

SUPPLEMENTARY FILE

Targeted multi-omics in childhood-onset SLE reveal distinct biological phenotypes associated with disease activity; results from an explorative study
Wahadat et al.

SUPPLEMENTARY METHODS

FIGURES

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Supplementary Methods

Study population details

Demographics, laboratory parameters and clinical characteristics including disease activity parameters and medication usage at previous and subsequent clinical visits from time of study blood sampling were collected. Disease activity was assessed by the clinical Safety of Estrogen in Lupus National Assessment (SELENA)-SLEDAI at each visit (2). Disease flares were indicated by an increase of >3 in the clinical SELENA-SLEDAI from the previous visit and/or an intensification of treatment, respectively. Low Lupus Disease Activity State (LLDAS) was defined as described before (3).

Cytokine measurement details

Serum samples were thawed on ice and centrifuged at $1300 \times g$ for 5 min at room temperature. Twofold dilutions were prepared in low-protein-binding plates according to the manufacturer's instructions. For TGF β 1 detection, samples were first activated with 1 N HCl and then neutralized with 1.2 N NaOH/0.5 M HEPES. Subsequently, these samples were 1:15 diluted and analyzed in the ELLA Simple Plex system (SPCKB-PS-1369/237/934/230/1108, SPCKC-PS-3649, SPCKE-PS-3426).

RT-PCR of genes from four gene modules

Blood was collected in PAXgene RNA tubes and stored in -80°C until use for whole blood RNA purification. RNA was isolated from PAXgene Blood RNA Tubes according to manufacturer's protocol (PreAnalytiX GmbH). cDNA was synthesized using the High-Capacity Reverse Transcription Kit (Applied Biosystems, Bleiswijk, The Netherlands). RT-PCR was performed on a QuantstudioTM 5 Real-Time PCR System using predesigned primer/probe sets (Applied Biosystems). Genes were selected based on four previously described gene modules (4). For calculation of

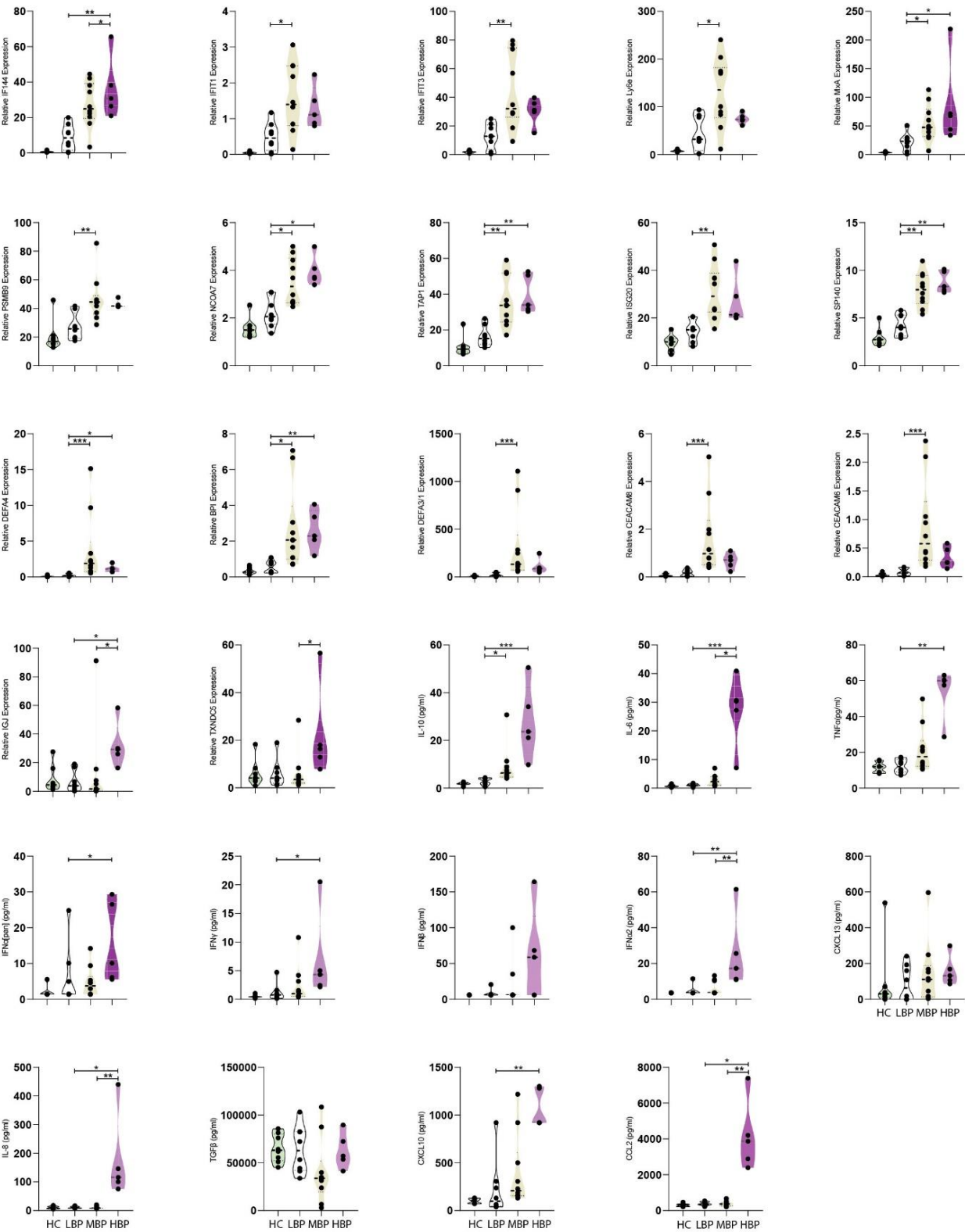
28 relative gene expressions, samples were normalized to expression of the
29 housekeeping gene Abl. Relative expression values were determined from
30 normalized CT values using the $2^{-\Delta CT}$ method (4).

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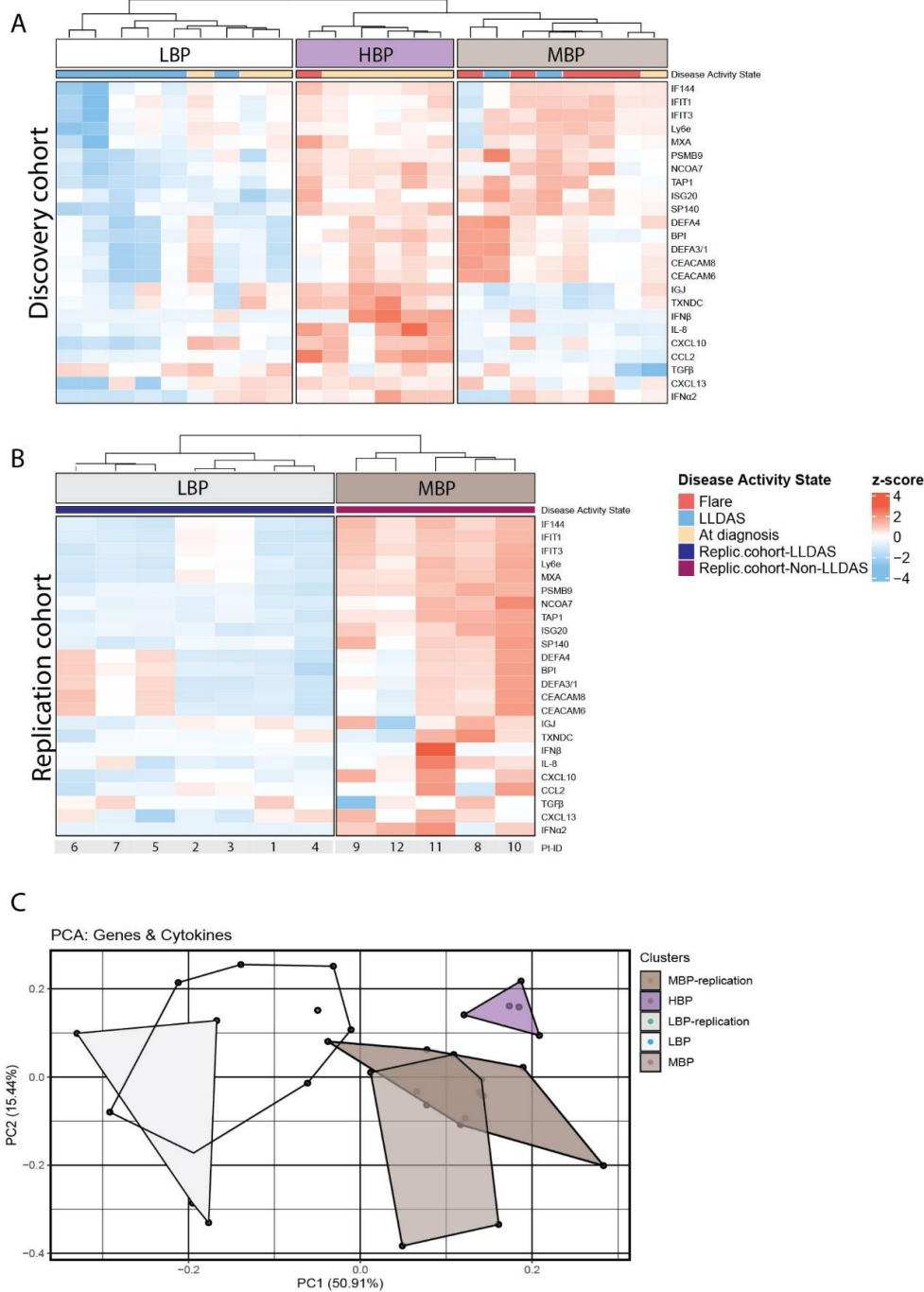
34 **Supplementary figure S1 Biological phenotypes show distinct gene expression**
35 **and cytokine levels.** Violin plots indicating different gene expression ($2^{\Delta\Delta CT}$) and
36 cytokines levels (pg/ml). Each dot represents one patient. Kruskal-Wallis test with
37 Dunn's multiple comparisons posthoc test was used for relation between the clusters.
38 HC, healthy controls, LBP, low biological phenotype; HBP, high biological phenotype;
39 MBP, mixed biological phenotype.



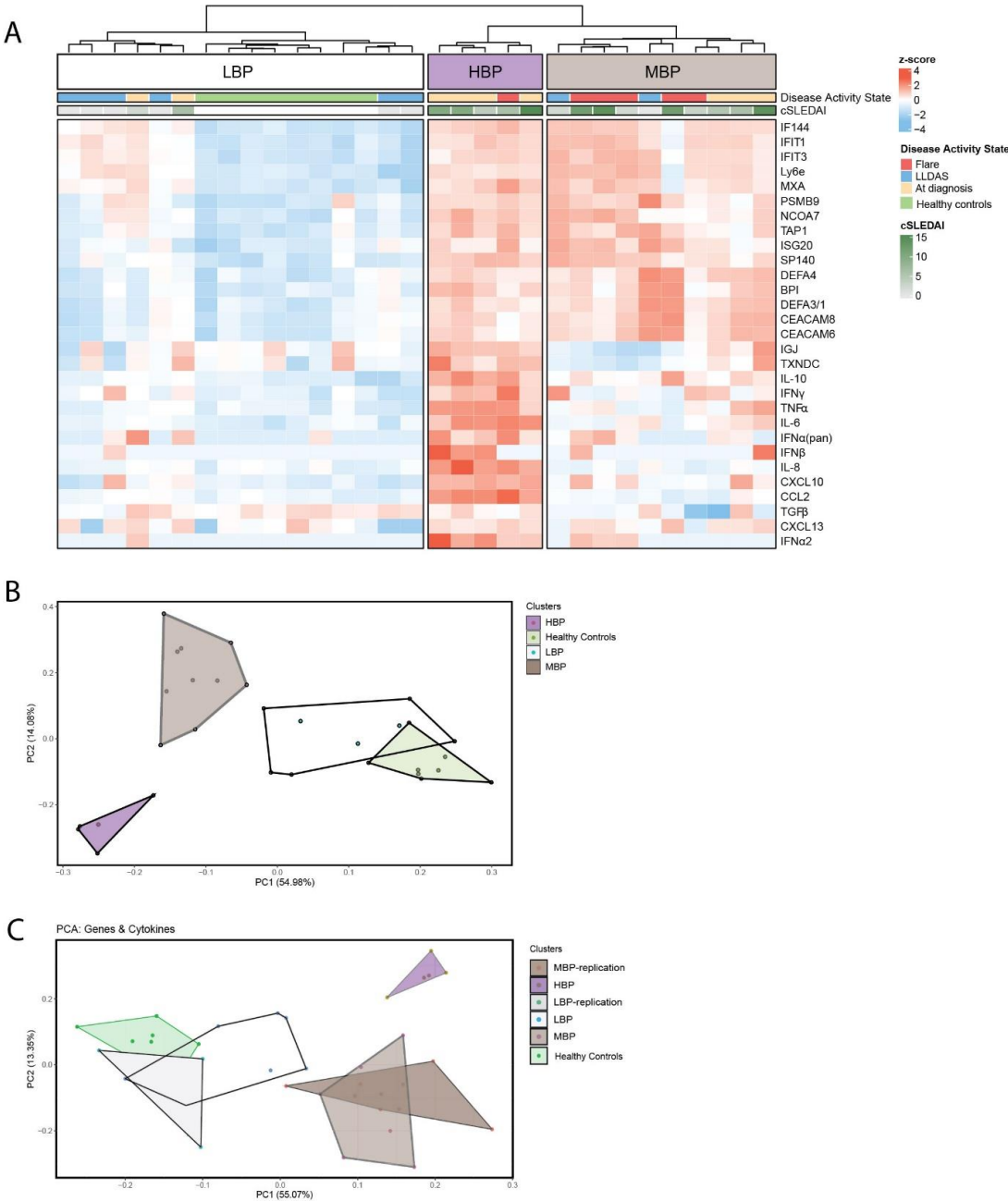
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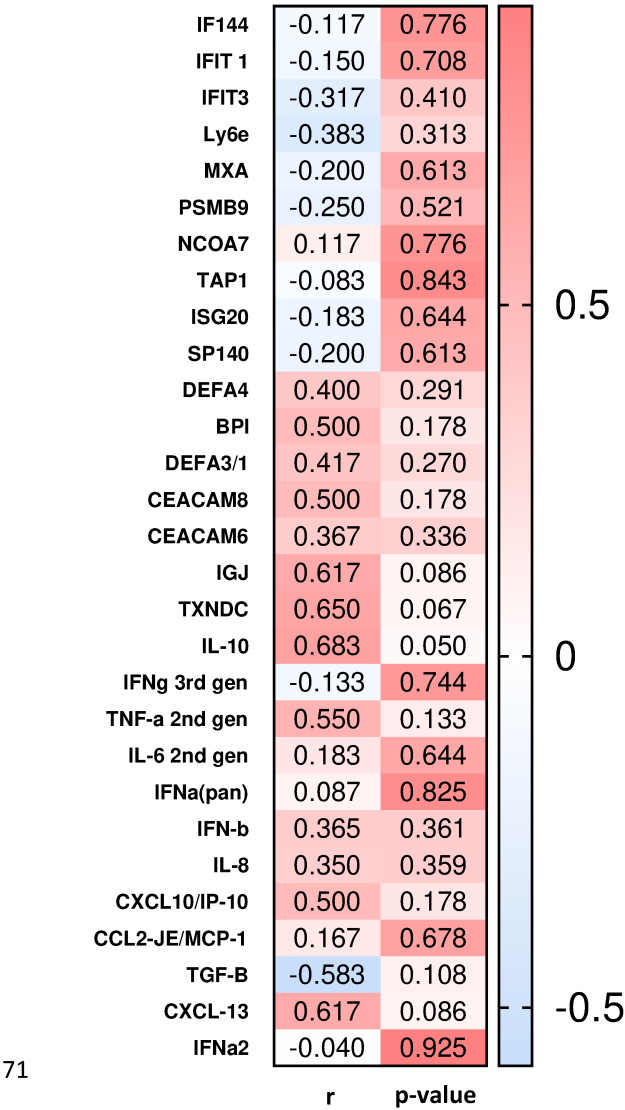
Supplementary figure S3 Validation of biological phenotypes in an independent cSLE cohort. A/B). Unsupervised hierarchical clustering using Ward’s agglomerative method and passing the Euclidean distance between samples, using row-based log-transformed z-scores, identified three clusters in the discovery cohort and two clusters in the replication cohort. **C).** Principal component analysis (PCA) showing that there is overlap between the LBP group from the discovery cohort and LBP group of the replication cohort. Patients in the MBP from the discovery cohort and replication cohort show overlap. The first two components with their percentage of variance are shown in parentheses.



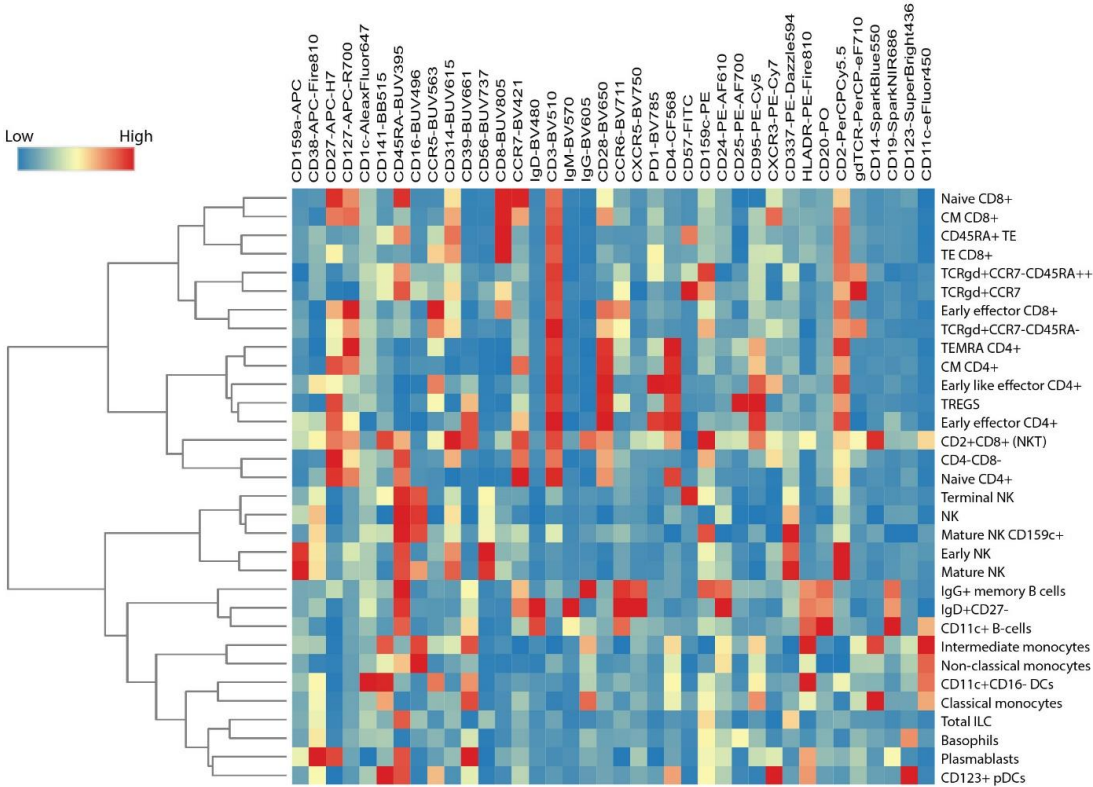
Supplementary figure S4. Unsupervised hierarchical clustering groups healthy controls within the low biological phenotype cluster of cSLE patients A) Unsupervised hierarchical clustering using Ward’s agglomerative method, passing the Euclidean distance between samples, using row-based log-transformed z-scores, identified 3 clusters. Healthy controls cluster in the LBP group. **B)** PCA shows overlap between the LBP and healthy control group. **C)** PCA shows overlap between healthy controls and patients in the LBP group from the discovery and replication cohort. Red-blue color indicates the z-scores. LBP, low biological phenotype; HBP, high biological phenotype; MBP, mixed biological phenotype.



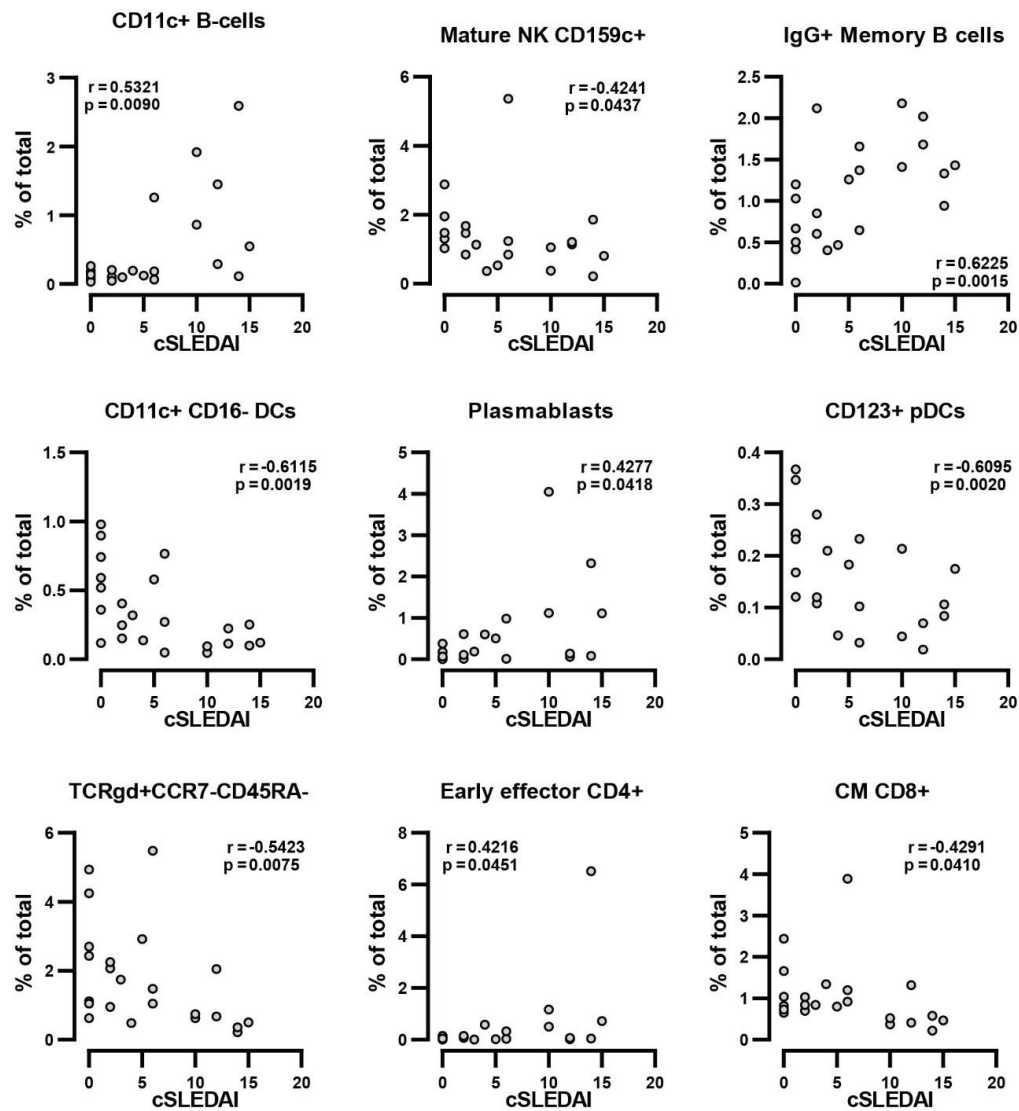
67 **Supplementary figure S5 Biological phenotypes are not influenced by the use**
68 **of corticosteroids.** Heatmap indicating the Spearman's rho and p-value between
69 prednisone use in mg/kg/day vs gene expression or cytokine levels. r represents
70 Spearman's Rho.



Supplementary figure S6 FLOWSom analysis identified 32 distinct immune cell subsets. Clustered heatmap indicating the expression of various immune cell marker. Red indicates a high expression while blue indicates a low expression.



79 **Supplementary figure S7 Clinical SELENA-SLEDAI is significantly associated**
80 **with 9 immune cell populations** Correlation plots depicting correlations between 9
81 immune cell populations and the clinical SELENA-SLEDAI (N=23). r represents
82 Spearman's rho and p indicates the p-value.



Supplementary table T1 Characteristics of replication cohort and healthy controls

ID	Gender	Age (years)	Ethnicity	Disease duration (years)	Clinical SELENA- SLEDAI	BILAG-2004	Anti-dsDNA (IU/mL) [#]	Auto-antibodies	Treatment at visit				Disease Activity State	Biological Phenotype ^{\$}
									HCQ	Prednisone (mg/kg/day)	NSAIDs	DMARD		
Pt1	Male	10	Non-White	5.44	0	0	0.9	-	Yes	.	No	No	LLDAS	LBP
Pt2	Male	13.4	Non-White	1.17	0	6	0.7	Anti-SSA	Yes	.	No	Yes	LLDAS	LBP
Pt3	Male	16.8	Non-White	4.56	0	1	41	Anti-SSA	Yes	.	No	Yes	LLDAS	LBP
Pt4	Female	17.8	White	2.81	0	1	0.4	-	Yes	.	No	No	LLDAS	LBP
Pt5	Female	16.7	White	3.93	2	10	4.1	-	Yes	.	No	Yes	LLDAS	LBP
Pt6	Female	18.1	Non-White	2.05	0	2	2.9	-	Yes	0.16	No	Yes	LLDAS	LBP

*Active refers to patients with a cSELENA-SLEDAI >4 who are not at diagnosis or have a flare
#Measured with a fluorometric enzyme immunoassay, cut-off is 10 IU/mL
**DMARD refers to mycophenolate mofetil, azathioprine, cyclophosphamide, methotrexate
\$LBP; Low Biological Phenotype, HBP; High Biological Phenotype, MBP; Mixed Biological Phenotype

Pt7	Female	17.6	White	2.42	0	6	0.1	-	Yes	.	No	Yes	LLDAS	LBP
Pt8	Female	16.4	White	0.1	5	11	0.9	Anti-SSA	Yes	0.17	No	Yes	Active*	MBP
Pt9	Female	17.8	White	3.48	5	10	10	Anti-Sm	Yes	.	No	Yes	Active*	MBP
Pt10	Female	16.5	White	0.73	4	6	0.5	Anti-SSA/B	Yes	0.12	Yes	Yes	Active*	MBP
Pt11	Female	16.2	White	0.64	8	11	93	Anti-RNP, Anti-Sm	Yes	0.08	No	Yes	Active*	MBP
Pt12	Female	16.9	White	2.18	4	5	9.5	AntiSSA/B, Anti-Sm	Yes	0.05	No	Yes	Active*	MBP
HC1	Female	17.9	White	-	-	-	-	-	-	-	-	-	-	LBP
HC2	Female	17.1	White	-	-	-	-	-	-	-	-	-	-	LBP
HC3	Male	18.3	White	-	-	-	-	-	-	-	-	-	-	LBP
HC4	Male	16.5	White	-	-	-	-	-	-	-	-	-	-	LBP
HC5	Female	14	White	-	-	-	-	-	-	-	-	-	-	LBP
HC6	Female	14.7	White	-	-	-	-	-	-	-	-	-	-	LBP
HC7	Male	12.4	White	-	-	-	-	-	-	-	-	-	-	LBP
HC7	Female	12.4	White	-	-	-	-	-	-	-	-	-	-	LBP
HC8	Female	17.9	White	-	-	-	-	-	-	-	-	-	-	LBP

Supplementary table T2. A positive logFC illustrates higher number of immune cell subsets in cSLE patients compared to HC, while a negative value indicates a higher number of immune cell subsets in the HC. The bold line indicates the threshold of 0.1.

HC vs cSLE			
	<i>logFC</i>	<i>p-value</i>	<i>FDR</i>
<i>Central memory CD8+</i>	-1.74711	7.20E-08	2.30E-06
<i>Mature NK CD159c+</i>	-1.63086	2.63E-07	4.21E-06
<i>TCRgd+CCR7-CD45RA-</i>	-1.84315	1.39E-06	1.49E-05
<i>NK</i>	-1.09615	0.000176	0.001411
<i>Terminal NK</i>	-2.05502	0.000638	0.003103
<i>CD123+ pDCs</i>	-1.29871	0.000664	0.003103
<i>CD11c+ B cells</i>	2.515655	0.000679	0.003103
<i>early like effector CD4+</i>	1.666455	0.000931	0.003723
<i>Early effector CD4+</i>	3.789902	0.001646	0.005854
<i>Plasmablasts</i>	3.038351	0.003022	0.009388
<i>CD4-CD8-</i>	0.921128	0.003259	0.009388
<i>TEMRA CD4+</i>	-0.7998	0.003521	0.009388
<i>CD11c+CD16- DCs</i>	-1.06455	0.010298	0.024734
<i>Total ILC</i>	-1.11639	0.010821	0.024734
<i>Non-classical monocytes</i>	-0.84832	0.060216	0.122393
<i>Early effector CD8+</i>	-1.21033	0.061197	0.122393
<i>Naive CD4+</i>	0.531037	0.078319	0.144305
<i>CD2+CD8+ (NKT)</i>	0.711644	0.081171	0.144305
<i>CD45RA+ Terminal effector</i>	1.318206	0.088535	0.149111
<i>IgD+CD27-</i>	0.476732	0.148027	0.23457
<i>TREGs</i>	0.50859	0.155947	0.23457
<i>Intermediate monocytes</i>	0.802366	0.161267	0.23457
<i>TCRgd+CCR7-CD45RA++</i>	-0.78278	0.24337	0.333149
<i>Naïve CD8+</i>	0.297481	0.256112	0.333149
<i>Basophils</i>	-0.38973	0.260273	0.333149
<i>Terminal effector CD8+</i>	-0.35758	0.47405	0.582145
<i>Central memory CD4+</i>	-0.15357	0.495708	0.582145
<i>IgG+ memory B cells</i>	0.356027	0.509377	0.582145
<i>Early NK</i>	0.291065	0.580484	0.640535
<i>Classical monocytes</i>	-0.14209	0.62904	0.670976
<i>Mature NK</i>	0.082463	0.917493	0.94709
<i>TCRgd+CCR7-</i>	0.065349	0.983709	0.983709

Supplementary table T3. A positive logFC illustrates higher number of immune cell subsets in the HBP group compared to LBP group, while a negative value indicates a higher number of immune cell subsets in the LBP group. The bold line indicates the threshold of 0.1.

HBP vs LBP			
	<i>logFC</i>	<i>p-value</i>	<i>FDR</i>
<i>CD11c+CD16- DCs</i>	-2.54453	1.36E-07	4.34E-06
<i>Early effector CD4+</i>	3.032646	1.68E-06	2.69E-05
<i>CD11c+ B cells</i>	2.80641	2.54E-06	2.71E-05
<i>Non-classical monocytes</i>	-2.44421	2.15E-05	0.000172
<i>Terminal NK</i>	-3.19247	0.000272	0.001493
<i>TCRgd+CCR7-CD45RA-</i>	-1.99941	0.00028	0.001493
<i>TCRgd+CCR7-</i>	-3.79634	0.002806	0.012827
<i>Total ILC</i>	-1.64627	0.004236	0.016945
<i>Mature NK CD159c+</i>	-1.18301	0.006739	0.02396
<i>Plasmablasts</i>	2.517105	0.009603	0.03073
<i>Early effector CD8+</i>	-2.55299	0.01336	0.038865

<i>Intermediate monocytes</i>	-2.10203	0.015138	0.040369
<i>Classical monocytes</i>	-0.97833	0.01767	0.043496
<i>Mature NK</i>	-1.48163	0.019892	0.045467
<i>Early NK</i>	-1.41406	0.025771	0.054978
<i>CD123+ pDCs</i>	-1.1826	0.046643	0.093287
<i>CD2+CD8+ (NKT)</i>	0.868515	0.058235	0.109619
<i>Central memory CD8+</i>	-0.83291	0.076628	0.136228
<i>Naive CD4+</i>	0.566811	0.098094	0.165211
<i>TEMRA CD4+</i>	-0.68883	0.106759	0.165652
<i>CD4-CD8-</i>	0.562335	0.108709	0.165652
<i>early like effector CD4+</i>	0.569636	0.325099	0.472871
<i>TREGs</i>	-0.4511	0.341199	0.474711
<i>IgG+ memory B cells</i>	0.671926	0.416964	0.555952
<i>NK</i>	-0.31752	0.487388	0.623857
<i>Terminal effector CD8+</i>	0.362942	0.551063	0.678231
<i>Central memory CD4+</i>	0.111494	0.750093	0.888999
<i>Naive CD8+</i>	-0.10091	0.792288	0.905472
<i>Basophils</i>	-0.13687	0.854158	0.942519
<i>TCRgd+CCR7-CD45RA++</i>	-0.14647	0.925646	0.987355
<i>IgD+CD27-</i>	-0.03022	0.966962	0.993822
<i>CD45RA+ Terminal effector</i>	-0.04186	0.993822	0.993822

Supplementary table T4. A positive logFC illustrates higher number of immune cell subsets in the HBP group compared to MBP group, while a negative value indicates a higher number of immune cell subsets in the MBP group. The bold line indicates the threshold of 0.1.

HBP vs MBP			
	<i>logFC</i>	<i>p-value</i>	<i>FDR</i>
<i>TCRgd+CCR7-</i>	-4.64398	0.000351	0.011237
<i>Non-classical monocytes</i>	-2.4343	0.000786	0.012582
<i>Classical monocytes</i>	-1.24328	0.005736	0.061188
<i>Plasmablasts</i>	2.307618	0.012353	0.075124
<i>Total ILC</i>	-1.3371	0.014008	0.075124
<i>Terminal NK</i>	-2.04466	0.018816	0.075124
<i>Early NK</i>	-1.75968	0.020078	0.075124
<i>TCRgd+CCR7-CD45RA-</i>	-1.79162	0.020948	0.075124
<i>Intermediate monocytes</i>	-1.37744	0.021129	0.075124
<i>Early effector CD8+</i>	-2.44316	0.025614	0.081965
<i>Mature NK</i>	-1.71238	0.038231	0.10636

<i>CD4-CD8-</i>	0.841503	0.039885	0.10636
<i>CD2+CD8+ (NKT)</i>	0.872938	0.051328	0.126346
<i>CD11c+CD16- DCs</i>	-1.07787	0.056967	0.13021
<i>Naive CD4+</i>	0.65101	0.136929	0.292114
<i>early like effector CD4+</i>	0.82462	0.170632	0.332529
<i>Central memory CD4+</i>	0.48435	0.176656	0.332529
<i>NK</i>	0.488394	0.193316	0.343674
<i>Mature NK CD159c+</i>	-0.61621	0.22644	0.381372
<i>CD11c+ B cells</i>	0.569034	0.448662	0.717858
<i>TEMRA CD4+</i>	-0.339	0.485386	0.739635
<i>Terminal effector CD8+</i>	-0.58677	0.558822	0.812831
<i>IgD+CD27-</i>	-0.27533	0.591283	0.822654
<i>CD45RA+ Terminal effector</i>	-0.5539	0.642539	0.856719
<i>CD123+ pDCs</i>	-0.27541	0.705776	0.863231
<i>TREGs</i>	-0.2265	0.71051	0.863231
<i>Early effector CD4+</i>	0.290938	0.751142	0.863231
<i>Central memory CD8+</i>	-0.16058	0.755327	0.863231
<i>Basophils</i>	-0.16103	0.819215	0.903961
<i>IgG+ memory B cells</i>	-0.07282	0.92338	0.969227
<i>Naive CD8+</i>	-0.03214	0.959364	0.969227
<i>TCRgd+CCR7-CD45RA++</i>	-0.02574	0.969227	0.969227

Supplementary table T5. A positive logFC illustrates higher number of immune cell subsets in the LBP group compared to MBP group, while a negative value indicates a higher number of immune cell subsets in the MBP group. The bold line indicates the threshold of 0.1.

LBP vs MBP			
	<i>logFC</i>	<i>p-value</i>	<i>FDR</i>
<i>CD11c+CD16- DCs</i>	1.466663	0.0008	0.025602
<i>CD11c+ B cells</i>	-2.23738	0.002541	0.040661
NK	0.80591	0.02366	0.244704
<i>Early effector CD4+</i>	-2.74171	0.030588	0.244704
<i>CD123+ pDCs</i>	0.907191	0.045984	0.294297
<i>Central memory CD8+</i>	0.672321	0.069403	0.370152
<i>Terminal NK</i>	1.147809	0.084308	0.385409
<i>Mature NK CD159c+</i>	0.566798	0.130962	0.523849
<i>Central memory CD4+</i>	0.372856	0.203745	0.637633
<i>Terminal effector CD8+</i>	-0.94971	0.216119	0.637633
<i>TCRgd+CCR7-CD45RA-</i>	0.621976	0.219186	0.637633
<i>IgG+ memory B cells</i>	-0.74475	0.27453	0.710676
<i>TEMRA CD4+</i>	0.349824	0.288712	0.710676
<i>TCRgd+CCR7-</i>	-0.84764	0.383948	0.870212
<i>CD4-CD8-</i>	0.279167	0.429992	0.870212
<i>Classical monocytes</i>	-0.26495	0.436739	0.870212
<i>Total ILC</i>	0.309179	0.520564	0.870212
<i>Intermediate monocytes</i>	-0.24511	0.552167	0.870212
<i>IgD+CD27-</i>	0.310412	0.552199	0.870212
<i>Early NK</i>	-0.34562	0.5531	0.870212
<i>TREGs</i>	0.224608	0.597906	0.870212
<i>CD45RA+ Terminal effector</i>	-0.51204	0.598271	0.870212
<i>early like effector CD4+</i>	0.254984	0.638872	0.888865
<i>Mature NK</i>	-0.23075	0.736046	0.970168
<i>Naïve CD4+</i>	0.0842	0.811962	0.970168
<i>Naïve CD8+</i>	0.068766	0.828235	0.970168
<i>Plasmablasts</i>	-0.20949	0.85335	0.970168
<i>TCRgd+CCR7-CD45RA++</i>	0.120722	0.877862	0.970168
<i>Early effector CD8+</i>	0.109827	0.879215	0.970168
<i>Basophils</i>	-0.02416	0.963336	0.985693
<i>Non-classical monocytes</i>	0.009906	0.974538	0.985693
<i>CD2+CD8+ (NKT)</i>	0.004424	0.985693	0.985693

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