Supplement to: Spatial characterization of interface dermatitis in cutaneous lupus reveals novel chemokine ligand-receptor pairs that drive disease

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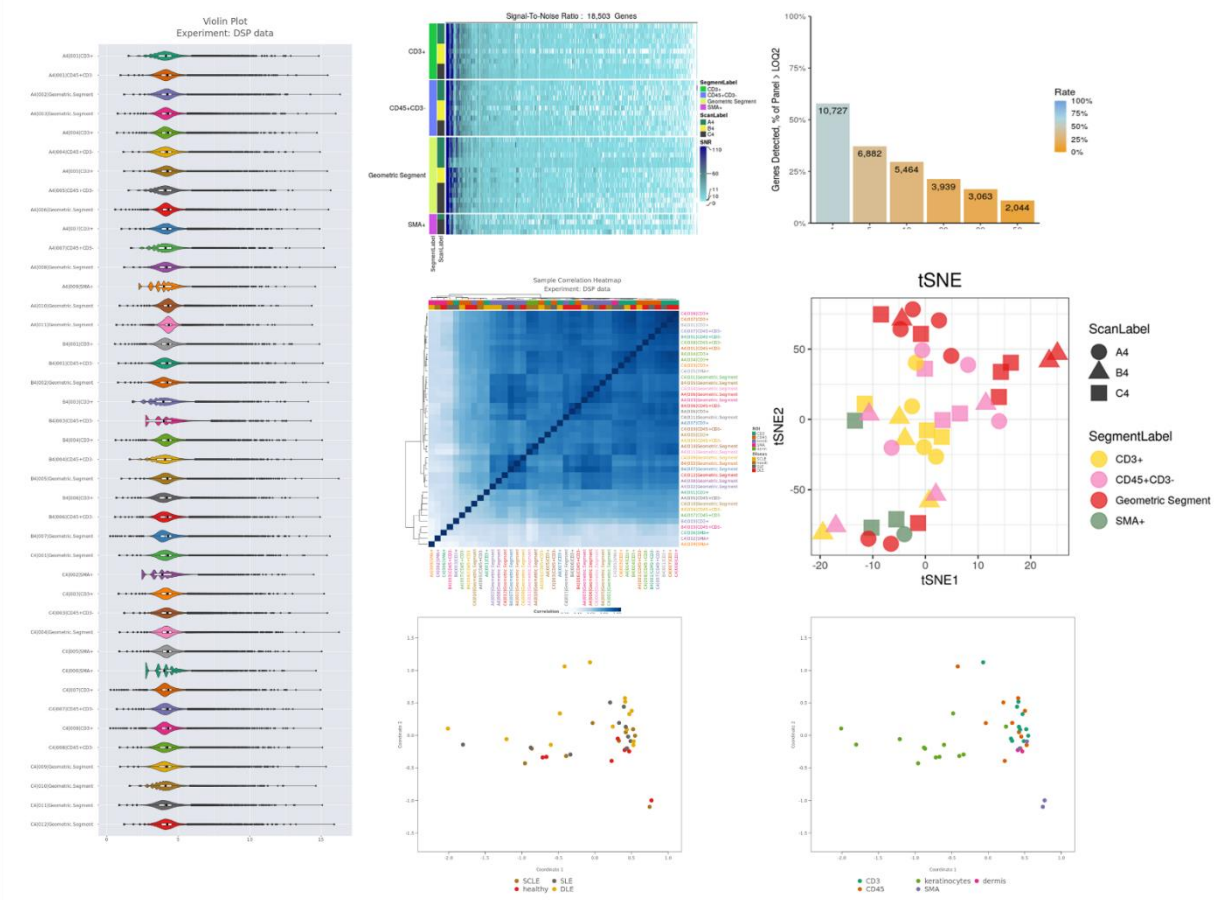

Fig. S1. Quality Control for Digital Spatial Profiling (DSP) Whole Transcriptome Atlas (WTA) spatial transcriptomics. (A). Violin Plot showing sample distribution. (B). Signal-to-noise heatmap grouped by ROI type and slide. (C). Histogram Chart for LOQ2 Values, presents a histogram chart that conveys the distribution of LOQ2 values. These values were employed to filter genes that are expressed near background levels, aiding in data quality assessment. (D). Sample Correlation Heatmap showed correlation heatmap is presented, depicting the pairwise correlation between samples within the DSP - RNA dataset. Notably, this heatmap highlights specific regions of interest (ROIs) in proximity to the respective antibodies used for staining. (E). t-distributed Stochastic Neighbor Embedding (t-SNE) Graph visualizes the data points with scan labels and segment labels, effectively illustrating the spatial distribution of data and the relationship between segments. (F). Principal Component Analysis (PCA) for the clustering of samples based on disease type (left) and cell types (right).

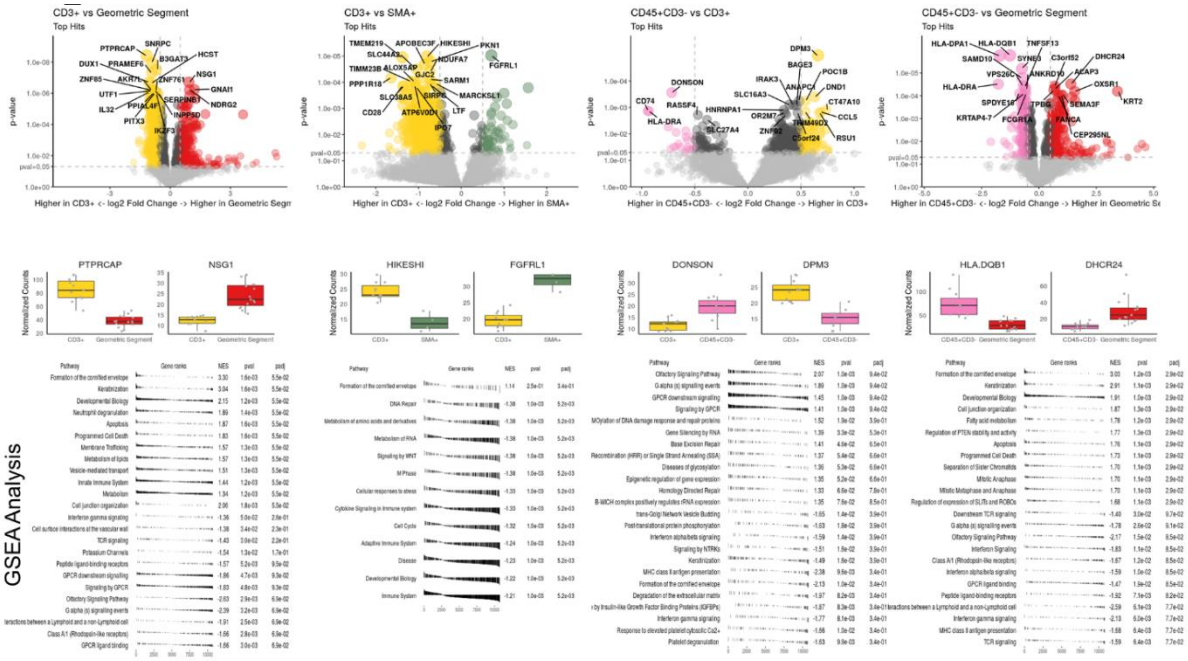

Fig S2. Quality Control and Pathway Analysis for Regions of Interest (ROIs). (A). Volcano Plot Comparisons present a comparing gene expression in different segments. The following pairwise segment comparisons are CD3+ vs. Geometric, CD3+ vs. SMA+, CD45+CD3- vs. CD3+, and CD45+CD3- vs. Geometric. Each volcano plot illustrates the distribution of gene expression across segments, with the log-fold change on the x-axis and the statistical significance on the y-axis. Additionally, for each volcano plot, a bar chart highlights the most highly expressed genes in the respective segments, providing valuable insights into the key drivers of variation. (B). Gene Set Enrichment and Analysis (GSEA) unveils significant pathways and functional annotations associated with the gene expression data within the ROIs.

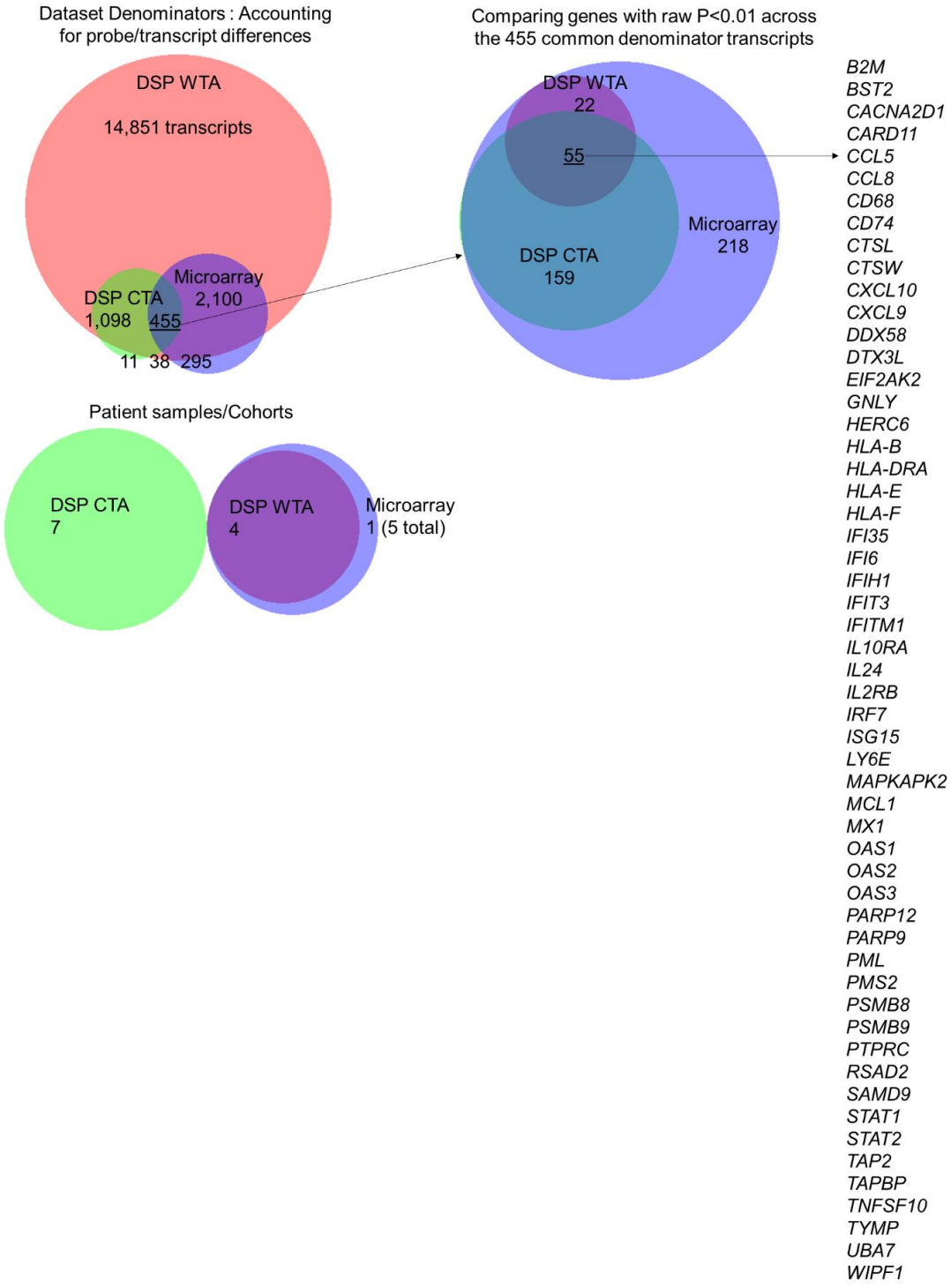

Fig S3. Validation of DSP dataset using historical microarray dataset and a cancer transcriptome atlas (CTA) dataset. We compared DEGs in DSP WTA (this dataset), DSP CTA (Vesely dataset) and microarray (Ko/Harris dataset). Among these datasets, 455 common probe/transcript denominators were identified. This figure focuses on the 55 genes present across all datasets with raw p-values < 0.01. This comparative analysis offers a robust assessment of the consistency and reliability of the DSP WTA dataset by examining the shared DEGs across multiple datasets. The presence of these 55 genes underscores their significance in the context of cutaneous lupus.

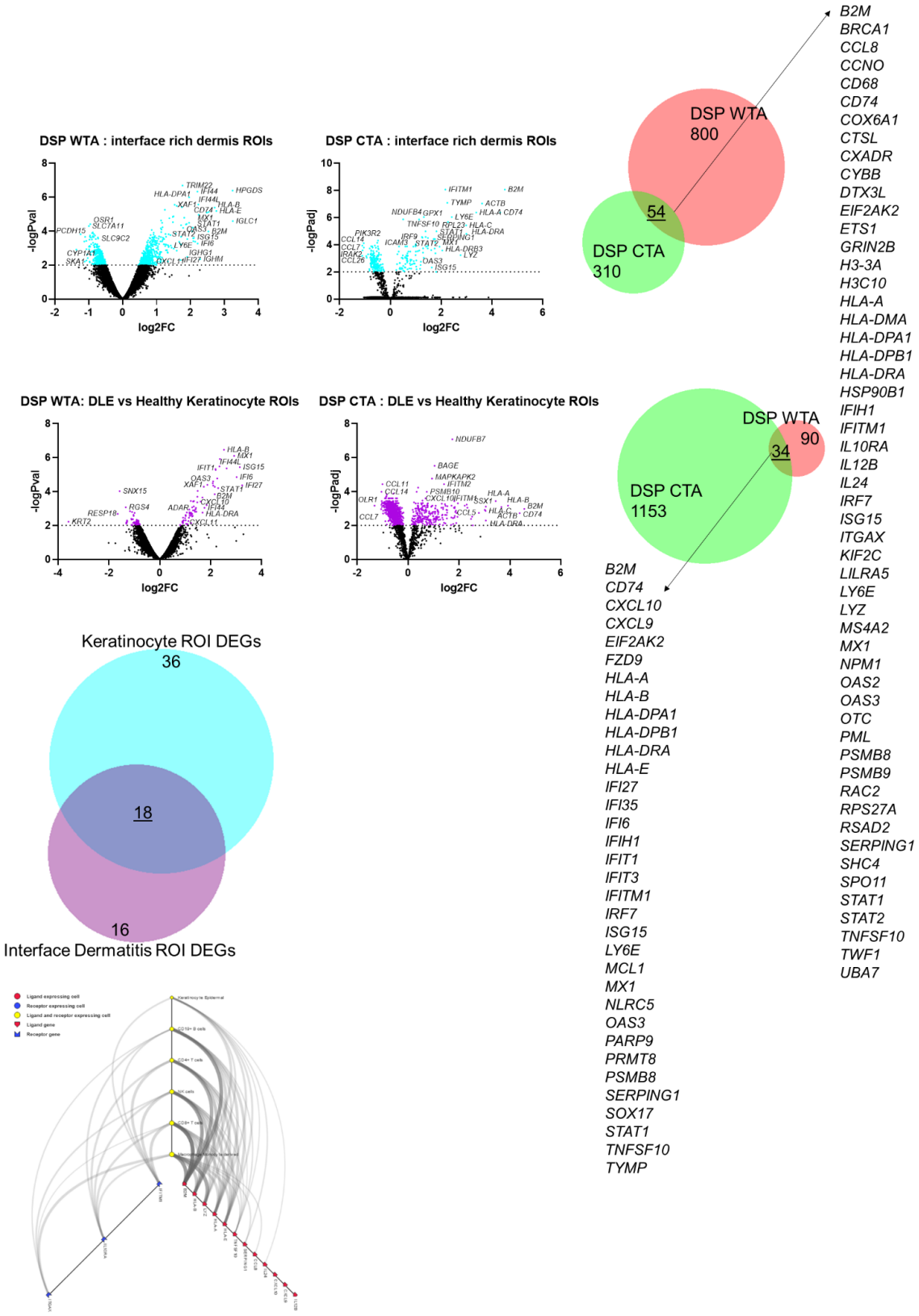

Fig S4. Validation of DLE dataset ROIs as compared to CTA. We compared DEGs in interface dermatitis rich ROIs (CD45+) in WTA (this dataset) and CTA (Vesely dataset) (teal volcanoes). Of these, we found 54 conserved DEGs in the inflammatory infiltrate. We also compared DEGs in the epidermal/keratinocyte geometric ROIs in the WTA and CTA datasets and found 34 overlapping DEGs (purple volcanoes). Last, we examined shared DEGs across ROI types and

found 18 DEGs that are expressed by both the stroma and the immune cells (purple vs teal BioVenn). We also examined receptor:ligand pairs across these ROI types to understand how the immune system communicates with keratinocytes in CLE using cellPhoneDB, which demonstrated multiple predicted interactions.

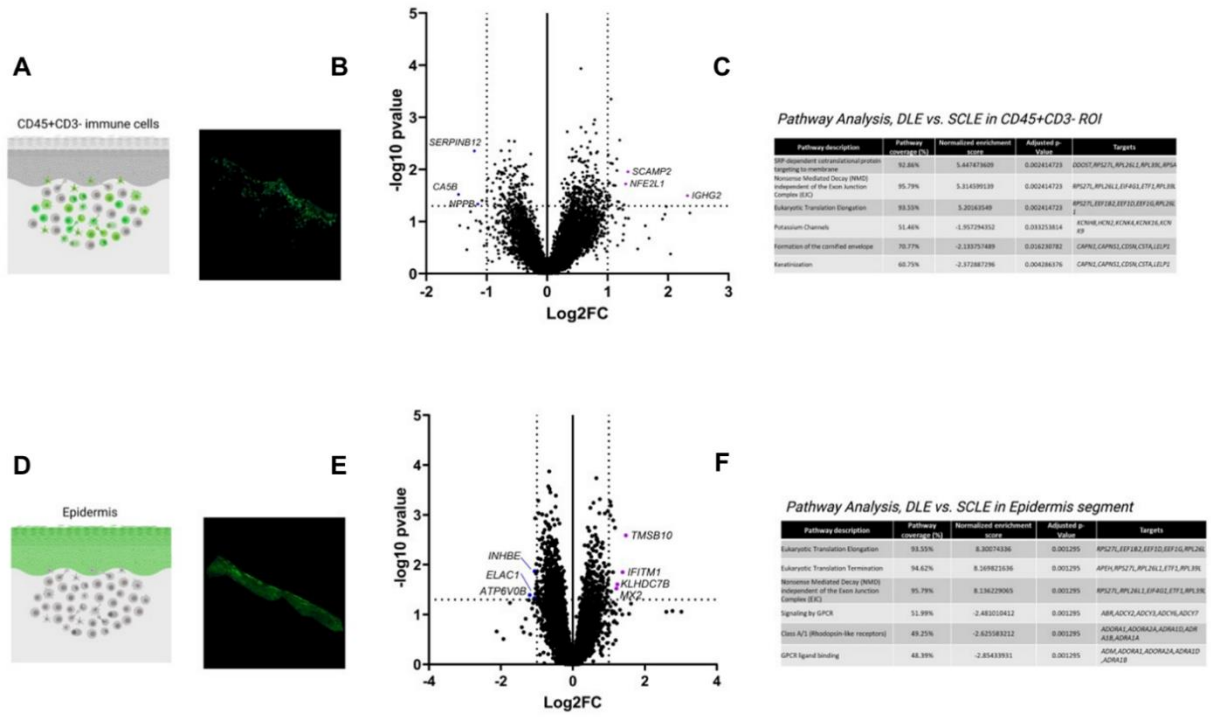

Fig S5. Examining CD45+ and epidermal ROIs in DLE vs SCLE reveals pathways and DEGs unique to each CLE subtype. We examined differential gene expression, pathway analysis, and the unique genes associated with Cutaneous Lupus Erythematosus (CLE) subtypes - Discoid Lupus Erythematosus (DLE) and Subacute Cutaneous Lupus Erythematosus (SCLE). (A) Schematic of immune cell ROIs (CD45+CD3-) and example image. (B) Volcano Plot of Differential Gene Expression (DLE vs. SCLE) showed highlights genes that are uniquely expressed or differentially regulated in each subtype. (C) Pathway Analysis of the CD45+CD3- ROIs in in both DLE and SCLE. (D) Schematic of geometric ROIs for epidermis and example image. (E) Volcano Plot of Differential Gene Expression (DLE vs. SCLE) highlights genes that are uniquely expressed or differentially regulated in keratinocytes/epidermis in each subtype. (F) Pathway Analysis of the epidermal ROIs in in both DLE and SCLE with pathways associated with immune cells in these clinical subtypes.

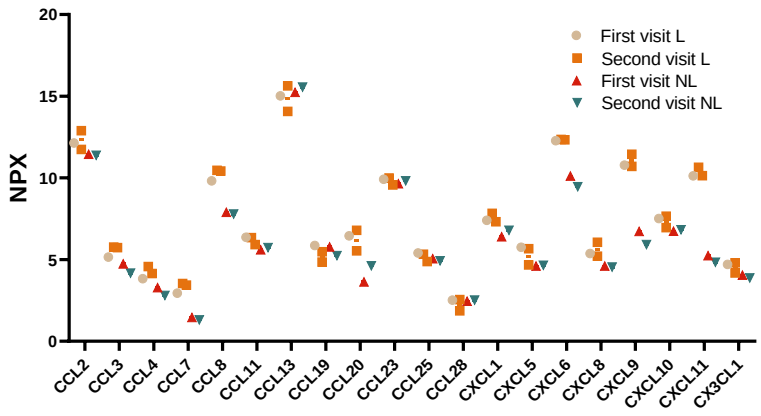

Fig S6. Stability of chemokines in lesional and nonlesional samples over time. We compared data obtained during the first and second visits from a repeat donor who was sampled approximately 6 months apart. The quantification of chemokines is based on Normalized Protein eXpression (NPX) values from Olink targeted proteomics.

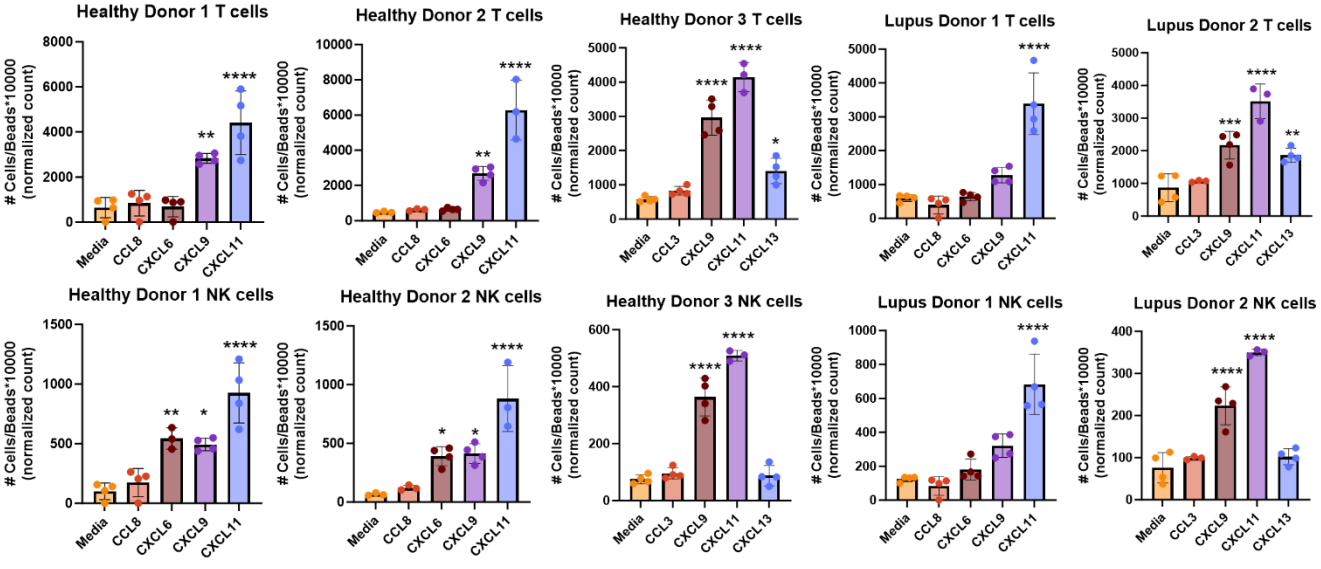

Fig S7. Additional donors for chemotaxis exhibit similar migratory patterns. We tested n=3 healthy and n=2 lupus donor PBMCs in triplicate or quadruplicate in chemotaxis assays. Top row depicts T cell migration, bottom row depicts NK cell migration. (*p<0.05, **p<0.01, ****p<0.0001 for one-way ANOVAs with Dunnet's post hoc tests compared to media control wells).

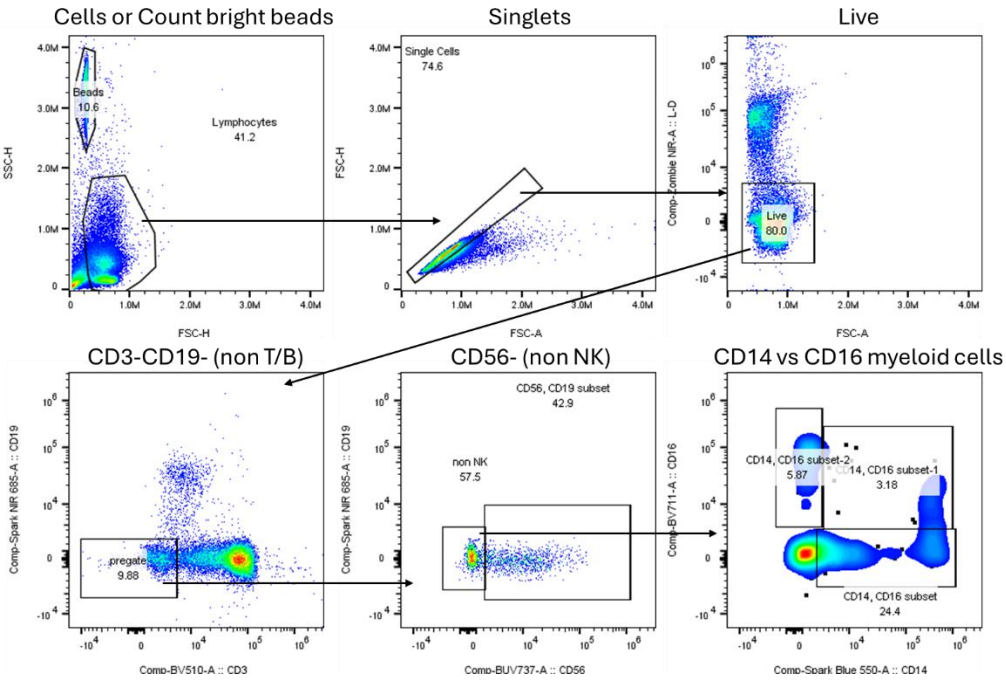

Fig S8. Flow gating strategy for assessing myeloid populations. Example flow gating strategy employed to assess CD14 vs CD16 myeloid cells using cells->singlets->live->CD3-CD19- and CD56- pregates, followed by CD14 vs CD16. This example is from PBMCs.

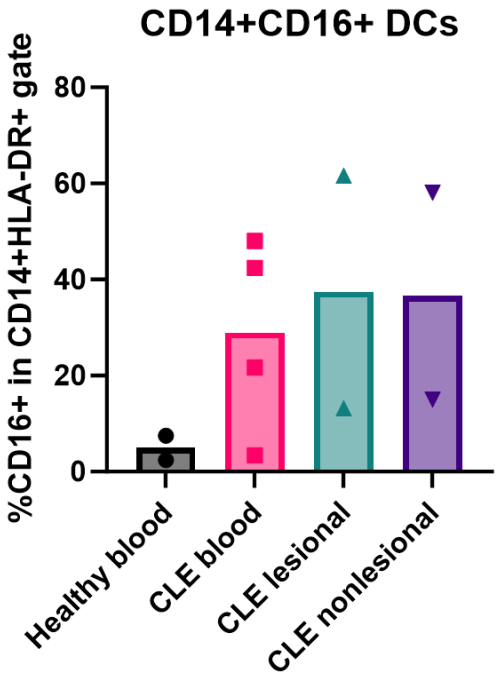

Fig S9. Examination of CD14+CD16+ cells in blister biopsies. Confirmation of CD16+ antigen presenting cell populations as described in Kahlenberg 10X spatial dataset. Note that not every blister and blood donor had significant cell populations as detected by flow cytometry.

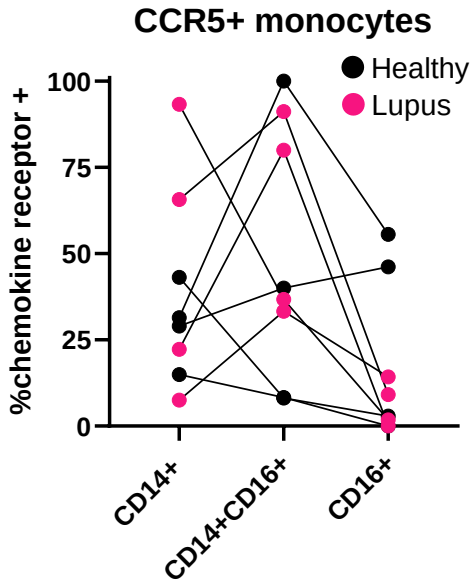

Fig S10. **CCR5 is not enriched on CD14 vs CD16 expressing myeloid cells**. CCR5 is an alternate receptor for CCL8 on monocytes. We noted no specific trend in expression in different quadrants for CD14 vs CD16 expressing myeloid cells, which could not account for chemotactic differences observed in response to CCL8.

Table S1. Blister biopsy and blood donation patient info & characterization (UMass Chan).

Subject #	Diagnosis	Age	Sex	Race/Ethnicity	Treatment status	Pertinent medical history	Blister biopsy	Blister site L	Blister site NL	Blood sample
1	SCLE	45	M	White	Hydroxychloroquine (Plaquenil), last used 2 years prior to the biopsy; triamcinolone 0.1% ointment BID, PRN 1 year prior; Steroids Creams Last applied few months prior to the biopsy	none	Y	upper back	forearm, inner aspect	Y
2	Active SLE	44	F	White, Hispanic Latina	On Lyrica	none	Y	cheek	forearm	Y
3	SCLE	75	F	White	On Plaquenil. Prescribed just prior to biopsy: Protopic 0.1% ointment BiD PRN itching or rash on the face; triamcinolone 0.1% cream BID PRN itching or rash on the trunk and limbs; fluocinonide scalp solution	Raynaud's	Y	outer arm	forearm, inner aspect	Y
4	SCLE with SLE	55	F	White	On hydroxychloroquine	Fibromyalgia, photosensitivity	Y	arm	arm	Y
5	Healthy	34	F	White	none	none	N	N/A	N/A	Y
6	Healthy	54	F	Asian	none	none	N	N/A	N/A	Y
7	Healthy	28	M	White, Middle Eastern	none	none	Y	N/A	arm	N
8	Healthy	60	F	White	none	none	Y	N/A	arm	N
9	Healthy	52	F	White	none	none	Y	N/A	arm	Y

SCLE = subacute cutaneous lupus erythematosus; SLE = systemic lupus erythematosus; BID = twice daily; PRN = taken as needed; FH = family history; L = lesional; NL = nonlesional; N/A = not applicable, ND = not determined, Y = yes, N = no

Table S2. Archival CLE biopsies used in WTA Digital Spatial Profiling (UMass Chan).

Disease status	Systemic symptoms	Age (yr)	Sex	Ethnicity	Age at diagnosis (yr)	Age at skin biopsy (yr)	Site of Bx	Positive antibodies	CLE-related medications at the time of Bx
SCLE	Bilateral chronic hand pain	68	M	White, not Hispanic Latino	68	68	Shoulder+ Forearm	+ANA (speckled), +SSA, +dsDNA	None
DLE	Swollen salivary glands	42	M	White, not Hispanic Latino	?	42	Face	+Anti-SM/RNP, +ANA	HCTZ (new forearm rash). Cheek rash predated HCTZ
SCLE (drug induced)	None	76	F	White, not Hispanic Latino	76	76	Shoulder	+ANA (speckled), +SSA, +SSB; Later diagnosed with Sjogren's	HCTZ, glipizide, omeprazole
SCLE	None (until 04/2020, joint pain and blue toes)	37	M	White, not Hispanic Latino	37	37	Abdomen	+ANA, +SSA, +SSB	Oral ketoconazole (previous hx of tinea versicolor)
DLE	None	68	F	White, not Hispanic Latino	68	68	Scalp	+ANA (speckled and homogeneous),+SSA, +Sm/RNP	Topical clobetasol, T-Gel shampoo (initially thought psoriasis)
DLE	None	45	F	White, not Hispanic Latino	45	?	Neck (Posterior auricular region)	Borderline +ANA	?
DLE	vague arthralgia, no other features to meet criteria for SLE	51	F	White, not Hispanic Latino	51	51	Forehead	+ANA, +dsDNA	None
Healthy	N/A	64	F	White, not Hispanic Latino	N/A	N/A	Forehead	N/A	None
Healthy	N/A	32	M	White, not Hispanic Latino	N/A	N/A	Forehead	N/A	None
Healthy	N/A	41	M	White, not Hispanic Latino	N/A	N/A	Forearm	N/A	None

Table S3. Validation archival CLE biopsies used in CTA Digital Spatial Profiling (Yale).

Sample Name	Sex	Age (years)	Location	Treatment at time of biopsy	ROIs
CTA panel					
DLE #1	F	45	Cheek	None	1-4
DLE #2	M	53	Nose	None	5-8
DLE #3	F	40	Cheek	None	9-12
Control #1	F	28	Neck	-	1-4
Control #2	M	52	Back	-	5-8
Control #3	M	33	Back	-	9-12

Table S4. Olink DEPs calculated by NPX software and 2-way ANOVA.

Significant for CLE lesional versus nonlesional and healthy					
CXCL11					
CLE Lesional vs. CLE Nonlesional	2.878	0.2467 to 5.508	Yes	*	0.0351
CLE Lesional vs. Healthy	5.395	1.531 to 9.259	Yes	*	0.0127
CLE Nonlesional vs. Healthy	2.518	-2.698 to 7.733	No	ns	0.1869
CXCL9					
CLE Lesional vs. CLE Nonlesional	2.74	1.189 to 4.291	Yes	**	0.0023
CLE Lesional vs. Healthy	5.385	0.2335 to 10.54	Yes	*	0.0456
CLE Nonlesional vs. Healthy	2.644	-1.693 to 6.981	No	ns	0.167
CXCL6					
CLE Lesional vs. CLE Nonlesional	1.97	0.2310 to 3.710	Yes	*	0.0275
CLE Lesional vs. Healthy	5.917	0.08853 to 11.75	Yes	*	0.0482
CLE Nonlesional vs. Healthy	3.947	-2.539 to 10.43	No	ns	0.137
IFN-gamma					
CLE Lesional vs. CLE Nonlesional	2.358	0.2003 to 4.516	Yes	*	0.0331
CLE Lesional vs. Healthy	4.2	1.447 to 6.953	Yes	**	0.0075
CLE Nonlesional vs. Healthy	1.842	-0.8639 to 4.548	No	ns	0.1426
CASP-8					
CLE Lesional vs. CLE Nonlesional	1.746	0.1036 to 3.389	Yes	*	0.0374
CLE Lesional vs. Healthy	3.206	0.6071 to 5.804	Yes	*	0.0235
CLE Nonlesional vs. Healthy	1.459	-1.249 to 4.168	No	ns	0.2286
CTF1					
CLE Lesional vs. CLE Nonlesional	0.7969	0.08803 to 1.506	Yes	*	0.0287
CLE Lesional vs. Healthy	1.008	0.2062 to 1.810	Yes	*	0.0177
CLE Nonlesional vs. Healthy	0.2112	-0.4943 to 0.9167	No	ns	0.6099
Significant for CLE lesional or nonlesional versus healthy					
HGF					
CLE Lesional vs. CLE Nonlesional	1.419	-0.3617 to 3.200	No	ns	0.1258
CLE Lesional vs. Healthy	4.07	2.410 to 5.730	Yes	***	0.0004
CLE Nonlesional vs. Healthy	2.651	0.8788 to 4.423	Yes	**	0.007
Flt3L					
CLE Lesional vs. CLE Nonlesional	0.005873	-0.7783 to 0.7900	No	ns	0.9998
CLE Lesional vs. Healthy	2.871	0.6221 to 5.121	Yes	*	0.0265
CLE Nonlesional vs. Healthy	2.865	0.5416 to 5.189	Yes	*	0.0299
CCL28					

CLE Lesional vs. CLE Nonlesional	0.1427	-0.3112 to 0.5966	No	ns	0.687
CLE Lesional vs. Healthy	0.5747	0.1965 to 0.9529	Yes	**	0.0063
CLE Nonlesional vs. Healthy	0.432	0.04441 to 0.8196	Yes	*	0.0311
CCL25					
CLE Lesional vs. CLE Nonlesional	-0.1353	-1.333 to 1.062	No	ns	0.951
CLE Lesional vs. Healthy	2.122	0.3620 to 3.881	Yes	*	0.027
CLE Nonlesional vs. Healthy	2.257	0.5139 to 4.000	Yes	*	0.0192
IFNL1					
CLE Lesional vs. CLE Nonlesional	1.479	-0.5759 to 3.534	No	ns	0.1587
CLE Lesional vs. Healthy	2.646	0.5894 to 4.703	Yes	*	0.0164
CLE Nonlesional vs. Healthy	1.167	0.2188 to 2.116	Yes	*	0.0203
CEACAM3					
CLE Lesional vs. CLE Nonlesional	0.1451	-0.02555 to 0.3157	No	ns	0.096
CLE Lesional vs. Healthy	-0.296	-0.5794 to -0.01256	Yes	*	0.043
CLE Nonlesional vs. Healthy	-0.4411	-0.7595 to -0.1226	Yes	*	0.0218

Table S5. Chemotaxis and chemokine receptor staining blood donor information (UMass Chan and Dartmouth Hitchcock).

Subject	Gender	Age	race	Skin color	Diagnosis	Treatment	Chemotaxis ligands	Chemokine Receptor staining
A	Female	32 y/o	Hispanic	white	SLE	Plaquenil 300mg daily	N/A	CCR1, CXCR3, CCR5, CCR7, CXCR1, CXCR5, CXCR2, CCR2
B	Female	64 y/o	Hispanic	white	DLE/SCLE	6 (15mg) pills MTX/week for past 8 mo, synthroid, albuterol	CXCL9, 11,13, CCL3	CCR1, CXCR3, CCR5, CCR7, CXCR1, CXCR5, CXCR2, CCR2
C	Male	42 y/o	Hispanic	White	DLE with SLE	Benlysta 200mg Qweekly, methotrexate 25mg Qweekly, plaquenil 200mg, Kenalog, folic acid 1mg QD, betamethasone	CXCL9, 11, 13, 6, CCL3, 25, 8	CCR1, CXCR3, CCR5, CCR7, CXCR1, CXCR5, CCR9, CCR2
D	Female	43 y/o	White	White	CLE/Sjorgens	Plaquenil	N/A	CCR1, CXCR3, CCR5, CCR7, CXCR1, CXCR5, CXCR2, CCR2
E	Female	48 y/o	White	White	CCLE with SLE, also has non scarring alopecia	Triamcinolone 0.1% PRN, protopic 0.1% PRN, hydroxychloroquine 300mg QD	N/A	CCR1, CXCR3, CCR5, CCR7, CXCR1, CXCR5, CCR9, CCR2
F	Female	35 y/o	Middle eastern	White	Healthy		N/A	CCR1, CXCR3, CCR5, CCR7, CXCR1, CXCR5, CXCR2, CCR2
G	Female	34 y/o	Middle eastern	White	Healthy		CXCL9, 11,13, CCL3	CCR1, CXCR3, CCR5, CCR7, CXCR1, CXCR5, CXCR2, CCR2
H	Female	36 y/o	Hispanic	White	Healthy		CXCL9, 11, 13, 6, CCL3, 25, 8	CCR1, CXCR3, CCR5, CCR7, CXCR1, CXCR5, CCR9, CCR2
I	Male	28 y/o	Middle eastern	White	Healthy; Hypothyroidism	Levothyroxine	CXCL9, 11, 6, CCL3, 8	CCR1, CXCR3, CCR5, CCR7, CXCR1, CXCR5, CCR9, CCR2
J	Female	28 y/o	Middle eastern	White	Healthy		CXCL9, 11, 13, 6, CCL3, 25, 8	CCR1, CXCR3, CCR5, CCR7, CXCR1, CXCR5, CCR9, CCR2
K	Male	26 y/o	Asian	White	Healthy		CXCL6 dose curve	CCR1, CXCR3, CCR5, CCR7, CXCR1, CXCR5, CCR9, CCR2

DLE = discoid lupus erythematosus; SCLE = subacute cutaneous lupus erythematosus; SLE = systemic lupus erythematosus; N/A = not applicable

Table S6. Antibody information & RRIDs.

Marker	Company	Catalog No.	Lot No.	Dose	RRID
CD3 (BV510)	Biolegend	344828	B370758	5ul/test	RRID:AB_2563704
CD8 (BV605)	Biolegend	344742	B370756	5ul/test	RRID:AB_2566513
CD4 (Alexa Flour 700)	Biolegend	344622	B365788	5ul/test	RRID:AB_2563150
CD14 (Spark Blue 550)	Biolegend	367148	B361330	5ul/test	RRID:AB_2832724
CD56 (BUV737)	BD Biosciences	612766	2332923	5ul/test	RRID:AB_2813880
CD19 (Spark Nir 685)	Biolegend	302270	B358744	5ul/test	RRID:AB_2832581
CD16 (BV711)	Biolegend	360732	B397395	5ul/test	RRID:AB_2800992
HLA-DR (PE)	Biolegend	307606	B373145	5ul/test	RRID:AB_314684
CCR1 (PerCP/Cyanine 5.5)	Biolegend	362912	B323489	5ul/test	RRID:AB_2728353
CXCR3 (PECy7)	Biolegend	353720	B322685	5ul/test	RRID:AB_11219383
CCR5 (BUV563)	BD Biosciences	741401	3198452	5ul/test	RRID:AB_2870893
CCR7 (PE/Fire 640)	Biolegend	353262	B351674	5ul/test	RRID:AB_2876669
CXCR1 (APC)	Biolegend	320612	B397637	5ul/test	RRID:AB_2126475
CXCR5 (BV421)	Biolegend	356920	B381006	5ul/test	RRID:AB_2562303
CXCR2 (PE/Dazzle 594)	Biolegend	320722	B381595	5ul/test	RRID:AB_2750215
CCR2 (BV785)	Biolegend	357234	B366953	5ul/test	RRID:AB_2800972
CCR9 (PE/Dazzle 594)	Biolegend	358918	B384511	5ul/test	RRID:AB_2715935
Live/Dead (Zombie NIR Dye)	Biolegend	77184	B369131	5ul/test	N/A

Table S7. Chemokine information.

Reagent	Company	Catalog #	Lot #	Dose (s) tested in chemotaxis assay
CCL3	Biolegend	759504	B364645	10, 50ng/mL
CCL19	Biolegend	582104	B361963	30 ng/ml
CCL23	Biolegend	587002	B389072	10 ng/ml
CCL25	Biolegend	586804	B272484	200 ng/ml
CXCL6	R&D Systems	333-GC-025/CF	AMM0222041	5, 50, 100, 200, 500 ng/ml
CXCL9	Biolegend	578104	B366484	100 ng/ml
CXCL11	Biolegend	574904	B312963	200ng/mL
CXCL13	Biolegend	574704	B255322	500 ng/ml
CX3CL1	R&D Systems	365-FR-025/CF	AF50521041	100 ng/ml
CCL2	Biolegend	571404	B365559	50 ng/ml
CCL8	Biolegend	581604	B370430	50, 100 ng/ml