 21. Gaydosik AM et al. Single-cell transcriptome analysis identifies skin-specific T-cell
399 responses in systemic sclerosis [Internet]. *Ann. Rheum. Dis.* [published online ahead of print:
400 May 24, 2021]; doi:10.1136/annrheumdis-2021-220209

401 22. Joller N et al. Treg cells expressing the coinhibitory molecule TIGIT selectively inhibit
402 proinflammatory Th1 and Th17 cell responses. *Immunity* 2014;40(4):569–581.

403 23. Zhang F et al. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues
404 by integrating single-cell transcriptomics and mass cytometry. *Nat. Immunol.* 2019;20(7):928–
405 942.

406 24. Smith SL et al. Diversity of peripheral blood human NK cells identified by single-cell RNA
407 sequencing. *Blood Adv* 2020;4(7):1388–1406.

408 25. Braunstein I, Klein R, Okawa J, Werth VP. The interferon-regulated gene signature is
409 elevated in subacute cutaneous lupus erythematosus and discoid lupus erythematosus and

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410 correlates with the cutaneous lupus area and severity index score. *Br. J. Dermatol.*
411 2012;166(5):971–975.

412 26. Berthier CC et al. Molecular Profiling of Cutaneous Lupus Lesions Identifies Subgroups
413 Distinct from Clinical Phenotypes [Internet]. *J. Clin. Med. Res.* 2019;8(8).
414 doi:10.3390/jcm8081244

415 27. Turnier JL, Kahlenberg JM. The Role of Cutaneous Type I IFNs in Autoimmune and
416 Autoinflammatory Diseases. *J. Immunol.* 2020;205(11):2941–2950.

417 28. Tabib T et al. Myofibroblast transcriptome indicates SFRP2hi fibroblast progenitors in
418 systemic sclerosis skin. *Nat. Commun.* 2021;12(1):4384.

419 29. Rao DA. T Cells That Help B Cells in Chronically Inflamed Tissues. *Front. Immunol.*
420 2018;9:1924.

421 30. Korsunsky I et al. Fast, sensitive and accurate integration of single-cell data with Harmony.
422 *Nat. Methods* 2019;16(12):1289–1296.

423 31. Butler A, Hoffman P, Smibert P, Papalexi E, Satija R. Integrating single-cell transcriptomic
424 data across different conditions, technologies, and species. *Nat. Biotechnol.* 2018;36(5):411–420.

425

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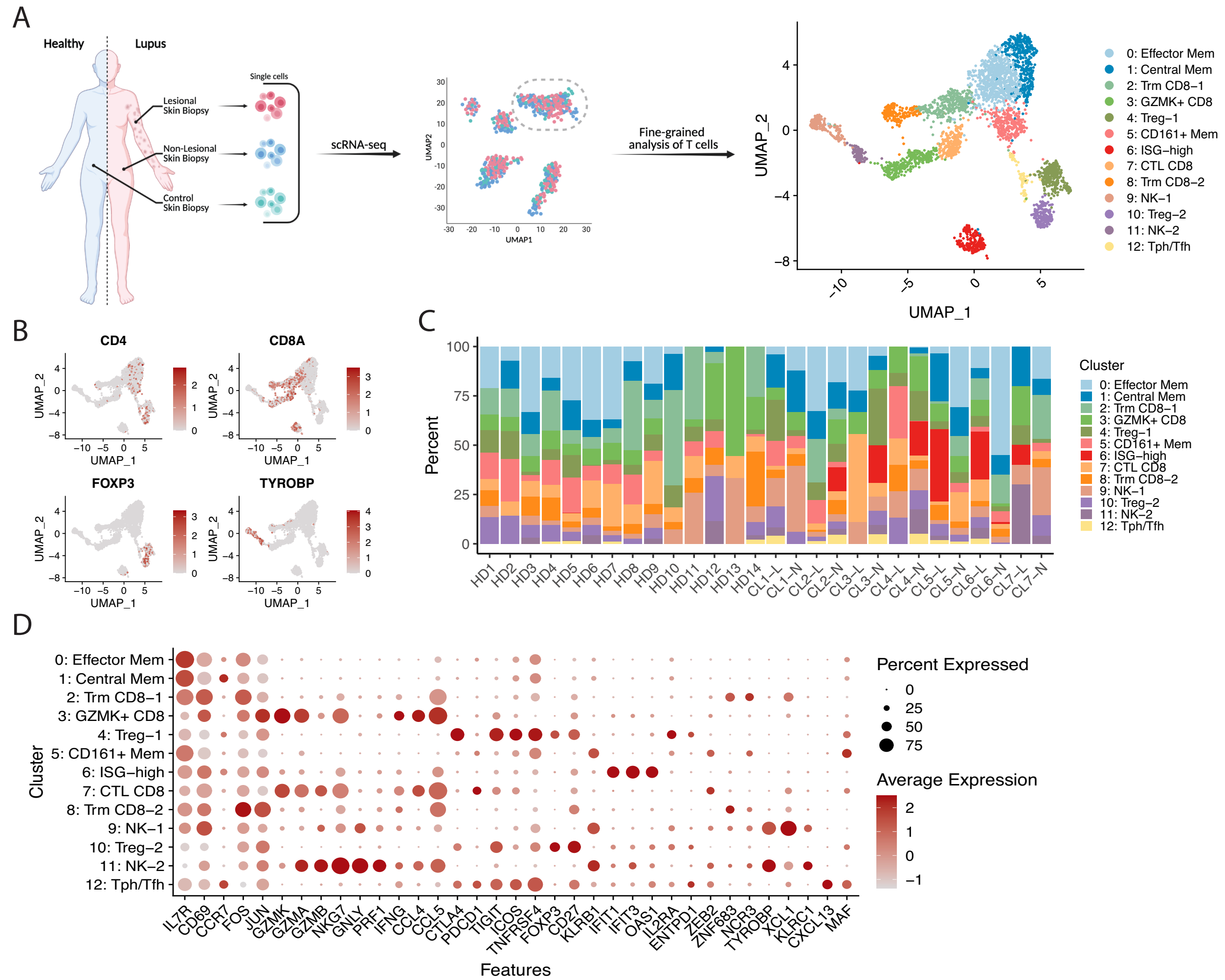

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428 **Figure 1. Identification of T and NK cell states in lesional and non-lesional skin biopsies of**
429 **cutaneous lupus patients. A.** Schematic diagram of study, including sample collection, initial
430 scRNA-seq, and fine clustering of T cell subsets. **B.** UMAP plots of main T cell lineage marker
431 expression. **C.** Bar plot of cluster assignments for captured cells in each sample. **D.** Dot plot of
432 differentially expressed genes in each identified cluster. HD, healthy donor. CL, cutaneous lupus.
433 L, lesional. N, non-lesional.
434

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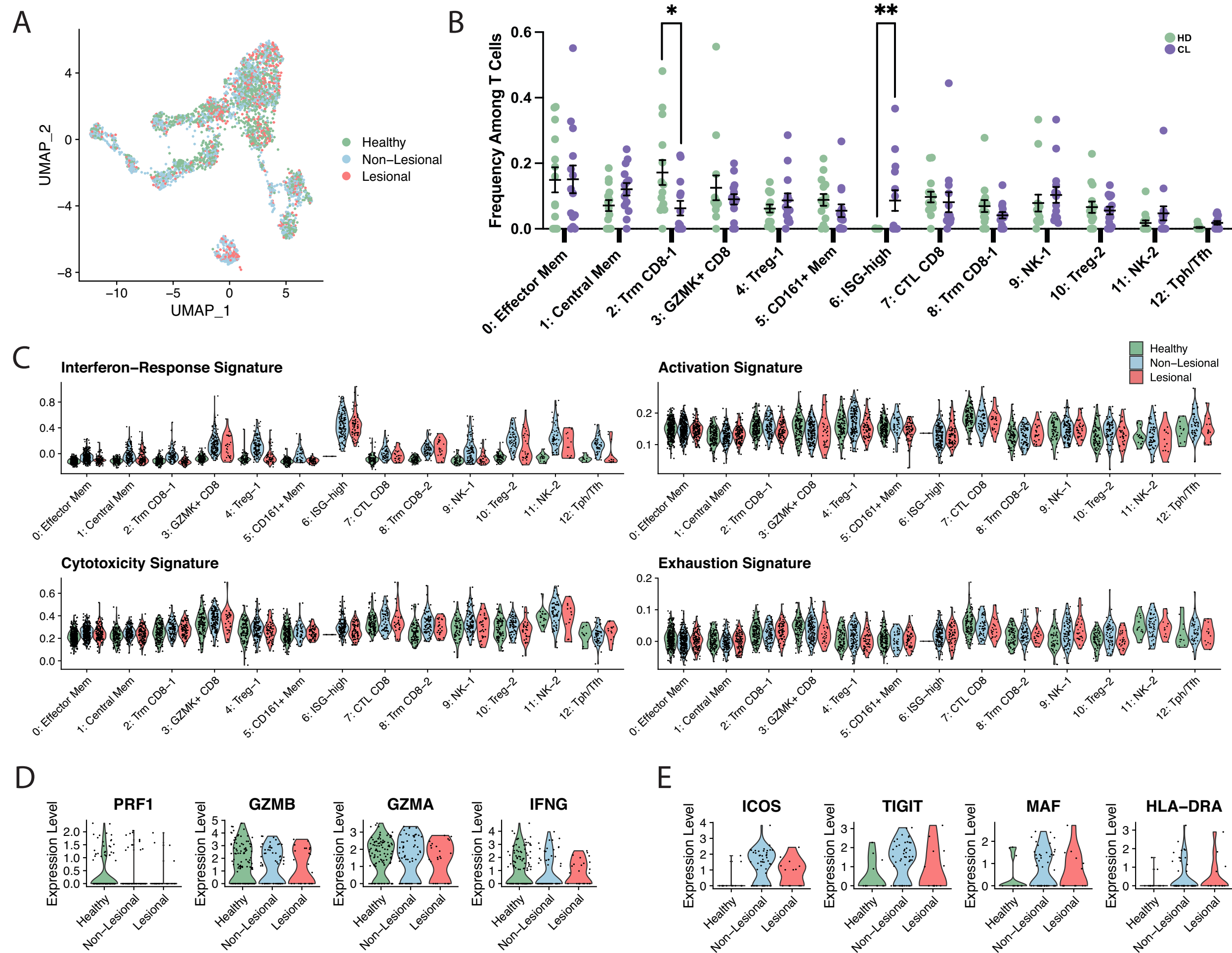

435
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437 **Figure 2. Examination of differences between lesional, non-lesional, and healthy skin**
438 **biopsies. A.** UMAP plot of cells colored by sample type. **B.** Comparison of the frequencies of T
439 cells per cluster between cutaneous lupus (CL) patients and healthy donors (HD). **C.** Violin plots
440 of signature scores between healthy, lesional, and non-lesional cell components of each cluster.
441 **D.** Violin plots of the expression of select markers between healthy, lesional, and non-lesional
442 cell components of the CTL CD8 cluster. **E.** Violin plots of the expression of select markers
443 between healthy, lesional, and non-lesional cell components of the Tph/Tfh cluster. Mean ± SEM
444 shown. *p<0.05, **p<0.01 by Mann-Whitney U test in (B).

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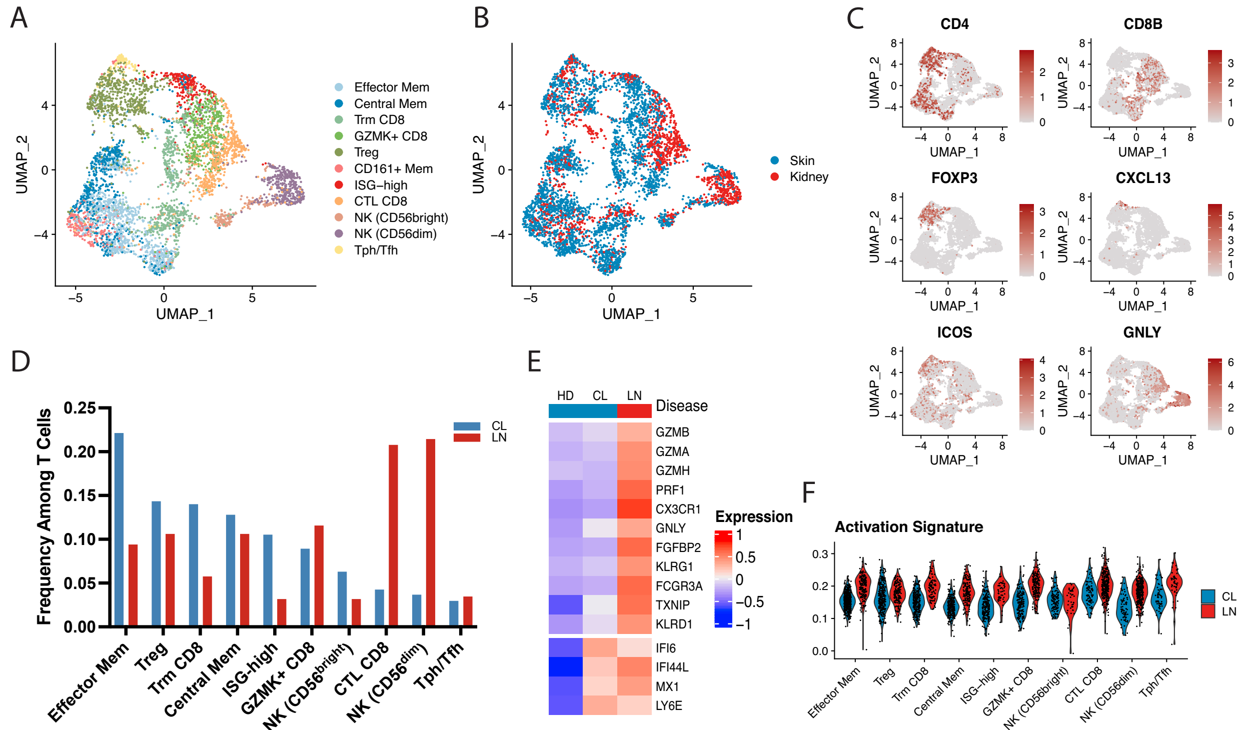

445
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447 **Figure 3. Integration of cutaneous lupus and lupus nephritis single-cell datasets reveals**
448 **decreased cytotoxic profiles in the skin T cells. A.** UMAP plot of the cluster identifications
449 resulting from canonical correlation analysis (CCA) integration. **B.** UMAP plot of the location of
450 cells from each study. **C.** Representative UMAP plots of select major lineage markers. **D.**
451 Comparison of the frequencies of T cells per cluster between cells from cutaneous lupus (CL)
452 and lupus nephritis (LN) datasets. **E.** Scaled heatmap of cytotoxic genes (top) and select
453 interferon-stimulated genes (bottom). **F.** Violin plot of the activation signature scores between
454 CL and LN cells for each cluster.

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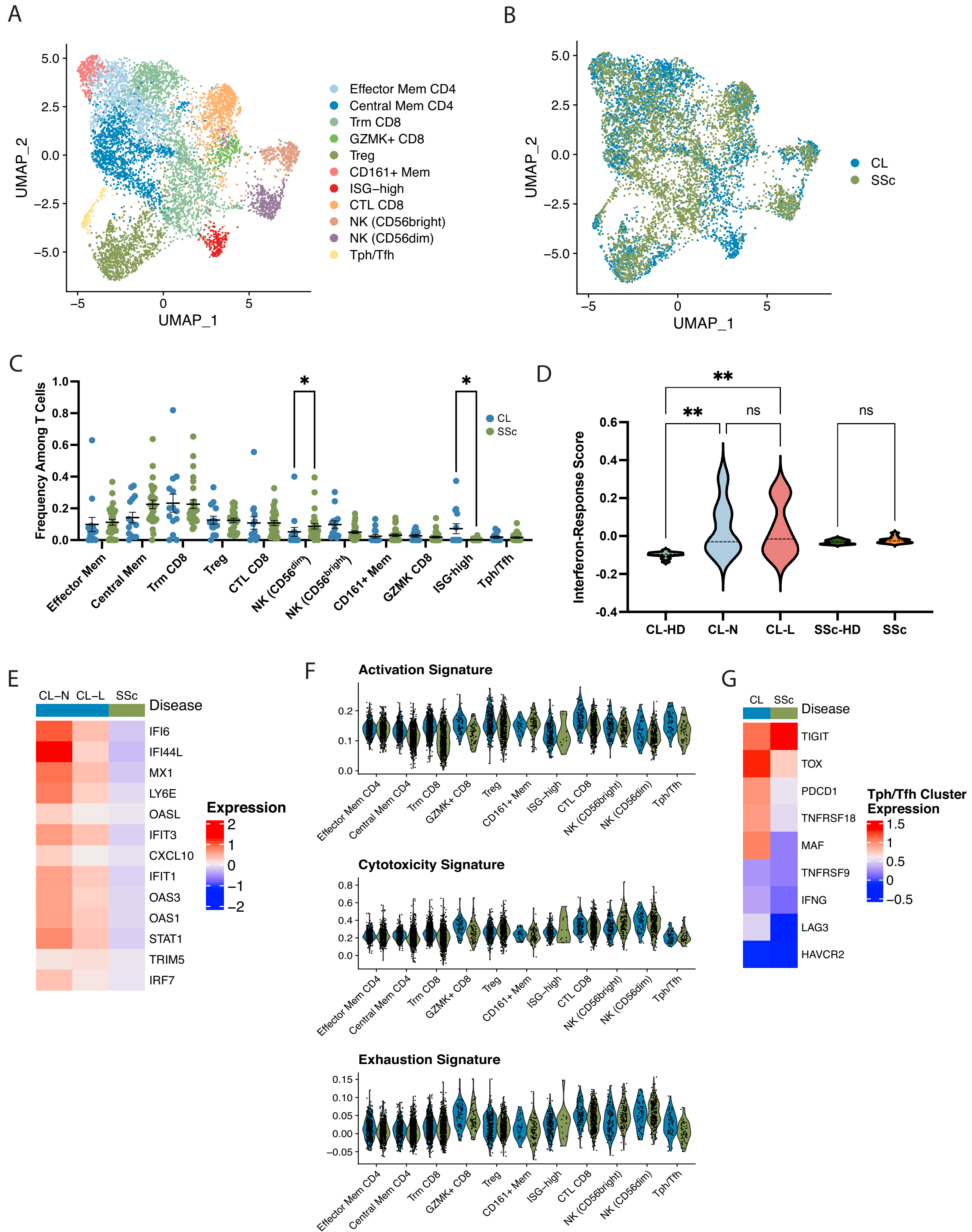

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457 **Figure 4. Integration of cutaneous lupus and systemic sclerosis single-cell datasets**
458 **demonstrates selective ISG upregulation in lupus. A.** UMAP plot of the cluster identifications

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459 resulting from CCA integration. **B.** UMAP plot of the location of cells from each study. **C.**
460 Comparison of the frequencies of T cells per cluster between cells from cutaneous lupus (CL)
461 and systemic sclerosis (SSc) datasets. **D.** Violin plot of interferon response signatures between
462 study and sample types. **E.** Scaled heatmap of interferon-stimulated genes. **F.** Violin plots of
463 signature scores between CL and SSc cells for each cluster. **G.** Scaled heatmap of select
464 activation and exhaustion markers in the Tph/Tfh cluster. Mean ± SEM shown. *$p<0.05$,
465 **$p<0.01$ by Kruskal-Wallis test in (C) and (D).

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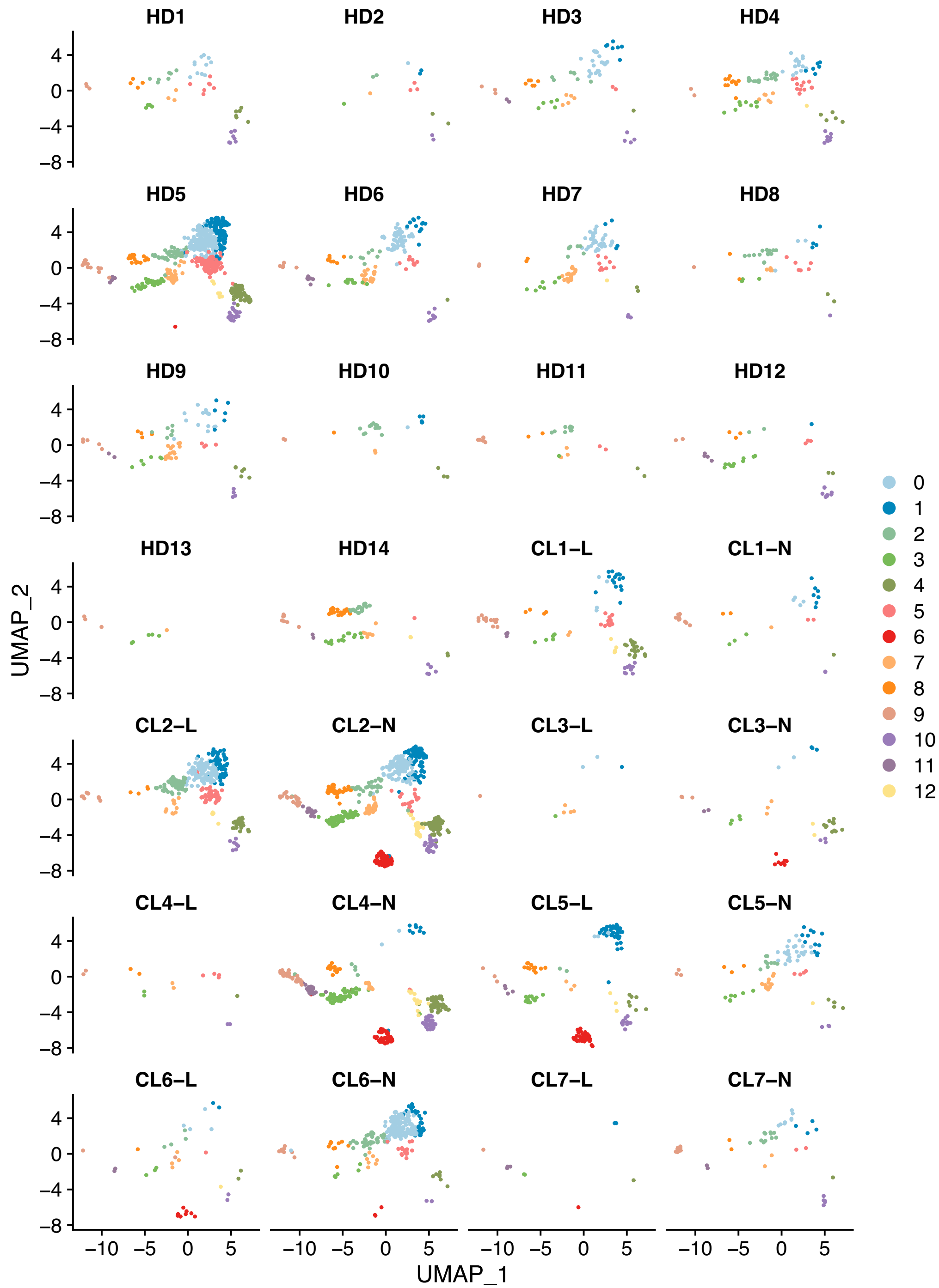

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467 **Supplementary Figure 1.** UMAP plot locations of the cells from each sample.

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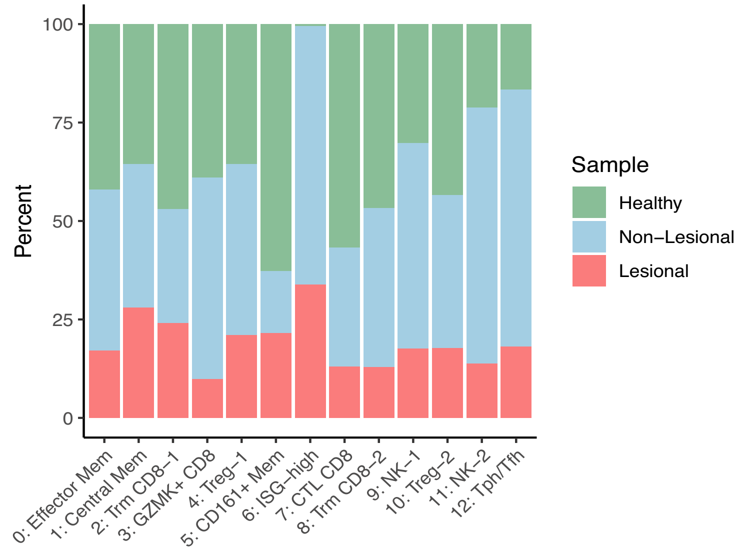

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470 **Supplementary Figure 2.** Barplot of the healthy, non-lesional, and lesional components of each
471 cluster.

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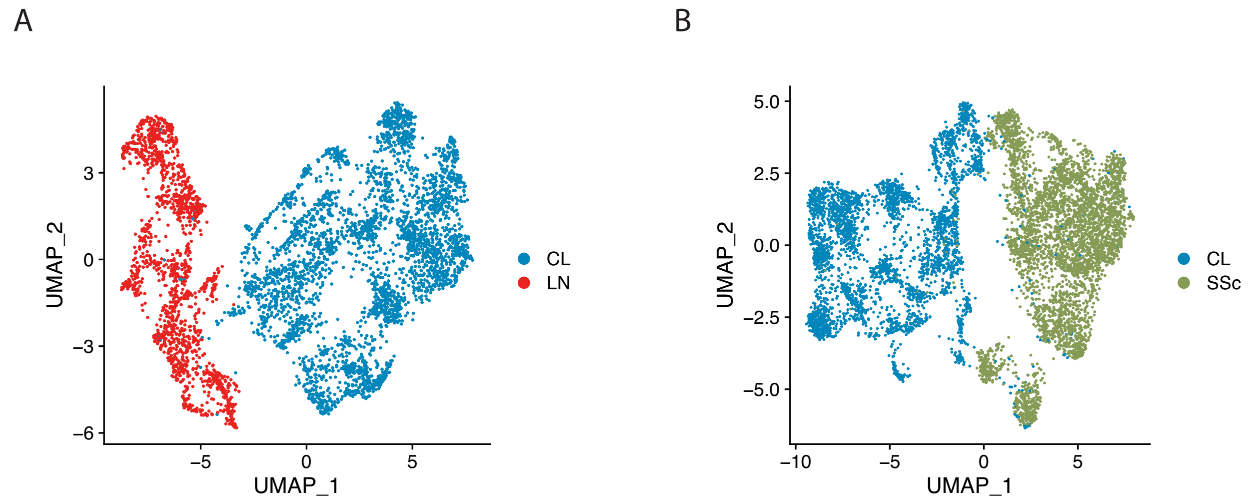

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474 **Supplementary Figure 3. A.** UMAP plot of the merged cutaneous lupus (CL) and lupus
475 nephritis (LN) datasets before CCA integration. **B.** UMAP plot of the merged CL and systemic
476 sclerosis (SSc) datasets before CCA integration.

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477 [See uploaded file]
478
479 **Supplementary Table 1.** Clinical characteristics of cutaneous lupus patients included in this
480 study.

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481 [See uploaded file]
482
483 **Supplementary Table 2.** Genes differentially expressed across T/NK clusters in skin biopsy
484 samples.

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485 [See uploaded file]
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487 **Supplementary Table 3.** Signature gene lists for activation, cytotoxicity, exhaustion, and
488 interferon response.