Supplemental Methods

Gene expression meta-analysis

We used R package MetaIntegrator for integrating discovery cohorts as described previously(125) to identify differentially expressed genes between healthy controls and patients with SLE. First, we computed an effect size for each gene in each study as Hedges' adjusted g. Next, we summarized the effect sizes across all studies for each gene using DerSimonian-Laird method for random effects inverse variance model, where each effect size is weighted by the inverse of the variance in that study. Finally, we corrected the p-values for the summary effect size of each gene for multiple hypothesis testing using Benjamini-Hochberg false discovery rate (FDR)(126).

Briefly, we downloaded gene expression microarray data from 40 independent experiments with 7,471 samples from the Gene Expression Omnibus (GEO)(127). Accession numbers, tissue of origin, data center, sample size, and PubMed identifiers (IDs) for all datasets are listed in Table 1. We randomly selected 6 datasets composed of 370 whole blood and PBMC samples as "Discovery" datasets, based on our previous finding that 4-5 datasets with 250-300 samples have sufficient statistical power to find a robust reproducible disease gene signature using our multi-cohort analysis framework. The six experiments used in the discovery set were required to have both SLE patients and healthy volunteers for use as cases and controls, respectively, and were limited to analyses of PBMCs or blood. Based on previous analyses, a combined sample size of 370 patients from six datasets was considered sufficient for the discovery phase of the meta-analysis(26). We used eight gene expression datasets with 2,407 samples from individuals who have SLE and healthy volunteers in PBMCs or whole blood as a validation set. The remaining 26 datasets and 4,694 samples, which include sorted cell and tissue data, were assigned to the extended validation set.

We used the following thresholds in our meta-analysis of the discovery set to select genes in the SLE MetaSignature: absolute value of effect size greater than one; false discovery rate less than five percent; and measurements of individual genes in the identified signature in at least four datasets. We then used the genes in the SLE MetaSignature to calculate an SLE MetaScore for each patient sample. To avoid an overrepresentation of up or down-regulated genes in the SLE MetaScore, we modified the default MetaIntegrator calculateScore function. The modified function calculated the geometric mean of the up-regulated genes and the inverse of the down-regulated genes (rather than subtracting the geometric mean of the down-regulated genes from the geometric mean of the up-regulated genes). We then used the z-score to scale the geometric means across samples in the same study.

SLEDAI correlation significance

We generated 100 random gene sets with the same number of positive and negative genes as the SLE MetaSignature. We evaluated the correlation for each of these gene sets with SLEDAI using the same method as the SLE MetaScore. For each randomization, we evaluated the median correlation across all 5 studies with SLEDAI measurements.

IFN meta-analysis

We downloaded gene expression microarray data from 15 datasets [Table S2] of primary human cells stimulated with Type I IFN from GEO(25) using the MetaIntegrator R package(15). Using MetaIntegrator, we ran meta-analysis with unstimulated cells treated as controls and type I IFN stimulations as cases. In our analysis, we utilized the effect size and effect size FDR estimates for IFN based on our meta-analysis.

Neutrophil, NK cell, and heavy metal datasets

We identified 7 relevant datasets for our follow-on analyses of the SLE MetaSignature and downloaded them from GEO(25) using the MetaIntegrator R package(15). These datasets included stimulations to induce NETosis, intermediate cell populations from neutropoeisis, and heavy metal exposures in cell lines.

Pathway analysis

Pathway analysis was performed using the Differential Expression Analysis for Pathways (DEAP) tool(28). As input, gene effect size measurements were used from all discovery and validation datasets. 1000 random rotations of the data were performed to assess statistical significance. Pathways were downloaded from the PANTHER pathway database(128).

Stanford pSLE patient cohort

All subjects were recruited and all samples were collected following protocols approved by the Stanford University Institutional Review Board (IRB protocol #13952, 14734). Patients who fulfilled American College of Rheumatology (ACR) revised diagnostic criteria for SLE were consented at the Pediatric Rheumatology Clinic at Stanford Children's Health Lucile Packard Children's Hospital (LPCH), Stanford(129). Age-appropriate consent and assent was obtained. A total of 43 new-onset SLE patients were recruited along with 12 patients diagnosed with Juvenile Idiopathic Arthritis (JIA) as disease controls. Initial whole blood samples were obtained within a mean of 5 days of diagnosis. One patient (93) initially only had 3 ACR criteria, but was monitored and diagnosed with SLE (meeting 4 ACR criteria) ~3 years after the initial sample was obtained. This patient was not included in the comparison of SLE versus JIA. Clinical assessment of disease activity and treatment was conducted using a modified Safety of Estrogens in Lupus Erythematosus National Assessment Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI(130)), calculated for each visit. Whole blood samples were collected from 10 healthy volunteers.

Stanford pSLE cohort sample collection and RNA processing

At each patient visit, approximately 3ml of whole blood was collected into a Tempus™ Blood RNA Tube (Life Technologies #4342792) and frozen at -20°C for a minimum of 30 days. Batched samples were thawed and processed for RNA extraction using a Tempus™ RNA Isolation Kit (Life Technologies #4380204). All RNA samples were stored at -80°C prior to use. Samples were analyzed

using a Bioanalyzer (Agilent Technologies) to confirm RNA quality prior to assay. All samples had RNA Integrity Numbers (RIN) > 7.9.

Fluidigm Transcript Analysis

A panel of 33 genes was selected from the genes in the SLE MetaSignature, based on availability of TaqMan probes and meta-analysis effect size in a preliminary version of the SLE MetaSignature. Once we finalized the SLE MetaSignature, we used those genes which were in the final SLE MetaSignature, and for which we had acquired probes based on the preliminary analysis. TaqMan probes are listed in Table S4.

The Human Immune Monitoring Center (HIMC) at Stanford University performed transcript analysis. Reverse transcription was conducted at 50°C for 15 min using the High Capacity Reverse Transcription kit (ABI) and 10-50 ng total RNA. Pre-amplification of cDNA was performed using the TaqMan PreAmp Master Mix Kit (Invitrogen). TaqMan probes are listed in Table S1. Reverse transcriptase was inactivated and Taq polymerase reaction was initiated by bringing samples to 95°C for 2 min. The cDNA was preamplified by denaturing for 10 cycles at 95°C for 15 s, then annealing at 60°C for 4 min. The cDNA product was diluted 1:2 with 1x TE buffer (Invitrogen). 2X Applied Biosystems Taqman Master Mix, Fluidigm Sample Loading Reagent, and preamplified cDNA were mixed and loaded into the 48.48 Dynamic Array (Fluidigm) sample inlets, followed by 10X assays. Real-time PCR was carried out with the following conditions: 10 min at 95°C, followed by 50 cycles of 15 s at 95°C and 1 min at 60°C. All reactions were performed in duplicate. Gene Ct values were normalized to GAPDH, Beta-actin, and Beta-2-microglobulin. Average DCt values of controls were then subtracted from target gene DCt values to give ddCT. Relative gene expression levels were calculated as 2^{-ddCt}.

Statistical power analysis

We computed statistical power for the meta-analysis of gene expression between healthy controls and patients with SLE in both discovery and validation analysis(131) under no, low, moderate, or high heterogeneity assumption. In discovery datasets, we had >90% statistical power at p-value of 0.01 to detect effect size >0.44 and >0.9 when assuming no heterogeneity or high heterogeneity, respectively. In validation cohorts, we had >90% statistical power at p-value of 0.01 to detect effect size >0.3 and >0.59, when assuming no or high heterogeneity, respectively. In the cohort of whole blood samples from pediatric patients with SLE used for validation using Fluidigm, we had statistical power >99%.

Code and data availability

All code and data necessary for reproducing this analysis are available at https://wiki.khatrilab.stanford.edu/sle.

Statistics

The statistics related to gene expression meta-analysis, correlation analysis, pathway analysis, and statistical power analysis are all described in the appropriately title sections above. In general, a Benjamini-Hochberg False Discovery Rate (FDR) threshold of 0.05 was used to label any findings as "significant" in this manuscript.

Supplemental Figures

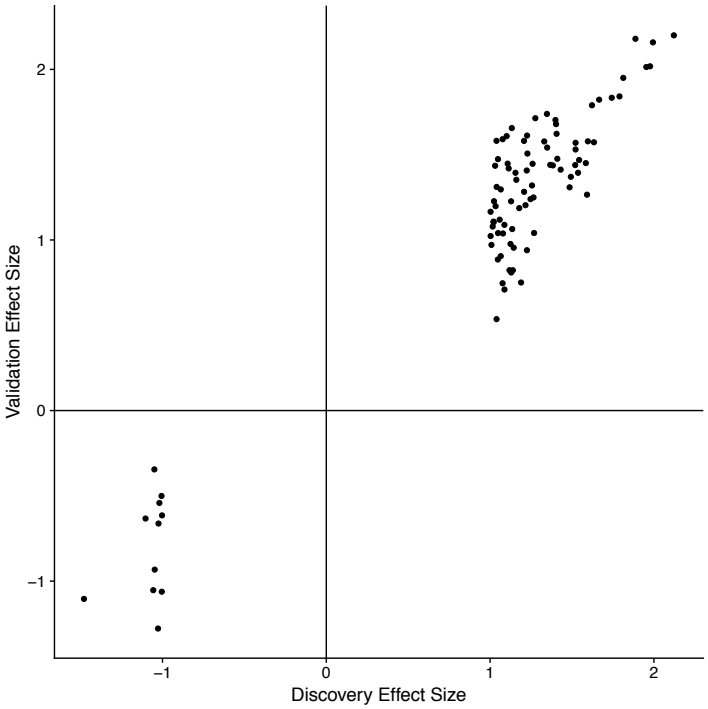

Figure S1. Discovery vs. validation effect sizes for the SLE MetaSignature. Effect sizes for all 93 genes from the SLE MetaSignature are displayed in both the discovery and validation data.

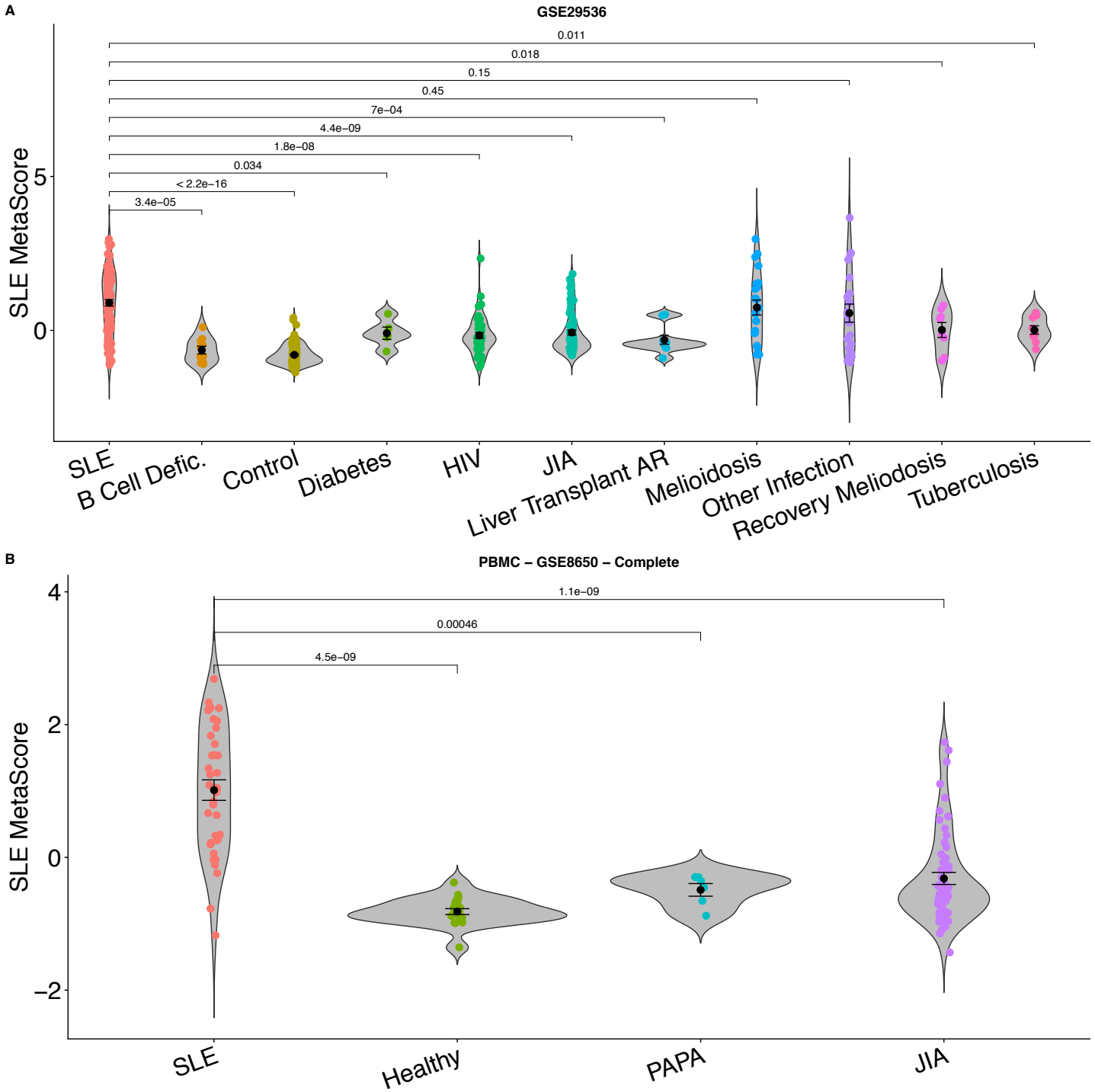

Figure S2. SLE compared to other diseases. Related to Figure 3. (A and B) Violin plots for additional datasets comparing SLE to non-SLE diseases. For all panels: B cell deficiency (B Cell Defic.); type 2 diabetes (Diabetes); human immunodeficiency virus (HIV); juvenile idiopathic arthritis (JIA); liver transplant acute rejection (Liver Transplant AR); pyogenic arthritis, pyoderma gangrenosum and acne (PAPA).

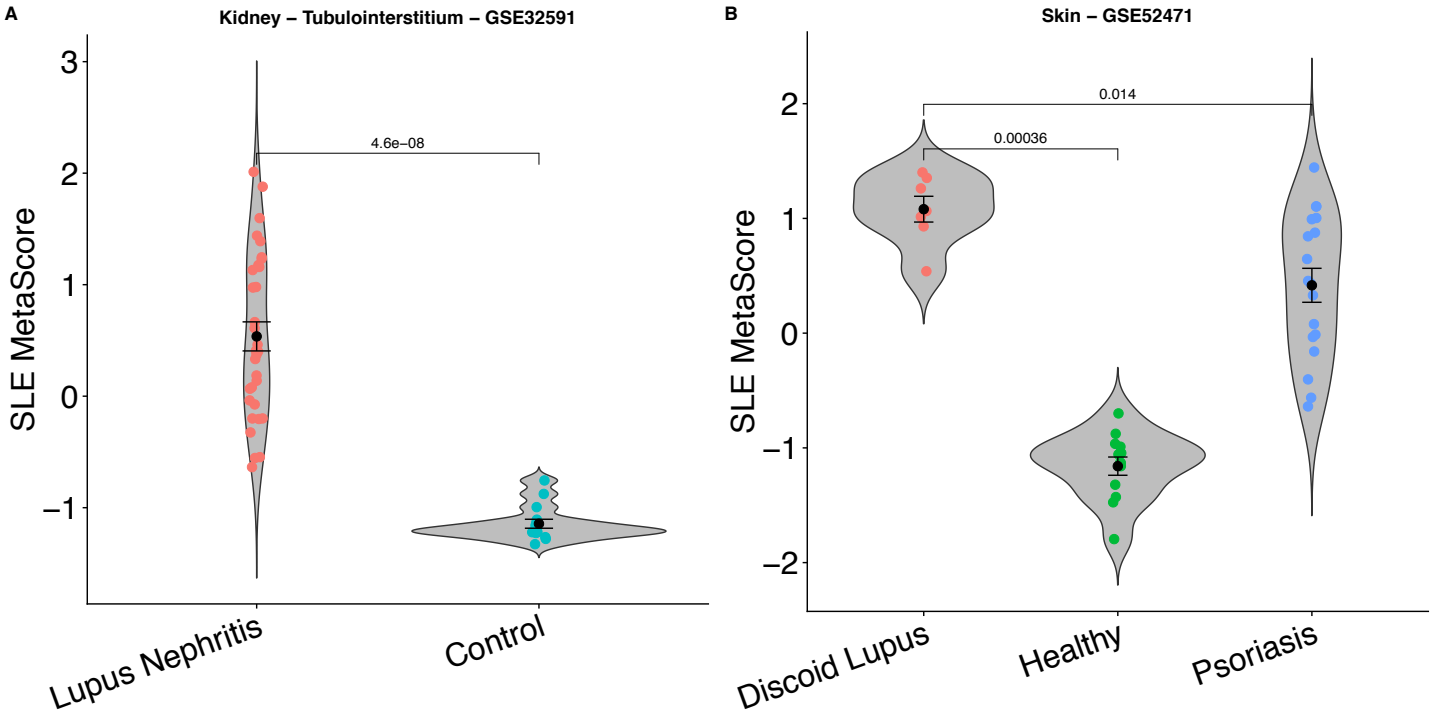

Figure S3. SLE MetaSignature in other tissues. Related to Figure 3. Violin plots for additional datasets examining SLE in kidney tubulointerstitium (A) and skin (B) tissues.

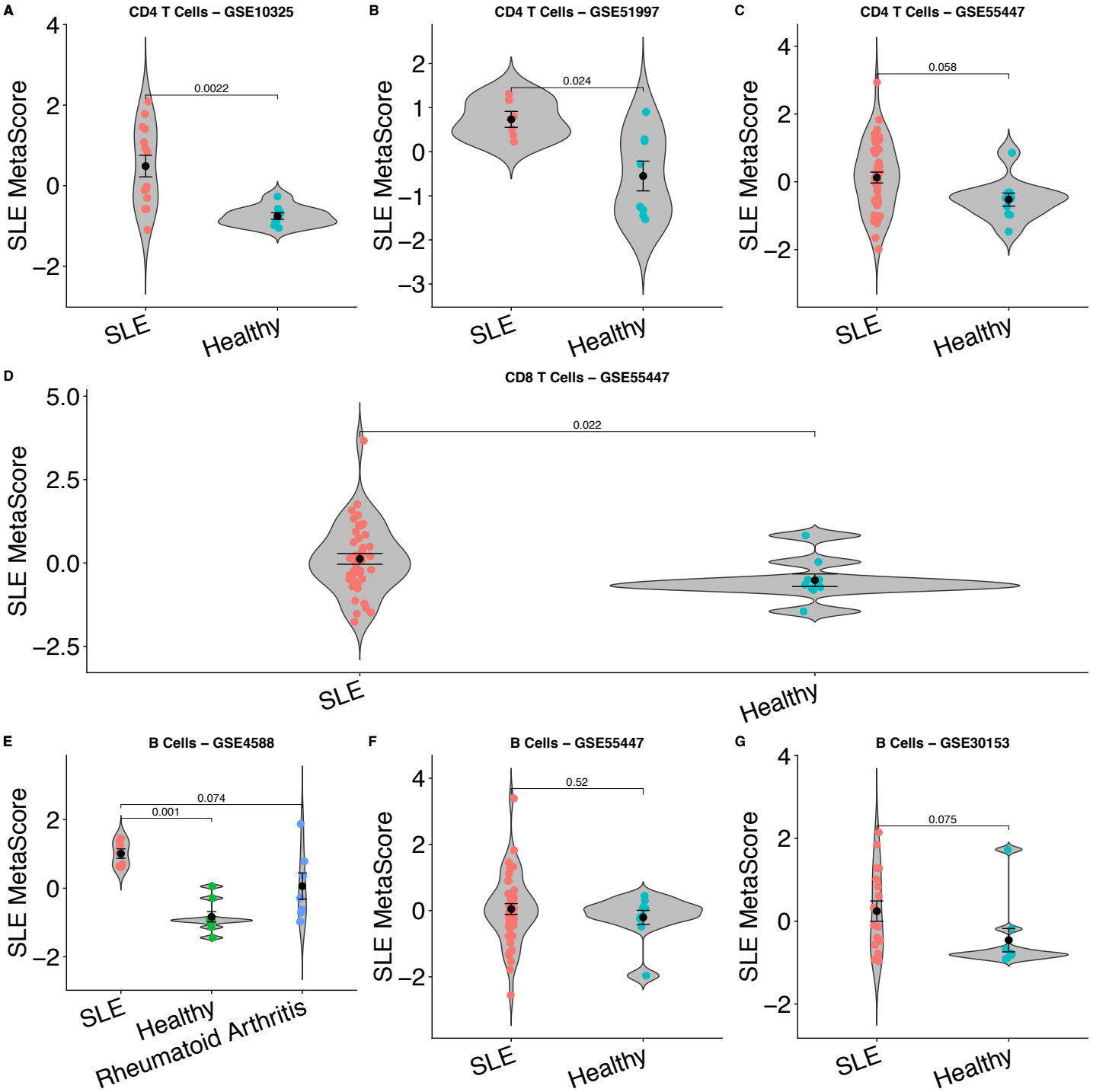

Figure S4. SLE MetaSignature in other cell types. Related to Figure 3. Violin plots for additional datasets examining the SLE MetaSignature in sorted cell populations, including CD4 T cells from different datasets (A-C), CD8 T cells (D), and B cells in different datasets (E-G).

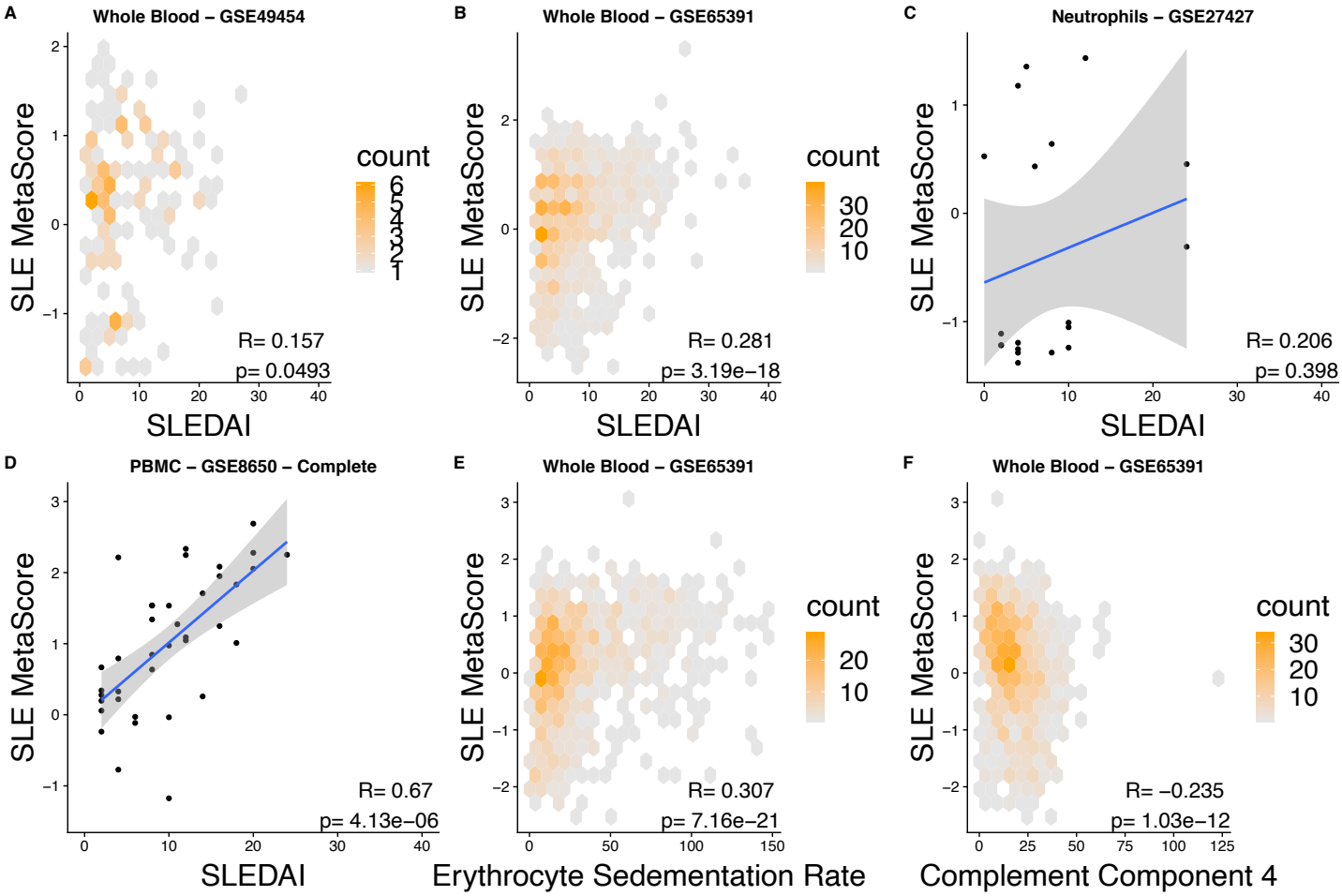

Figure S5. SLE disease activity. Related to Figure 4. (A-D) SLE MetaScore vs. SLEDAI in whole blood (A-B), neutrophil (C), and PBMC (D) samples from SLE patients. (E) SLE MetaScore vs. erythrocyte sedimentation rate. (F) SLE MetaScore vs. complement C4 levels in SLE patients.

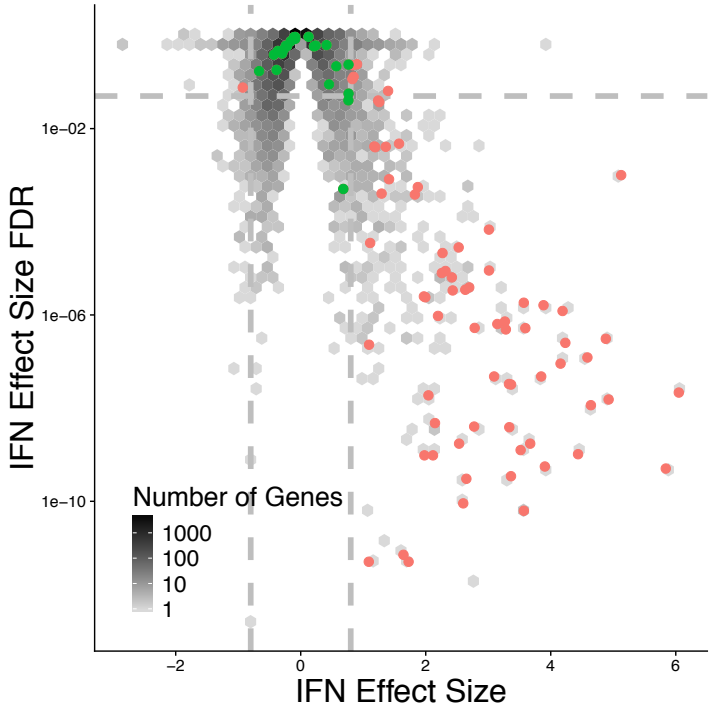

Figure S6. IFN volcano plot. IFN effect size vs. IFN effect size false discovery rate. Red points indicate the 70 genes which are in the SLE MetaSignature and are significantly different in response to IFN. Green points indicate the 23 genes which are in the SLE MetaSignature and are not significantly different in response to IFN.

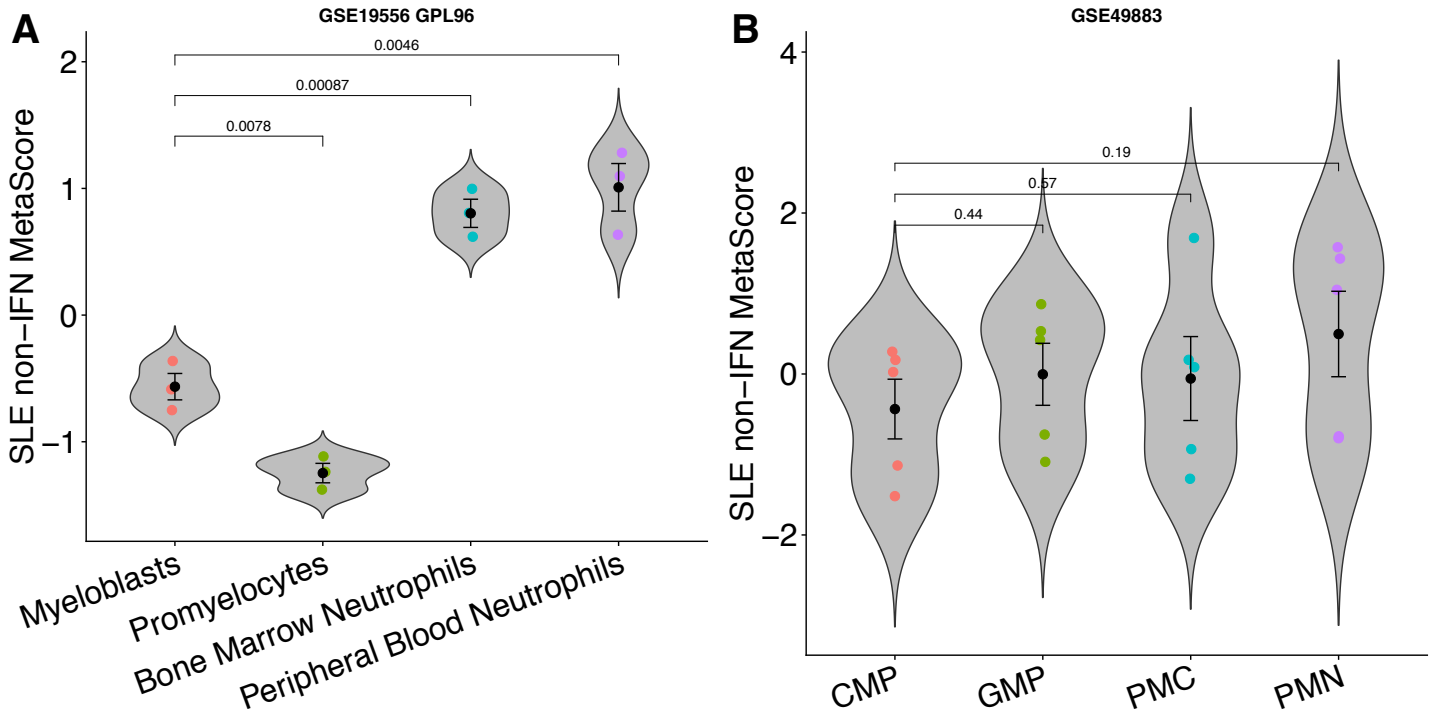

Figure S7. Neutrophil development. Related to Figure 7. SLE non-IFN MetaScore is elevated in mature neutrophils in different datasets of neutropoeisis (A, B).

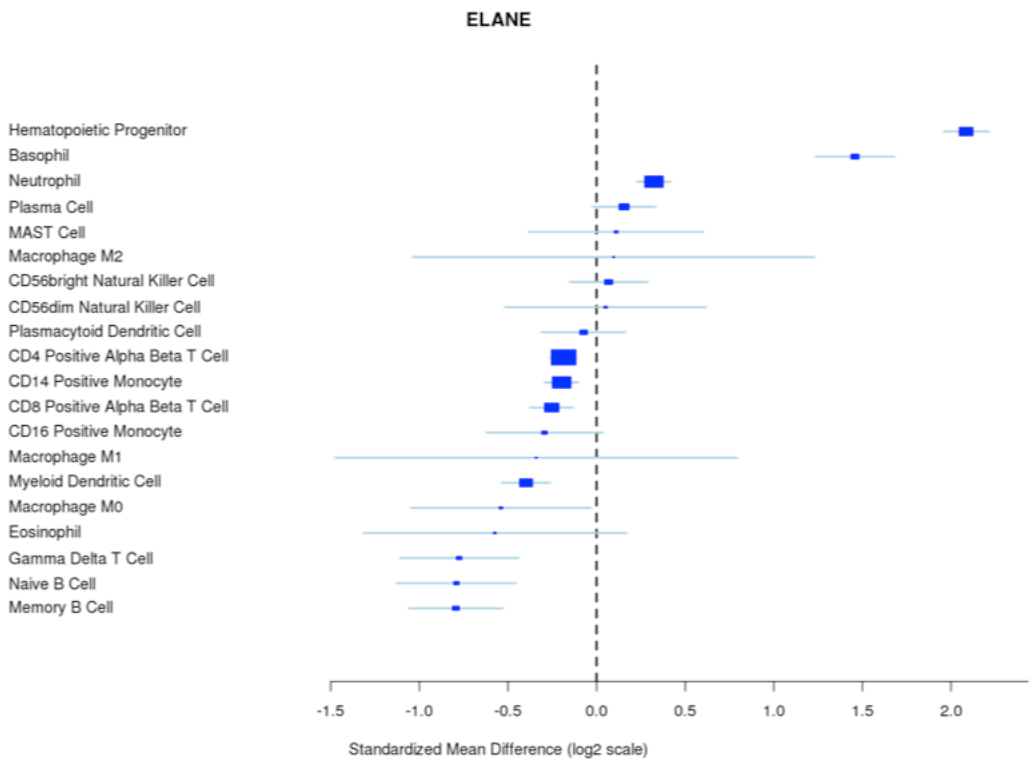

Figure S8. *ELANE* gene effect sizes across cell types. *ELANE* is most differentially expressed in hematopoietic progenitor cells compared to all other cell populations.

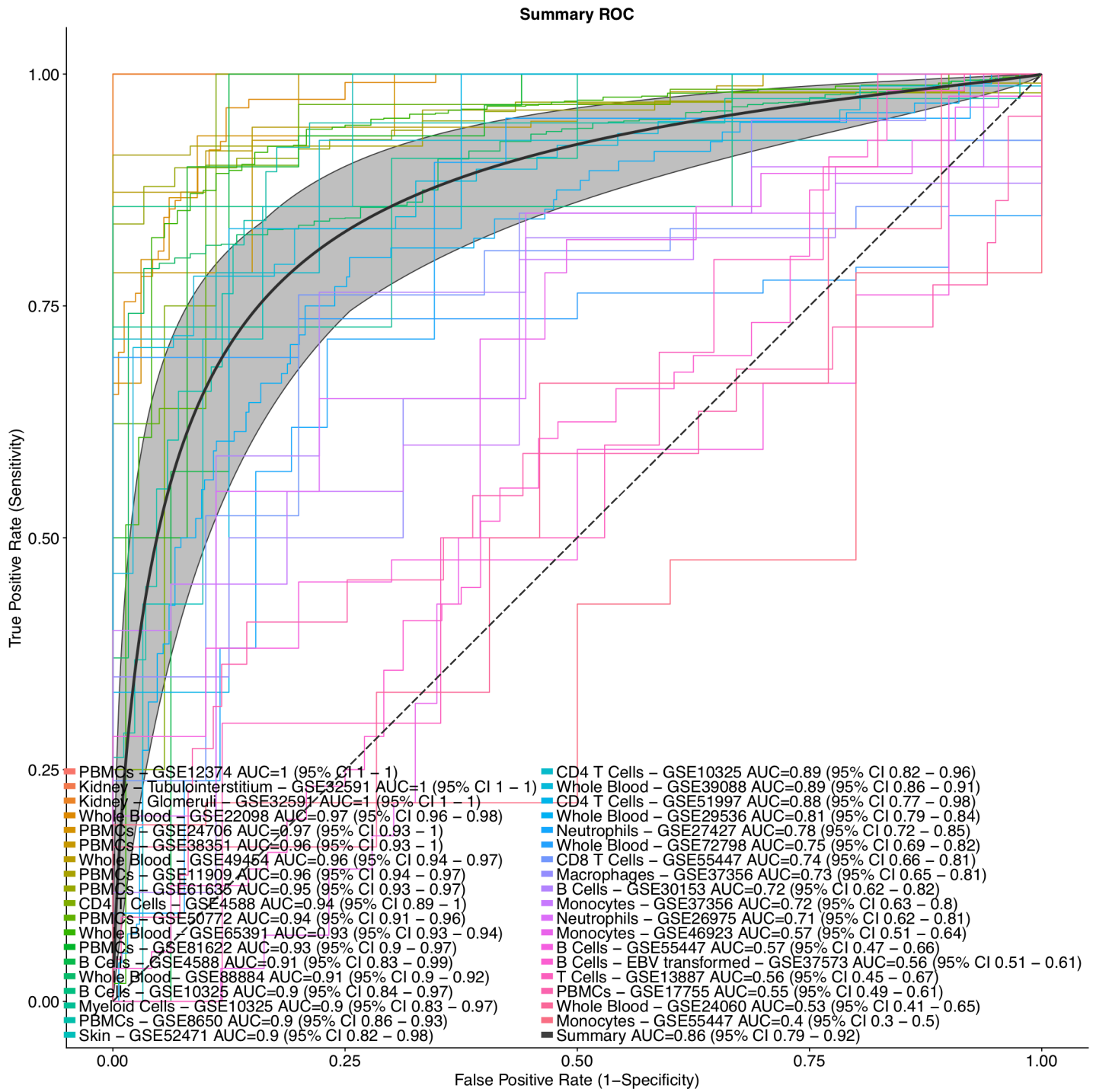

Figure S9. Extended validation ROC. ROC curves for data in the extended validation set. Some datasets also appear in the discovery and validation ROC plots, but may have different AUC estimates, because we have included additional patients which were excluded from the discovery and validation plots due to failure to match our discovery/validation criteria (in which subjects with other diseases were included/excluded, and follow up longitudinal visits from the same subject were included/excluded).

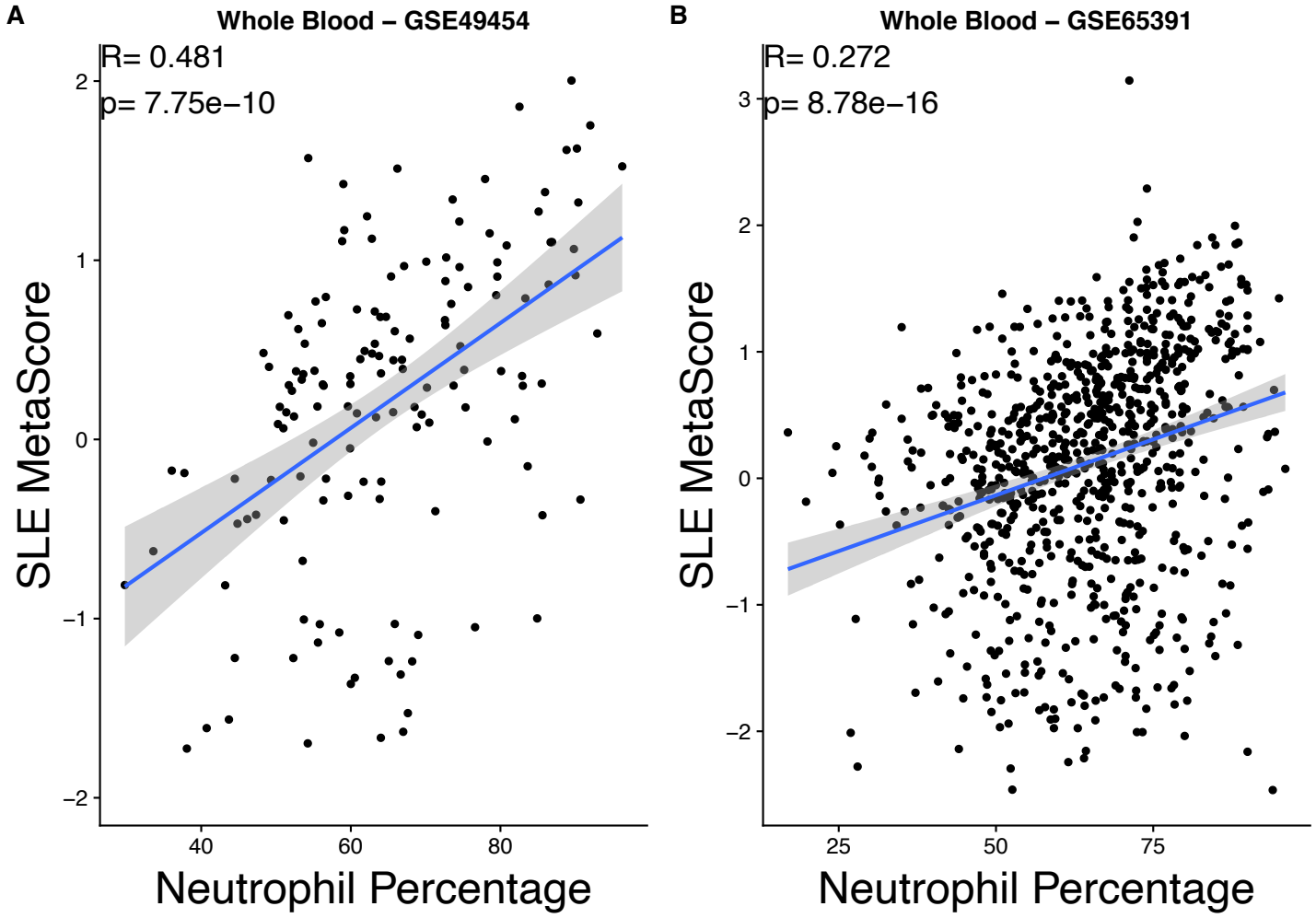

Figure S10. Neutrophil abundance correlation with SLE. In both datasets where neutrophil percentage was provided, we observed a significant correlation between the SLE MetaScore and neutrophil abundance.

Supplemental Tables

Table S1. SLE MetaSignature gene names. All genes in the SLE MetaSignature are listed, with their effect sizes and false discovery rates in SLE, IFN, and neutrophils. Additionally, it is indicated whether these genes were considered as IFN or neutrophil genes in our analysis.

Gene Name	Effect Size SLE	Effect Size IFN	Effect Size Neutrophils	Effect Size FDR SLE	Effect Size FDR IFN	Effect Size Standard Error Neutrophils	IFN Gene	Neutrophil Gene
IFI44L	2.12	3.59	0.039	5.08E-17	5.21E-07	0.044	X	
EPSTI1	1.99	2.42	-0.048	2.17E-33	6.40E-06	0.051	X	
HERC5	1.98	3.88	0.638	1.50E-19	1.62E-06	0.047	X	
IFI44	1.95	3.34	-0.047	2.25E-25	3.89E-09	0.044	X	
IFI27	1.89	1.99	-0.22	3.24E-20	2.39E-06	0.045	X	
RSAD2	1.81	6.05	1.16	7.43E-54	2.16E-08	0.046	X	X
ISG15	1.79	4.59	0.408	3.54E-22	1.22E-07	0.047	X	
OASL	1.74	5.84	0.929	1.16E-34	5.05E-10	0.045	X	X
IFIT3	1.67	4.44	1.52	4.99E-31	1.03E-09	0.046	X	X
CMPK2	1.63	5.13	0.838	2.76E-29	0.001	0.057	X	X
IFIT1	1.62	4.64	1.25	1.03E-21	1.16E-08	0.046	X	X
USP18	1.6	3.28	-0.257	2.30E-25	4.89E-07	0.047	X	
SIGLEC1	1.59	0.832	0.074	2.27E-10	0.229	0.05	X	
MX2	1.58	4.88	0.813	6.41E-12	3.08E-07	0.045	X	X
MX1	1.54	4.16	0.202	0.00212	9.03E-08	0.044	X	
LY6E	1.54	2.53	-0.572	2.36E-04	1.73E-09	0.045	X	
PLSCR1	1.52	1.73	0.788	1.66E-35	5.05E-12	0.045	X	
OAS2	1.52	3.36	-0.445	1.34E-33	3.46E-10	0.045	X	
SPATS2L	1.52	1.29	-0.525	1.23E-43	4.03E-04	0.051	X	
OAS1	1.49	3.57	-0.125	7.24E-19	6.27E-11	0.044	X	
OAS3	1.49	3.09	0.315	2.76E-08	4.78E-08	0.047	X	
PARP12	1.43	3.01	-0.036	9.79E-17	9.00E-06	0.047	X	
SCO2	1.41	1.09	0.363	2.83E-07	2.30E-07	0.045	X	
IFIH1	1.41	2.78	0.493	7.87E-18	5.27E-07	0.045	X	
IFITM3	1.4	2.15	0.664	1.81E-16	4.78E-09	0.045	X	
IFITM1	1.4	3.91	0.613	1.40E-08	5.58E-10	0.045	X	
DDX60	1.38	3.15	0.385	3.89E-09	6.39E-07	0.051	X	
TRIM22	1.37	2.2	0.109	6.80E-05	9.53E-07	0.044	X	
RTP4	1.35	4.19	-0.281	9.19E-11	1.22E-06	0.048	X	
SAMD9L	1.35	2.7	0.714	1.98E-05	3.90E-06	0.052	X	
XAF1	1.33	2.65	-0.078	1.70E-11	3.05E-10	0.047	X	
IFI35	1.28	3.67	0.01	6.59E-08	1.73E-09	0.044	X	
TNFAIP6	1.27	0.763	2.35	1.44E-08	0.0404	0.05		X
MT2A	1.27	1.24	-0.07	3.94E-26	0.0401	0.045	X	
LAP3	1.26	2.32	0.034	1.69E-09	8.78E-06	0.047	X	
HERC6	1.26	1.18	-0.102	2.52E-05	0.00414	0.047	X	

FBXO6	1.25	3.01	0.416	3.93E-15	6.79E-05	0.052	X	
TDRD7	1.23	4.23	0.479	2.77E-08	2.52E-07	0.045	X	
IFIT5	1.23	3.37	0.859	4.57E-12	3.21E-08	0.045	X	X
PHF11	1.22	1.97	-0.002	1.04E-04	2.50E-06	0.047	X	
IFIT2	1.22	4.93	1.33	5.53E-07	1.53E-08	0.046	X	X
TAP1	1.22	3.57	0.109	9.84E-07	1.83E-06	0.045	X	
TOR1B	1.21	1.36	0.958	1.89E-10	0.00407	0.045	X	X
TNFSF13B	1.21	2.27	1.48	6.00E-08	2.13E-05	0.054	X	X
ELANE	1.19	-0.106	0.324	0.0472	0.919	0.048		
IRF7	1.18	3.33	0.521	6.79E-07	3.30E-08	0.045	X	
DHX58	1.16	2.43	-0.56	3.79E-06	3.36E-06	0.051	X	
ZBP1	1.16	1.26	0.829	1.14E-08	0.0372	0.048	X	X
TYMP	1.14	1.11	0.786	1.15E-06	3.51E-05	0.048	X	
GRN	1.14	0.212	0.379	0.00969	0.582	0.044		
LAMP3	1.14	1.83	0.194	2.72E-10	3.85E-04	0.045	X	
IFI6	1.13	2.63	0.418	3.17E-08	3.48E-06	0.047	X	
NTNG2	1.13	0.765	1.71	7.38E-05	0.0564	0.054		X
SAT1	1.13	1.09	0.608	2.96E-07	5.05E-12	0.047	X	
SERPING1	1.12	1.19	0.979	9.08E-09	0.00399	0.046	X	X
DEFA4	1.12	-0.0884	0.93	0.00343	0.926	0.045		X
DDX58	1.11	3.52	1.29	4.19E-14	1.26E-09	0.048	X	X
SAMD9	1.11	3.27	0.688	7.53E-04	7.22E-07	0.047	X	
PARP9	1.1	2.26	1.31	2.45E-04	7.94E-06	0.053	X	X
MT1E	1.09	0.764	-0.044	2.17E-07	0.237	0.045		
IRF9	1.09	2.12	0.424	0.00942	9.69E-10	0.047	X	
TCN2	1.08	0.453	0.228	1.68E-07	0.089	0.044		
MT1HL1	1.08	NA	NA	1.05E-08	NA	NA		
HSH2D	1.08	1.87	1.37	0.00647	5.56E-04	0.053	X	X
ISG20	1.07	3.85	0.314	2.53E-06	4.78E-08	0.044	X	
ZCCHC2	1.06	1.41	0.625	0.0193	8.19E-04	0.047	X	
SP100	1.06	1.64	0.43	0.00811	7.11E-12	0.045	X	
LMO2	1.05	0.846	0.66	1.01E-04	0.13	0.045	X	
GBP1	1.05	2.53	0.582	5.09E-06	2.82E-05	0.045	X	
IFITM2	1.05	1.98	0.759	0.00517	9.69E-10	0.045	X	
LGALS3BP	1.04	1.57	-0.169	1.48E-20	0.00475	0.044	X	
REM2	1.04	-0.365	2.43	0.00159	0.464	0.062		X
TNFSF10	1.04	2.77	0.608	1.06E-04	4.02E-09	0.045	X	
HESX1	1.03	0.901	-0.09	2.35E-19	0.24	0.045	X	
MT1A	1.03	1.4	0.28	2.47E-05	0.0645	0.077	X	
CCR1	1.02	0.569	1.26	2.46E-06	0.217	0.046		X
CEACAM1	1.02	0.681	1.66	2.42E-07	5.06E-04	0.047		X
MYD88	1.02	2.04	0.765	0.00125	1.88E-08	0.045	X	
BST2	1.02	2.6	-0.553	2.10E-06	9.03E-11	0.045	X	
LHFPL2	1.01	0.239	0.124	5.75E-06	0.607	0.044		
FFAR2	1	0.826	2.76	1.25E-04	0.116	0.054	X	X
MT1F	1	0.413	-0.302	8.61E-05	0.621	0.045		
RPL10A	-1	-0.386	-1.41	2.14E-06	0.182	0.047		X

CD1C	-1	-0.297	-0.254	6.81E-19	0.409	0.044		
VSIG1	-1.01	-0.16	-0.553	3.21E-08	0.751	0.052		
GPR183	-1.02	0.125	-0.488	4.76E-09	0.934	0.048		
DSC1	-1.02	-0.218	-0.214	1.35E-06	0.592	0.045		
KLRB1	-1.03	-0.238	-0.006	1.50E-15	0.603	0.044		
FBL	-1.05	-0.43	-1.75	6.94E-05	0.385	0.047		X
EIF3E	-1.05	-0.665	-1.23	0.00265	0.171	0.049		X
ABCB1	-1.06	-0.0877	-0.256	5.47E-11	0.845	0.045		
NAP1L3	-1.1	-0.27	-0.178	4.38E-10	0.5	0.044		
EIF3L	-1.48	-0.927	-1.9	2.55E-17	0.076	0.055	X	X

Table S2. Results from pathway analysis of the SLE meta-analysis data. Pathways related to inflammation (green) and nucleic acid metabolism (orange).

Pathway Name	p-Value	False Discovery Rate	DEAP Statistic	Pathway Subpath
Formyltetrahydrofolate biosynthesis	2.00E-04	0.0101	2.13	(DHFR,TYMS,DHFRL1) --> (MTHFD2L,MTHFD1L,MTHFD2) --> (TYMS) --> (DHFR,DHFRL1)
Salvage pyrimidine deoxyribonucleotides	2.00E-04	0.0101	1.18	(DEADC1,CDA) --> (TK1)
Salvage pyrimidine ribonucleotides	4.00E-04	0.0135	0.967	(DEADC1,CDA) --> (UPRT) --> (UPP2,UPP1)
Purine metabolsim	0.0012	0.0242	-0.526	(AMPD3) --> (NT5E)
Inflammation mediated by chemokine and cytokine signaling pathway	0.0012	0.0242	12.2	(SOCS5,SOCS7,SOCS6,SOCS3,JAK2,CISH) --> (STAT3) --> (IFNAR1) --> (IFNG,IFNAR1) --> (IFNG,JAK2,IFNAR1,STAT3) --> (SOCS5,SOCS7,SOCS6,SOCS3,JAK2,CISH) --> (JAK2,IFNAR1,STAT3) --> (SOCS5,SOCS7,SOCS6,SOCS3,JAK2,CISH) --> (IFNG,IFNAR1,JAK2) --> (SOCS5,SOCS7,SOCS6,SOCS3,JAK2,CISH) --> (IFNG,IFNAR1,JAK2) --> (JAK2,IFNAR1,STAT3) --> (IFNG,IFNAR1) --> (JAK2)
Toll receptor signaling pathway	0.0022	0.037	1.31	(TRAM,TICAM1) --> (TRAF2,TBK1,IKBKE)
Plasminogen activating cascade	0.0038	0.0505	3.72	(PLAUR,SERPINE1,PLAU) --> (PLAT) --> (SERPINE1,PLAT) --> (PLAUR,PLAU) --> (PLAUR,SERPINE1,PLAU) --> (PLAUR,SERPINB2,PLAU) --> (SERPINE1)
Oxidative stress response	0.0040	0.0505	0.683	(MAPK14,MAPK11,MAPK12,MAPK13) --> (EEF2K)
Interleukin signaling pathway	0.0058	0.0606	2.34	(STAT6,STAT4,STAT3,STAT2,STAT1,STAT5B,STAT5A) --> (MAPK3,MAPK1,MAPK6,MAPK7,MAPK15)
Interferon gamma signaling pathway	0.0060	0.0606	6.28	(IFNG,IFNGR1,IFNGR2) --> (JAK1) --> (IFNG,IFNGR1,IFNGR2) --> (IFNG,JAK2,IFNGR1,IFNGR2,JAK1) --> (STAT1) --> (JAK2) --> (IFNG,IFNGR2,IFNGR1,STAT1,JAK1)
Pyruvate metabolism	0.0110	0.101	-1.13	(ME1) --> (PC) --> (CS) --> (ACLY,CLYBL) --> (CS)
Fructose galactose metabolism	0.0122	0.103	-0.746	(KHK) --> (ALDOA,ALDOC,ALDOB)

Table S3. Gene-level prospective validation results. Gene name, probe ID, fold change, p-value, and FDR for all genes from the SLE MetaSignature which were run on the prospective Fluidigm RT-qPCR assay.

Gene symbol	TaqMan Probe ID	Fold change	Wilcox p-value	FDR
IFI27	Hs01086373_g1	232	1.21E-13	3.99E-12
RSAD2	Hs00369813_m1	27.4	7.17E-13	1.18E-11
IFI44L	Hs00199115_m1	19.7	3.47E-12	3.82E-11
LY6E	Hs03045111_g1	13.9	4.68E-11	3.86E-10
RTP4	Hs00223342_m1	6.86	1.55E-10	9.90E-10
EPSTI1	Hs01566789_m1	5.16	2.07E-10	9.90E-10
HERC5	Hs00180943_m1	7.45	2.10E-10	9.90E-10
ISG15	Hs01921425_s1	11.3	6.47E-10	2.67E-09
MX2	Hs01550811_m1	3.51	1.03E-09	3.78E-09
IRF7	Hs01014809_g1	4.83	1.81E-09	5.97E-09
MX1	Hs00895608_m1	6.81	8.57E-09	2.57E-08
OAS3	Hs00196324_m1	6.23	1.90E-08	5.22E-08
SERPING1	Hs00163781_m1	5.98	1.07E-07	2.52E-07
EIF3L	Hs00275016_m1	0.521	1.07E-07	2.52E-07
OAS2	Hs00942643_m1	4.85	3.44E-07	7.36E-07
IFI6	Hs00242571_m1	4.17	3.57E-07	7.36E-07
IFI44	Hs00951349_m1	7.9	8.59E-07	1.67E-06
RPL10A	Hs03043870_g1	0.607	1.10E-05	2.02E-05
SIGLEC1	Hs00988063_m1	3.22	2.52E-05	4.38E-05
PLSCR1	Hs01062171_m1	10.9	3.29E-05	5.43E-05
OASL	Hs00984387_m1	3.6	5.19E-05	8.16E-05
IFIT3	Hs01922752_s1	3.98	8.78E-05	1.32E-04
IFI35	Hs00413458_m1	2.15	2.08E-04	2.98E-04
IFITM3	Hs04194512_g1	9.32	3.37E-04	4.64E-04
LGALS3BP	Hs00174774_m1	14.6	3.84E-04	5.06E-04
OAS1	Hs00973637_m1	1600	5.80E-04	7.36E-04
IFITM2	Hs00829485_sH	2.24	0.00194	0.00237
XAF1	Hs01550142_m1	0.815	0.0027	0.00318
MT1F	Hs00744661_sH	1.41	0.00756	0.0086
IFIT1	Hs01911452_s1	1.86	0.0189	0.0208
TAP1	Hs00388675_m1	3.57	0.686	0.731
FBXO6	Hs00218350_m1	1.42	0.864	0.89
MT1E	Hs01938284_g1	1.12	0.905	0.905

Table S4. Interferon datasets. Data downloaded from GEO.

Dataset	Center	Cell Type	IFN Type	Stim Length	Sample Size	Reference
GSE14386	University of North Carolina, Chapel Hill, NC	PBMCs	IFNβ -1a	24h	30	(1)
GSE16755	NIH, Bethesda, ND	Monocyte derived macrophages	IFNα	1-4h	6	(2)
GSE17301	Center for Applied Medical Research, Pamplona, Spain	CD8+CD45R0- T cells	IFNα-2b or IFNα-5	48h	15	(3)
GSE1740	The Hospital for Special Surgery, New York, NY	Macrophages	IFNα	3h	12	(4)
GSE23307	Cleveland Clinic, Cleveland, OH	Whole blood	IFNβ	3h	6	(5)
GSE27337	University of Texas Southwestern, Dallas, TX	CD8+CD45RA+ T cells	IFNα	84h	48	(6)
GSE30536	Universite Laval, Quebec, Canada	Macrophages	IFNα-2	18h	17	(7)
GSE34627	Technion Institute, Haifa, Israel	Monocytes and T cells	IFNβ	16h	12	(8)
GSE36287	Harvard Universty, Boston, MA	Keratinocytes	IFNα		24	(9)
GSE37715	Hiroshima University, Hiroshima, Japan	Hepatocytes	IFNα	6h	15	
GSE38147	University of Basel, Basel, Switzerland	Hepatocytes	IFNα	6-24h	10	(10)
GSE38351	Deutsches Rheuma-Forschungszentrum, Berlin, Germany	Monocytes	IFNα-2a	1.5h	74	(11)
GSE3920	Medical School of Turin, Turin, Italy	Endothelial cells and fibroblasts	IFNα, IFNβ	5h	23	(12)

GSE47616	Northwestern University, Chicago, IL	Fibroblasts	IFNα, IFNβ	24h	22	(13)
GSE7509	Yale University, New Haven, CT	Monocyte derived dendritic cells	IFNα	24h	26	(14)

Table S5. Summary AUC comparison. We compared the complete SLE MetaSignature, IFN SLE MetaSiganture, and non-IFN SLE MetaSignature in terms of summary AUC in discovery and validation.

Cohort	**SLE MetaSignature Genes**	**IFN SLE Genes**	**Non-IFN SLE Genes**
Discovery	0.947	0.940	0.944
Validation	0.937	0.921	0.913

Table S6. SLEDAI correlation comparison. We compared the complete SLE MetaSignature, IFN SLE MetaSiganture, and non-IFN SLE MetaSignature in terms of correlation with SLEDAI in 5 datasets.

Dataset	**SLE MetaSignature Genes**	**IFN SLE Genes**	**Non-IFN SLE Genes**
Neutrophils- GSE27427	0.284	0.254	0.354
PBMCs- GSE8650	0.670	0.545	0.782
PBMCs- GSE88884	0.157	0.113	0.247
Whole Blood- GSE49454	0.281	0.252	0.342
Whole Blood- GSE65391	0.250	0.236	0.233

Table S7. Neutrophil, NK cell, and heavy metal datasets. Data downloaded from GEO.

Data Type	Dataset	Center	Sample Size	Reference
NETosis Stimulations	GSE80489	All India Institute of Medical Science, New Delhi, India	18	(15)
Neutropoeisis	GSE42519	Copenhagen University, Copenhagen, Denmark	34	(16)
Neutropoeisis	GSE19556	Princeton University, Princeton, NJ	24	(17)
Neutropoeisis	GSE49883	Karolinska Institute, Stockholm, Sweden	20	(18)
Cadmium Exposure of MCF7 Cell Line	GSE52404	University of Skovde, Skovde, Sweden	7	
Zinc Exposure of A549 Lung Cells	GSE6972	University of Southern California, Los Angeles, CA	24	(19)

Table S8. Disease activity correlations of all SLE MetaScore and subsets. Correlation of SLE MetaScore or subset with the patient SLEDAI values. Partial data is presented in Figure 6C.

Dataset	SLE MetaSignature	IFN SLE MetaSignature	Non-IFN SLE MetaSignature	Neutrophil SLE MetaSiganture	Under-appreciated SLE MetaSignature
Neutrophils-GSE27427	0.284	0.254	0.354	0.387	0.197
PBMCs- GSE8650	0.67	0.545	0.782	0.77	0.712
Whole Blood-GSE49454	0.157	0.113	0.247	0.197	0.283
Whole Blood-GSE65391	0.281	0.252	0.342	0.295	0.339
PBMCs- GSE88884	0.25	0.236	0.233	0.193	0.243

Supplement S1. Dataset descriptions.

Discovery datasets

GSE17755 (22 SLE, 22 healthy; 44 total)
Lee et al. profiled peripheral blood samples from 21 patients (all women, median age: 35 years, range: 26-72 years) with SLE according to the diagnostic criteria of the American College of Rheumatology (ACR) and 45 healthy individuals (23 males, 22 females). Because all patients with SLE were women, we only used healthy females as controls in this cohort, and discarded data from healthy males.

GSE8650: (38 SLE, 21 healthy; 59 total)
Allantaz et al. profiled PBMC samples from 19 pediatric patients with system juvenile idiopathic arthritis (sJIA) during the systemic phase of the disease (fever and/or arthritis), 25 sJIA patients with no systemic symptoms (arthritis only or no symptoms), 39 healthy controls, 94 pediatric patients with acute viral and bacterial infections (available under GSE6269), 38 pediatric patients with Systemic Lupus Erythematosus (SLE), and 6 patients with a second IL-1 mediated disease known as PAPA syndrome. We used transcriptome data only from healthy controls and pediatric patients with SLE in the discovery analysis. Rest of the samples were used in downstream analysis after the signature for SLE was derived.

GSE50635 (33 SLE, 16 healthy; 49 total)
Ko *et al.* profiled whole blood samples from 33 female patients with SLE and 16 matched controls from European-American (EA) and African-American (AA) ancestral backgrounds. This dataset is not associated with any publication in the NCBI GEO database.

GSE39088 (26 SLE, 34 healthy; 60 total)
Lauwerys *et al.* profiled 28 female patients with SLE (aged 18–50 years; recruited in a multicenter, randomized, double-blind placebo-controlled, phase I/II staggered dose-escalation trial of IFN-K (ClinicalTrials.gov registry number NCT01058343). Patients were randomized to receive three or four injections of placebo (n = 7) or 30 μg (n = 3), 60 μg (n = 6), 120 μg (n = 6) or 240 μg (n = 6) IFN-K. We only used samples prior to treatment initiation in the analysis.

GSE22098 (28 adult and 12 pediatric SLE, 42 healthy; 83 total)
Berry et al. collected whole blood sample from 12 pediatric streptococcus, 40 pediatric staphylococcus, 31 still's disease, 82 pediatric systemic lupus erythematosus (SLE) and 28 adult SLE patients. RNA was extracted and globin reduced. Labeled cRNA was hybridized to Illumina Human HT-12 Beadchips. Healthy controls were included to match patients' demographic data. Genespring software was used to analyze active TB transcript signatures, comparing with healthy controls and other inflammatory and infectious diseases.

GSE11909: (63 SLE, 12 healthy; 75 total) First visit patient data
Chaussabel et al. profiled 239 PBMC samples from individuals with one of the following conditions: systemic juvenile idiopathic arthritis (n=47), systemic lupus erythematosus (n=63), type I diabetes (n=20), metastatic melanoma (n=39), acute infections (*Escherichia coli* (n=22), *Staphylococcus aureus* (n=18), Influenza A (n=16)), or

liver transplant recipients undergoing immunosuppressive therapy (n=37). We only used data only from the first visit.

Validation datasets

GSE12374 ()

: Lee et al. obtained peripheral blood from female SLE patients (n = 11 median age 35 years, range 27 to 72 years) and healthy women (n=6). Gene expression profile was analyzed using DNA microarray covering 30,000 human genes. Differentially expressed immune response-related genes were selected and analyzed by using Expression Analysis Systemic Explorer (EASE) based on Gene Ontology (GO) followed by network pathway analysis with Ingenuity Pathways Analysis (IPA).

GSE24706: Li et al. obtained 10 healthy control (HC) samples with high ANA, 7 first degree relative of SLE patient (FDR) with high ANA, 10 HCs with low ANA, 6 FDR with low ANA, 15 SLE patients (SLE).
The overall study group included 1,159 individuals from DRADR: 401 healthy controls (HC) who were negative for current or past autoimmune disease, 116 first-degree relatives (FDR), 294 patients with SLE, 151 patients with less than 4 SLE criteria and considered as having incomplete lupus (ILE), 154 with rheumatoid arthritis (RA) and 43 with other miscellaneous conditions including scleroderma, Sjogren's syndrome, ankylosing spondylitis and vasculitis. More detailed analyses were carried out on a subset of HC individuals with high ANA values (n = 18) and these were compared to gender- and age-matched HC with negative ANA values (n = 16) and to SLE patients with high ANA levels of >100 E.U. (n = 14).
Measurements carried out on serum samples included ANA, extractable nuclear antibodies (ENA) and autoantibody profiling using an array with more than 100 specificities. Whole blood RNA samples from a subset of individuals were used to analyze gene expression on the Illumina platform. Data were analyzed for associations of high ANA levels with demographic features, the presence of other autoantibodies and with gene expression profiles.

GSE49454: Chiche el al. enrolled sixty-two consecutive patients with SLE fulfilling the 1997 ACR criteria were enrolled between 2009 and 2011 in the Departments of Internal Medicine and Nephrology at a French reference center for autoimmune diseases (Hôpital de la Conception, Marseille, France) and followed-up them prospectively.

SLE patients were split into three groups (Table S1). The “at inclusion” group included all SLE patients at their first visit, irrespective of SLE disease activity at that time. The “quiescent” group included SLE patients at their first available visit with low disease activity, defined by no flare or treatment modifications for at least 60 days prior to the visit, and a SLEDAI of ≤4. The “longitudinal” group included SLE patients who had at least three consecutive visits during the study.

GSE61635: The goal of this study was to characterize gene expression profiles in RNP autoantibody+ SLE versus healthy blood donors with a focus on select cytokines that may be important in B cell activation and differentiation, including BAFF, IL-21, and IL-33. Affymetrix microarrays were used to characterize the global program of gene expression in the SLE patients, and to identify differentially expressed genes in patients

compared to healthy controls. mRNA from the blood of a SLE cohort (79 patients with some repeat visits for a total of 99 arrays) and 30 healthy volunteers (one array per volunteer) were analyzed.
There were 73 female and 6 male subjects. Disease duration ranged from 0 to 453 months with a median of 37.5 months. SLE Disease Activity Index (SLEDAI) ranged from 0 to 31 with a median of 6.

GSE65391: Banchereau R et al. longitudinally profiled the whole blood transcriptomes of 158 SLE patients by microarray for up to 4 years, yielding 924 SLE samples and 48 matched pediatric healthy samples. The transcriptional data are complemented by demographic, laboratory and clinical data. They confirmed a prevalent IFN signature and identified a plasmablast signature as the most robust biomarker of DA.They also detected gradual enrichment of neutrophil transcripts during progression to active nephritis, and distinct signatures in response to treatment in different nephritis subclasses.

GSE72798: This cohort was generated to validate if IFNalpha kinoid induces neutralizing anti-IFNalpha antibodies that decrease the expression of IFN-induced and B cell activation associated transcripts: analysis of extended follow-up data from the IFN-K phase I/II study
Cohort has 82 total samples with 10 healthy and rest SLE, we have only included patient data for first visit for SLE patients.

GSE81622: This cohort was generated to perform whole genome transcription and DNA methylation analysis in PBMC of 30 SLE patients, including 15 with LN (SLE LN+) and 15 without LN (SLE LN-), and 25 normal controls (NC) using HumanHT-12 Beadchips and Illumina Human Methy450 chips. The serum pro-inflammatory cytokines were quantified using Bio-plex human cytokine 27-plex assay.

Extended validation :

GSE10325 : Becker et al. enrolled SLE patients fulfilled at least 4 of 11 American College of Rheumatology classification criteria for SLE. Disease activity assessed at the time of blood acquisition was calculated using the systemic lupus erythematosus disease activity index (SLEDAI). They compared PBMC subsets from a total of fifteen female SLE patients (mean age 39±12 years) and eleven female HC (mean age 37±10 years). Although patients were on a variety of disease modifying agents, patients on high dose immunocytotoxic therapies or steroids were excluded from the study. However, patients on lower doses of prednisone (10–20 mg/day; and 1 patient on 40 mg/day) were included.

GSE13887: Fernandez et al. investigated a total of 44 Caucasian female patients with systemic lupus erythematosus (SLE) in their cohort. Disease activity was assessed by the SLEDAI score[72]. Six patients were treated with rapamycin 2 mg/day (age: 40 ± 8.3 years; SLEDAI: 0.8). Among the 38 remaining SLE patients treated without rapamycin, 28 were receiving prednisone (5–50 mg/day) and immunosupppresive drugs including hydroxychloroquine (400 mg/day), mycophenolate mofetil (3 g/day), cyclosporin A (50–100 mg/day). Their mean age was 36.3 ± 4.3 years, ranging between 18–60; SLEDAI: 1.3 ± 0.9. Furthermore, ten patients (age: 38.5 ± 6.4) SLEDAI: 4.8 ± 3.8) were freshly diagnosed and had not been treated with prednisone or cytotoxic drugs.

These patients and five additional patients that have received prednisone or cytotoxic drugs provided cells for microarray analysis. As controls, 23 age-matched healthy female subjects and 8 female patients with rheumatoid arthritis (RA; age: 51.3 ± 6.7 years) [73] were studied. RA patients were treated with methotrexate, cyclosporin A, leflunomide, etanercept, or adalimumab. The study has been approved by the Institutional Review Board for the Protection of Human Subjects.

GSE24060: O'Hanlon et al. enrolled five adult (at least 18 years of age) and 15 juvenile MZ twin pairs discordant for SAID and 40 unrelated control subjects (two controls per twin pair) matched on age within 6 years, gender, and ethnicity in this study. These subjects were enrolled between 2001 and 2006 in the National Institutes of Health (NIH) investigational review board-approved Twins-Sib study assessing the pathogenesis of SAID. Twin pairs enrolled within 4 years of probands' diagnoses included 19 non-Hispanic Caucasian twin pairs and a single Hispanic twin pair (with SLE). Probands fulfilled American College of Rheumatology criteria for adult or juvenile SLE (n = 4 and 2, respectively), RA or JRA (n = 1 and 5, respectively), juvenile dermatomyositis (JDM) (n = 7), or juvenile polymyositis (JPM) (n = 1); they excluded patients with inherited, metabolic, infectious, or other causes of disease. The juvenile probands ranged in age from 3 to 18 years (mean of 11.2 years), whereas adults ranged from 19 to 43 years (mean of 29.2 years). Twins included 14 female and 6 male pairs. Monozygosity was confirmed by short tandem repeat analysis of genomic DNAs (Proactive Genetics, Inc., Augusta, GA, USA). Unrelated, matched controls were free of infections, trauma, vaccines, and surgeries for 8 weeks and had no first-degree family members with SAID.

GSE26949: Thacker et al. enrolled the patients and obtained peripheral blood, from the university of Michigan outpatient Rheumatology clinic who fulfilled the revised American College of Rheumatology criteria for SLE. Age- and gender- matched healthy controls were recruited by advertisement. Lupus disease activity was assessed by the SLE Disease Activity Index (SLEDAI). In overall experiment , Human healthy EPCs and CACs from PBMCs were isolated and cultured under proangiogenic stimulation; after IFNa incubation or not, RNA was extracted and processed for hybridization on Affymetrix microarrays.

GSE26950: Thacker et al. enrolled the patients and obtained peripheral blood, from the university of Michigan outpatient Rheumatology clinic who fulfilled the revised American College of Rheumatology criteria for SLE. Age- and gender- matched healthy controls were recruited by advertisement. Lupus disease activity was assessed by the SLE Disease Activity Index (SLEDAI). In overall experiment, Human lupus EPCs and CACs from PBMCs were isolated and cultured under proangiogenic stimulation; after IFNa incubation or not, RNA was extracted and processed for hybridization on Affymetrix microarrays.

GSE26975: Villanueva et al. enrolled Lupus patients from the University of Michigan outpatient rheumatology clinic who fulfilled the revised American College of Rheumatology criteria for SLE. Disease activity was assessed by the SLE disease activity index. Gender-matched healthy controls were recruited by advertisement. Demographic and clinical information on the lupus patients. In overall experiment they isolated neutrophils and LDGs from PBMCs. RNA from 9 healthy neutrophils, 10 lupus neutrophils and 10 lupus LDGs was extracted and processed for hybridization on Affymetrix microarrays. Lupus patients SLEDAI score was between 0 and 20. Most of the patients were on antimalarials, PDN or on MMF drugs.

GSE27427: Garcia-Romo et al. obatained blood samples from patients fulfilling the diagnosis of SLE according to the criteria established by the American College of Rheumatology. Disease activity was assessed by the SLEDAI as measured on the day of blood collection. Healthy pediatric controls were children visiting the clinic either for reasons not related to autoimmunity or infectious diseases or for surgery not associated with any inflammatory diseases. They ran to experiments, (Expt 1) Neutrophils from 21 SLE samples (19 patients) and 12 healthy donors were isolated, and extracted RNAs were used generate microarray data. (Expt 2) Neutrophils isolated from 2 healthy children (not used in the first experiment) were cultured with autologous sera (control), Interferon alpha (100U and 1000U), and 4 SLE sera and 6 SLE sera for 6 hours and RNAs were extract for microarray experiment.

GSE29536: This dataset was used to establish whole blood transcriptional modules (n=260) that represent groups of coordinately expressed transcripts that exhibit altered abundance within individual datasets or across multiple datasets. This modular framework was generated to reduce the dimensionality of whole blood microarray data processed on the Illumina Beadchip platform yielding data-driven transcriptional modules with biologic meaning.

This series combines nine independent datasets representing a spectrum of human pathologies expected to result in changes in gene abundance related to changes in expression or cellular composition of whole blood. These nine datasets are composed of 410 individual whole blood profiles generated from patients with HIV, tuberculosis, sepsis, systemic lupus erythematosus, systemic arthritis, B-cell deficiency and liver transplant. For each dataset healthy controls are also included. Each dataset's expression data was preprocessed independently.

GSE30153: Garaud et al. selected 17 patients (15 females and 2 males) ageing from 23 to 59 with the diagnosis of SLE for the study. The SLE diagnosis was based on the presence of at least 4 criterias among those defined by the American College of Rheumatology. The lupus was inactive in these patients for more than 6 months, with a Systemic Lupus Erythematosous Disease Activity Index (SLEDAI) score less than 4 , and they did not receive any immunosuppressive drug. If they needed steroids, the patients were not treated with more than 10 mg of prednisone per day (4 patients). 10 patients were treated with hydroxychoroquine. The 10 control subjects were healthy individuals, (8 females and 2 males) ageing from 23 to 53 years, with no personal nor familial history of autoimmune disease.
They compared the peripheral B cell transcriptomes of quiescent lupus patients to normal B cell transcriptomes in this cohort.

GSE32591: Berthier et al. collected renal a total of 47 samples from the European Renal cDNA Bank (ERCB), they were processed and used for microarray analysis: 15 pre-transplant healthy living donors (LD) and 32(25 female/7 male) lupus nephritis (LN) patients median age was . For real-time PCR, 11 LD and 9 LN samples were used from an independent cohort (of the ERCB).
RNA from glomeruli and tubulointerstitial compartments was extracted and processed for hybridization on Affymetrix microarrays.

GSE36700: Nzeusseu et al. obtained synovial biopsy tissue (15–20 synovial samples per patient) by needle arthroscopy of the affected knee of patients with SLE (n = 6), patients with RA (n = 7), and patients with OA (n = 6).

All patients with SLE met the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) revised classification criteria for SLE, all were female, and the mean age was 32 years (range 19–40 years). All SLE patients had active articular disease at the time of synovial tissue sampling, and none had received immunosuppressive therapy; some of the SLE patients were receiving nonsteroidal antiinflammatory drugs. All patients with RA met the ACR classification criteria for RA and all had early (<1 year's duration) active disease at the time of tissue sampling. Among the patients with RA, 2 were female and 5 were male, and the mean age was 51 years (range 37–69 years). In these patients, the mean C-reactive protein (CRP) level was 25 mg/liter (range 9–96 mg/liter), and the mean Disease Activity Score in 28 joints (including the CRP) was 5.08 (range 3.76–5.82). None of the RA patients had received any treatment, except with nonsteroidal antiinflammatory drugs. Among the patients with OA, 5 were female and 1 was male, and the mean age was 63.2 years (range 51–73 years).

All patients had a swollen knee at the time of the needle arthroscopy procedure. The biopsy samples were harvested before initiation of disease-modifying antirheumatic drugs or any other immunosuppressive therapy. All of the RA patients were subsequently treated with methotrexate. Tumor necrosis factor (TNF) blocking agents were added to the regimen of 5 of these patients at a later stage. All of the SLE patients subsequently received antimalarial drugs. Combination therapy with methotrexate was later started because of persistent joint involvement in 3 of these patients. Azathioprine was started for severe hematologic manifestations in 2 other patients.

GSE36941:Terrier et al. evaluated the safety and the immunological effects of vitamin D supplementation in SLE patients with hypovitaminosis D using transcriptomic study at M0 and M2.

They assessed 24 SLE patients for eligibility (twenty-two women and two men, mean age ± SD, 31 ± 8 years). Their serum 25(OH)D level was measured. Hypovitaminosis D was defined as serum 25(OH)D < 30 ng/mL, while vitamin D sufficiency was defined as serum levels between 30 and 100 ng/mL [17]. Those with hypovitaminosis D (< 30 ng/mL) were placed on the following schedule of oral vitamin D supplementation: 100,000 IU of cholecalciferol per week for 4 weeks, followed by 100,000 IU of cholecalciferol per month for 6 months. All supplemented patients were screened before vitamin D supplementation (Day 0, or D0), and 2 and 6 months (M2 and M6) after the beginning of vitamin D supplementation. All but four patients received hydroxychloroquine (200 or 400 mg daily) and/or oral prednisone (≤ 15 mg/day, median dosage 5 mg/day). Three patients received a stable dosage of immunosuppressive agents. The study was approved by the institutional ethics committee, the Comité de protection des personnes Ile-de-France VI, in the Pitié-Salpêtrière Hospital (Paris, France) and informed consent was obtained from all patients.

GSE37356: In this cohort, monocytes were obtained from 20 patients with SLE and 16 healthy controls and were in vitro differentiated into macrophages. Subjects also underwent laboratory and imaging studies of the coronary arteries, carotid arteries, and aorta to evaluate for subclinical atherosclerosis.

GSE37573: In this cohort, Epstein-Barr virus (EBV) transformed B cells derived from two patients with systemic lupus erythematosus (SLE) and two normal unrelated controls were stimulated with a biologically relevant signal, co-crosslinking of the B cell antigen receptor (BCR) and FcγR2b. Total RNA was isolated at various timepoints post-stimulation. Gene expression data were used for analysis of differential gene expression and analysis of the dynamics of gene expression variations.

GSE38351: Smiljanovic et al. generated profiles of human peripheral blood monocytes activated in vivo and stimulated in vitro. There were 15 SLE patients ageing from 21-63 years, 14 RA ageing from 67-22 and 12 Healthy donors aging from 20-60 years were included in the cohort. Monocytes from patients with SLE, RA and from healthy donors were used for generating disease-specific gene-expression profiles, where these profiles represent in vivo activation of monocytes.
In addition, monocytes from healthy donors were stimulated in vitro by cytokines: TNFα, IFNα2a and IFNγ. Cytokine-specific gene-expression profiles were generated by comparing stimulated monocytes with unstimulated ones. TNFα, IFNα2a- and IFNγ as cytokine-specific gene-expression profiles were compared with RA and SLE, as disease-specific gene-expression profiles.

GSE4588: In this cohort, CD4 T and B cells were sorted by flow cytometry from PBMC of patients with SLE, RA and healthy controls. GeneChip® Human genome U133 Plus 2.0 arrays were hybridized in monoplicates and the genes differentially expressed among the three groups of patients were identified using ANOVA tests with corrections for multiple comparisons.

GSE46920: In this cohort, Monocytes from 3 healthy donors were cultured for 6 hours in the presence of 20% serum from three newly diagnosed, untreated SLE patients. Microarray analysis was then performed upon normalizing the gene expression levels of samples incubated with SLE sera to those incubated with autologous serum.

GSE46923: In this cohort**,** Rodriguez-Pla et al. collected samples from 51 SLE patients, including 7males and 44 females. The average age of the patients at the day of sample collection was 15 years (range: 8–19), and the average duration of SLE was 0.69 years (range: 0–2.06 years). The breakdown of the patient ethnicity was: 45% Hispanic, 27% African American, 18% Caucasian, 4% Asian, and 2% unspecified. Thirteen sera from pediatric SLE patients were used for the flow cytometry staining on cultured monocytes, most of them in more than one independent experiment. SLE sera were selected based on high Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), absence of immunosuppressive medication, and absence of high dose prednisone at the moment of blood draw, in addition to absence of intravenous prednisolone bolus administration in the two months previous to the date of blood draw. Patients were all females. Average age was 15.8 years (range: 13–17). The breakdown of ethnicity was: 61.5% Hispanic, 30.8% African American, and 7.7% Caucasian.

The control population consisted of 21 randomly selected healthy children, including 5 males and 16 females (average age: 12 years; range: 6–22). The ethnic breakdown of the healthy donors was: 42% Caucasian, 29% Hispanic, 19% African American, and 10% Asians. Some of the flow cytometry staining on cultured monocytes were done using monocytes from three adult healthy donors (two males and one female with ages ranging from 31 to 56 years.)

GSE50772: Kennedy et al. enrolled all patients met the American College of Rheumatology criteria for SLE. They registered this trial (NCT00962832) on the ClinicalTrials.gov website. For purposes of executing clinical trials with different end points, patients with SLE are characterised predominantly as patients with extrarenal lupus (ERL) or as patients with lupus nephritis (LN). The following cohorts were evaluated: 61 patients with extrarenal lupus (ERL) in the University of Michigan observational cohort, 60 patients with mild ERL enrolled in the rontalizumab Phase I trial,[16] 135 patients with moderate-severe ERL in the EXPLORER rituximab trial,[17] 80 patients with moderate-severe lupus nephritis (LN) in the LUNAR rituximab trial[18] and 238 patients with moderate-severe ERL in the ROSE rontalizumab (anti-IFN-α monoclonal antibody) trial.[19] Healthy control subjects (n=85) were recruited by the Genentech blood donation programme for research use of blood samples, and were age matched and gender matched to the lupus trial patients.

GSE51997: Kyogoku et al. collected cells from Systemic Lupus Erythematosus (SLE) patients as follows: For CD4pos T cells, six patients with SLE (average age: 29.0 +/- 7.6) and four normal healthy donors (ND; 24.8 +/- 0.5) were recruited. For CD16neg monocytes, four patients with SLE (26.5 +/- 1.7) and four NDs (24.8 +/- 0.5) were recruited. For CD16pos monocytes, four patients with SLE (26.5 +/- 1.7) and three NDs (24.7 +/- 0.6) were recruited. All patients and NDs were female. The same NDs were examined before and after immunisation with yellow fever vaccine (YFV). Collection of cells from yellow-fever vaccinated individuals: ND were immunised with a vaccine against the wild-type YF virus, which is a single-stranded RNA virus without adjuvants. This vaccine consists of a live but attenuated strain of the yellow fever virus (YFV-17D). Based on its vaccination-associated clinical and serological manifestations, this immunisation can be regarded as a real viral infection. A total of 50 ml peripheral blood was taken 7 days after immunisation, when sufficient numbers of CD19pos/CD27high plasmablasts were detected by flow cytometry. Cell sorting: A total of 50 ml peripheral blood was collected in Vacutainer heparin tubes and erythrocytes were lysed in EL buffer. Subsequently, granulocytes were depleted using CD15-conjugated microbeads (MACS). The CD15-depleted fraction was stained with a CD14-fluorescein isothiocyanate (FITC) antibody, a CD16-APC-Cy7 antibody, a CD3-Vioblue antibody and a CD4-FITC antibody. Using a FACSAria cell sorter, CD4pos T cells, CD16neg monocytes and CD16pos monocytes were isolated with purities and viabilities of >97%. After sorting, the cells were immediately lysed with RLT buffer and frozen at -70 °C. Total RNA was isolated using an RNeasy mini kit, and quality control was ensured by Bioanalyser measurements.Total RNA was extracted using the RNeasy Mini kit. The integrity and amount of isolated RNA was assessed for each sample using an Agilent 2100 Bioanalyzer and a NanoDrop ND-1000 spectrophotometer. Biotinylated complementary RNA (cRNA) was synthesized from 100 ng total RNA, using reagents as recommended in the technical manual from Affymetrix. Fifteen micrograms of fragmented cRNA of each sample were hybridized to HG-U133 plus 2.0 arrays. Hybridization was performed according to procedure 2 as described in the technical manual. Finally, the arrays were scanned with a GeneChip Scanner 3000 using the GCOS software. All relevant GCOS data of quality checked microarrays were analyzed

with High Performance Chip Data Analysis (HPCDA, unpublished), using the BioRetis database (www.bioretis-analysis.de), as described and validated previously.

GSE52471: Jabbari et al. profiled the transcriptome of Discoid lupus erythematosus (DLE) skin in order to identify signaling pathways and cellular signatures that may be targeted for treatment purposes. Further comparison of the DLE transcriptome with that of psoriasis, a useful reference given our extensive knowledge of molecular pathways in this disease, provided a framework to identify potential therapeutic targets. Although a growing body of data support a role for IL-17 and T helper type 17 (Th17) cells in systemic lupus, we show a relative enrichment of IFN-γ-associated genes without that for IL-17-associated genes in DLE. Extraction of T cells from the skin of DLE patients identified a predominance of IFN-γ-producing Th1 cells and an absence of IL-17-producing Th17 cells, complementing the results from whole-skin transcriptomic analyses. These data therefore support investigations into treatments for DLE that target Th1 cells or the IFN-γ signaling pathway.

Eleven patients with active DLE were enrolled in the study. Punch biopsy and shave biopsy specimens of psoriasis (n=5) and normal (n=3) skin samples were from patients with active moderate to severe disease or healthy subjects, respectively. Additional sample data from prior studies were added.

GSE55447: Sharma et al. collected Peripheral blood from 21 African-American (AA) and 21 European-American (EA) SLE patients, 5 AA controls, and 5 EA controls. CD4+ T-cells, CD8+ T-cells, monocytes and B cells were purified by flow sorting. Each cell subset from each subject was run on an Illumina HumanHT-12 V4 expression BeadChip array (n=208 arrays). Differentially expressed genes (DEGs) were determined by comparing cases and controls of the same ancestral background.

GSE72747: Ducreux et al. recruited Twenty-eight patients with SLE (aged 18–50 years), according to the ACR criteria for SLE, in a multicentre, randomized, double-blind placebo-controlled, phase I/II staggered dose-escalation trial of IFN-K (ClinicalTrials.gov registry number NCT01058343). Patients were randomized to receive three or four injections of placebo (n = 7) or 30 μg (n = 3), 60 μg (n = 6), 120 μg (n = 6) or 240 μg (n = 6) IFN-K.

Global gene expression studies were performed in serial whole blood samples from SLE patients with a renal BILAG A prior to, 3 months, and 6 months after initiation of conventional immunosuppressive therapy (induction with high-dose corticosteroids, IV cyclophosphamide or oral mycophenolate during the first 3 months, followed by maintenance with moderate- to low-dose corticosteroids, azathioprine or mycophenolate). The expression of IFN-induced genes was analyzed, in comparison to global and renal indices of disease activity.

GSE78193: Normal donor blood was incubated with or without IFN-g stimulation to establish an IFN-g gene signature. Twenty-six patients aging between 18-65 years, with mild-to-moderate and stable SLE were administered placebo or a single dose of AMG 811, ranging from 2 mg to 180 mg subcutaneously or 60 mg intravenously. Antimalarial agents, leflunomide, azathioprine, methotrexate, and up to 20 mg/day of prednisone (or equivalent) were permitted as concomitant therapies. A therapeutic anti-IFN--g antibody, and changes in the IFN--g signature in whole blood of these subjects was measured.

Whole blood PAXgene tube samples were collected from all cohorts at baseline, day-1 (pre-dose), and at days 15, 56, and end of study (EOS) after treatment Arrays were hybridized in a Loop design.

References for Supplemental Materials

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