ONLINE SUPPLEMENT

PATIENTS AND METHODS

Study design, patients and healthy controls

Biological samples were collected from 133 consecutive SLE patients included in the Swiss SLE Cohort Study (SSCS) between November 2017 and December 2018. Inclusion criteria were age ≥ 18 years, diagnosis of SLE according to the updated American College of Rheumatology classification criteria [1] or the SLICC 2012 [2]. The cohort study was approved by the SwissEthics review board (PB_2017-01434) and all patients gave written informed consent.

Thirty-five age- and sex-matched healthy controls (HCs) were selected from the Geneva Blood donation bank following classical guidelines on assessing donor suitability for blood donation. In particular, fever or viral symptoms the week before blood sampling were exclusion criteria. HCs could not be matched based on ethnicity and ancestry.

Patient and public involvement.

No patients nor public were involved in the conception or realization of the present study.

Data collection and definitions

We collected demographic data, SLE clinical characteristics, the Safety of Estrogens in Lupus Erythematosus National Assessment–Systemic Lupus Erythematosus Disease Activity Index (SELENA–SLEDAI) [3]. The therapeutic regimen was recorded on the day of sample collection. Biological data collected were complete blood count, serum creatinine level, proteinuria, and complement C3 and C4 levels. Testing for anti-dsDNA (Q'Flash dsDNA Kit, Ruwag Life Science, Switzerland), anti-U_1RNP, anti-SSA, anti-C1q antibodies (ELISA, Ruwag Life Science, Switzerland) was performed according to manufacturer protocols in a central laboratory in Geneva (Switzerland). Medication with disease-modifying drugs was classified in four groups: 1) no treatment versus active treatment, 2) antimalarial (AM) therapy only, 3) systemic glucocorticoids (GC) and 4) immunosuppressant agents (IS). Active clinical features were based on SELENA-SLEDAI and active skin disease included at least one active SELENA-

SLEDAI features among active lupus rash, mucosal ulcers and alopecia. Patients were classified in 2 groups active and inactive SLE according to a modified definition of active SLEDAI, named clinical SLEDAI, that excluded the contribution of low serum complement and elevated dsDNA autoantibodies with a cut-off > 0 to define active disease.

mRNA collection extraction and quality

Whole blood was collected in Tempus tubes (Thermo Fisher Scientific, Waltham, USA), and stored at -80°C until mRNA purification. Serum samples were collected concomitantly. Total RNA was extracted by using MagMA for the Stabilized Blood Tubes RNA Isolation Kit (Thermo Fisher Scientific) and RNA quality was assessed post-extraction in a subset of samples by bioanalyzer RNA chip (Agilent Technologies). RNA quality assessed by RNA Integrity Number (RIN) was good in representative samples, with values > 8.

Quantitative assessment of mRNA levels and gene normalization

Gene expression was assessed by mRNA expression profiling by using a NanoString nCounter gene expression system (NanoString Technologies, Seattle, WA) as previously described [4]. Briefly, 100 ng total RNA was hybridized to the probes (a reporter probe and a capture probe) at 67 °C for 16–21 h by using a thermocycler. Samples were then inserted into the nCounter Prep Station for removing excessive probes, purification and immobilization onto the internal surface of a sample cartridge for 2 to 3 h. Finally, the sample cartridge was transferred to the nCounter Digital Analyzer, where color codes were counted and tabulated for each target molecule. Background correction involved subtracting from the raw counts the mean+2 SD of counts obtained with negative controls (alien probes lacking spiked transcript for background calculation). Values <1 were fixed to 1 to avoid negative values after log transformation. Positive controls (alien sequences of RNA spiked at various concentrations to assess the overall assay performance) were used as quality assessment: the ratio between the highest and the lowest positive controls average among samples was < 3. IFN-I, PMN and PB gene counts were normalized to the geometric mean of 7 housekeeping gene counts selected as the most stable by using the geNorm algorithm [5].

Gene selection and computation of the IFN-I gene scores

A literature review was performed to identify genes previously used to assess IFN-I signature as part of modules of genes [6] or as quantitative scores[7]. A custom panel of 23 genes was

then developed allowing the computation of six IFN-I gene scores [7–12] as well as *CXCL10* which was used in the Module M1.2 of Banchereau et al[6]. The detailed gene list used to generate each score, is the following *IFI27, IFI6, RSAD2, IFI44, IFI44L, USP18, LY6E, OAS1, SIGLEC1, ISG15, IFIT1, OAS3, HERC5, MX1, LAMP3, EPSTI1, IFIT3, OAS2, RTP4, PLSCR1, DNAPTP6, TYK1, CXCL10*

Moreover, our gene selection allowed the computation of the following IFN-I gene scores:

- Yao Y 2009 [7]: *IFI27, IFI6, RSAD2, IFI44, IFI44L, USP18, LY6E, OAS1, SIGLEC1, ISG15, IFIT1, OAS3, HERC5, MX1, LAMP3, EPSTI1, IFIT3, OAS2, RTP4, PLSCR1, DNAPTP6*
-Petri M 2009 [11]: *IFI27, OAS3, IFI44*
- McBride 2012 [8]: *IFI27, IFI44, IFIT1, MX1, OAS1, OAS2, OAS3*
- Rice 2013 [12]: *IFI27, IFI44L, IFIT1, ISG15, RSAD2, SIGLEC1*
- Morimoto 2015 [9]: *EPSTI1, HERC5, TYK1*
- Khamashta 2016 [10]: *IFI27, IFI44, IFI44L, RSAD2*

- Banchereau 2016 [6] (Module M1.2) (gene included are highlighted in bold): *BATF2, BIRC4BP, **CXCL10, DNAPTP6, EPSTI1**, FLJ20035, **ISG15, HERC5**, HES4, **IFI44, IFI44L**, IFIT1, **IFIT3**, IFITM3, **LAMP3, LY6E, MX1, OAS1, OAS2, OAS3**, OASL, OTOF, **RSAD2, RTP4**, SERPING1, TRIM6*

The computation of the score was performed as previously described[7,12]. Briefly, for each individual, the relative expression of each gene was computed by dividing its normalized value by the median of the normalized HC expression. Then IFN-I gene scores were calculated as the median of the relative expression of all genes contributing to the score. The mean plus 2 SD of HC values was used as a threshold to define high scores. Unless otherwise stated, the IFN-I gene score refers to the score of Khamastha et al. (10) with a cut-off of high score of 17.5 UI as previously described[13]. In individual gene expression analysis, TYK1 was excluded because it was not expressed (count of 0) in most patients.

Single-molecule array (Simoa) IFN-α and IFN-γ digital ELISA

The Simoa™ (single molecule array) HD-1 analyzer (Quanterix, Lexington, MA, USA) was used for ultrasensitive immunodetection (digital ELISA)[14] of IFN-α and IFN-γ, using single-plex bead-based assays and procedures obtained from Quanterix Corporation.

According to the manufacturer's instructions, the working dilutions were 1:2 for all sera. At low cytokine concentration, the percentage of bead-containing wells in the array that have a positive signal is proportional to the amount of cytokine present in the sample (digital measurement). At higher concentration, when most of the bead-containing wells have one or more labeled-cytokine molecules, the total fluorescence signal is proportional to the amount of cytokine present in the sample (analog measurement). Calibrators were run in duplicate and fit with a four-parameter logistic (4PL) regression, with 1/y2 weighting. Cytokine concentrations in serum samples were interpolated from the standard curves. We used a serum-IFN-α level threshold value of 136 fg/mL to define high IFN-α levels based on 3 SD above the mean value from 68 HC as previously described[15] and an IFN-γ threshold of 2558 fg/mL based on 3 SD above the mean value from 74 HC to define elevated IFN-γ levels.

Statistical analysis

Data are presented as median (interquartile (IQR) range) or counts (percentage). Chi-square test and Fisher's exact test were used to compare categorical variables; Mann-Whitney test was used to compare non-paired variables, and Spearman correlation analysis (rho) was used for correlations. Agreement for classifying SLE as having high IFN levels between IFN-I gene scores and IFN-α and IFN-γ serum levels measured by SIMOA was assessed using Kappa coefficient, with values defined as follows: $0 < \kappa < 0.2$ slight; $0.2 \leq \kappa < 0.4$ fair agreement, $0.4 \leq \kappa < 0.6$ moderate $0.60 \leq \kappa < 0.80$ substantial agreement and $\kappa \geq 0.80$ almost perfect agreement[16]. In order to assess features associated with high IFN-I gene score and IFN-α or IFN-γ serum levels, parameters with $p < 0.2$ on univariable analysis were entered in multivariable logistic models. A 2-tailed $p < 0.05$ was considered statistically significant. The areas under (AUCs) the receiver operating characteristics (ROC) curves to differentiate active vs inactive SLE according to IFN-α and IFN-γ serum levels, IFN-I gene score, C3 levels and anti-dsDNA titers were compared[17]. compared Analyses were performed with JMP v15 (SAS Institute Inc, Cary, NC).

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RESULTS

Supplementary table s1. Baseline characteristics and active features at the time of sampling in SLE patients (n=133)

Features	
Female	111 (83)
Age, median (range), years	45.6 (19-78.8)
Ethnicity	
Caucasian	98 (74)
African	16 (12)
Asian	12 (9)
Others	7 (5)
Active manifestations at the time of study	
Fever	5 (4)
Arthritis	26 (20)
Cutaneous	28 (21)
Serositis	6 (5)
Neurological disorder	9 (7)
Leukopenia	6 (5)
Thrombocytopenia	8 (6)
Proteinuria > 0.5 g/24h	6 (5)
Immunological features (presence)	
ANA*	125 (94)
Anti-dsDNA	61 (46)
Anti-SSA	55 (41)
Anti-Sm*	34 (26)
Anti-U_1RNP	37 (28)
Anti-C1q	34 (26)
aPL*	53 (40)
Low complement	28 (21)
C3 (g/l) median, range*	0.90 (0.39-1.8)
C4 (g/l) median, range*	0.16 (0.01-0.45)
Activity	
Clinical SLEDAI > 0	75 (56)
Clinical SLEDAI, median (range)	2 (0-42)

SLEDAI, median (range)	4 (0-46)
Current treatment	
No treatment	14 (11)
Antimalarials	97 (73)
Systemic glucocorticoids	53 (40)
Immunosuppressant agents	64 (48)
B cell targeted agents	9 (7)

* historical data: Antinuclear autoantibodies (ANA), Sm and aPL (antiphospholipid), C3 and C4. Double stranded DNA (ds-DNA), U_1 ribonucleoprotein (U1RNP), anti-C1q were determined by Q'Flash or ELISA as described in the methods section in supplementary material. SLEDAI, Safety of Estrogens in Lupus Erythematosus National Assessment–Systemic Lupus Erythematosus Disease Activity Index (SELENA–SLEDAI).

Supplementary Table s2. Association of high IFN-I gene score and high serum IFN-α with active SLE features

Features N (%) ƚ	Low IFN-I score (n=73)	High IFN-I score (n=57)	p-value uni	p-value multi	OR [95 CI%] multivariable	Low serum IFN-α (n=73)	High serum IFN-α (n=57)	p-value uni	p-value multi	OR [95% CI] multivariable
SLE fever	0 (0)	5 (9)	0.01*	0.99	-	1 (1)	4 (7)	0.16	0.53	2.25 [0.18-28.7]
Arthritis	7 (10)	19 (33)	0.007*	0.007*	4.81 [1.53-15.09]*	10 (14)	16 (28)	0.04*	0.10	2.34 [0.83-6.56]
Pooled active skin[ʃ]	8 (11)	20 (35)	0.0009*	0.01*	4.12 [1.32-12.84]*	9 (12)	19 (33)	0.004*	0.04*	2.71 [1.01-7.31]*
Serositis	2 (3)	4 (7)	0.40	-	-	2 (3)	4 (7)	0.40	-	-
Neurological disorders	3 (4)	6 (11)	0.18	0.98	-	4 (5)	5 (9)	0.46	-	-
Leukopenia	0 (0)	5 (9)	0.01*	0.99	-	0 (0)	5 (9)	0.01*	0.99	-
Thrombocytopenia	5 (7)	3 (6)	1	-	-	5 (7)	3 (6)	1	-	-
Proteinuria > 0.5g/24h	3 (4)	3 (6)	1	-	-	2 (3)	4 (7)	0.40	-	-
Low complement	13 (18)	15 (27)	0.23			13 (18)	15 (27)	0.23		
Positive anti-dsDNA Abs	29 (39)	32 (55)	0.06	0.03*	2.59 [1.08-6.18]*	25 (34)	36 (60)	0.003*	0.004*	3.27 [1.45-7.38]*
Current vs No treatment	12 (17)	2 (4)	0.02*	0.02*	0.11 [0.02-0.73]*	10 (14)	4 (7)	0.26	-	-
AM only	17 (24)	15 (26)	0.72	-	-	19 (26)	13 (23)	0.64	-	-
GC use	25 (34)	28 (49)	0.08	0.82	1.10 [0.46-2.67]	24 (33)	29 (51)	0.04*	0.11	1.94 [0.86-7.38]
IS use	35 (49)	29 (51)	0.80	-	-	37 (51)	27 (47)	0.65	-	-

Values are expressed as n (%).

IFN-I score according to [10].

ƚ % Among available data (3 patients had missing data regarding active features and treatment),

[ʃ] including active cutaneous lupus, mucosal ulcers and alopecia,

* $p < 0.05$

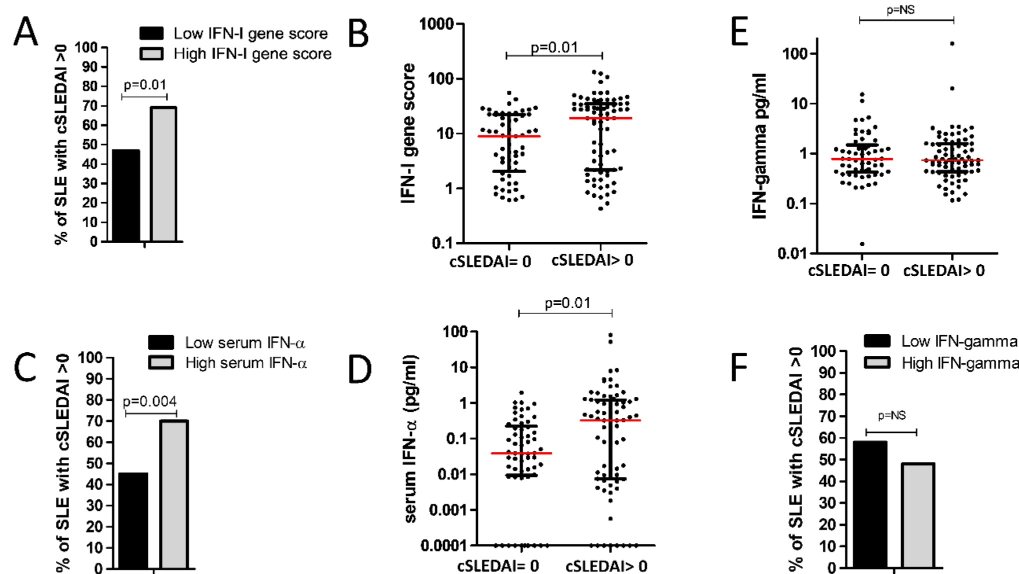
Variables with P<0.2 on univariable regression were entered in multivariable logistic models. Abs, antibodies; AM, antimalarials; GC, glucocorticoids; IS, immunosuppressant agents; multi, multivariable; uni, univariable.

Supplementary Table s3. Diagnostic performance of serum IFN-α and IFN-I gene score to predict clinical activity based on clinical SLEDAI> 0

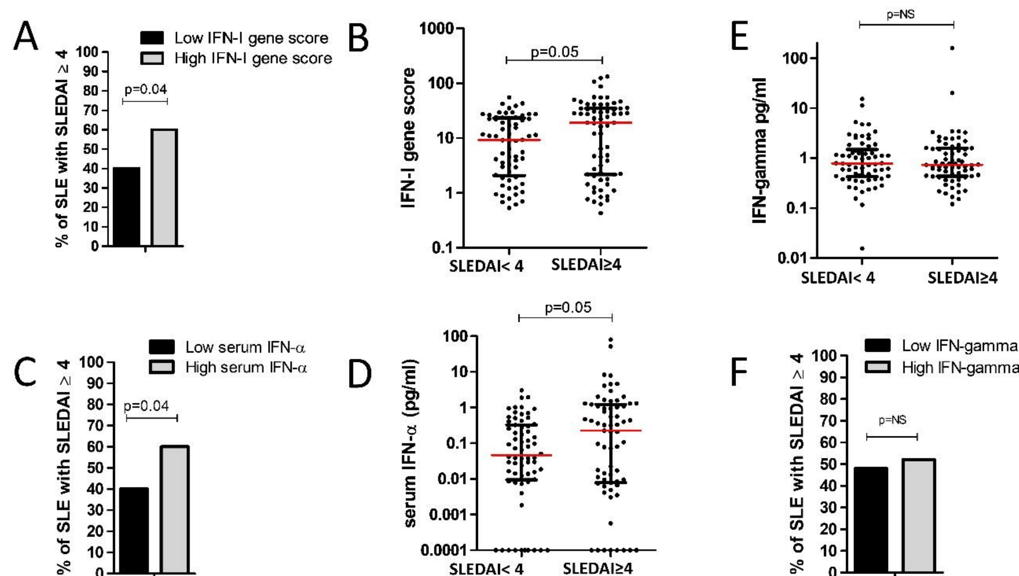
Features	**Serum IFN-α**		**IFN-I gene score**	
	Predefined cut-off 136 fg/mL	Optimal cut-off 319 fg/ml	Predefined cut-off 17.5 UI	Optimal cut-off 24.4 UI
Sensitivity	56%	52%	53%	47%
Specificity	67%	81%	69%	86%
PPV	69%	78%	69%	81%
NPV	54%	57%	53%	56%

PPV: Positive predictive value; NPV: Negative Predictive Value, Optimal cut-off were determined by the Youden Index (the sum of sensitivity and specificity – 1). By using an optimal cutoff computed using the Youden Index, only the specificity and the positive predictive value increased substantially, for both IFN-I gene scores and serum IFN-α levels.

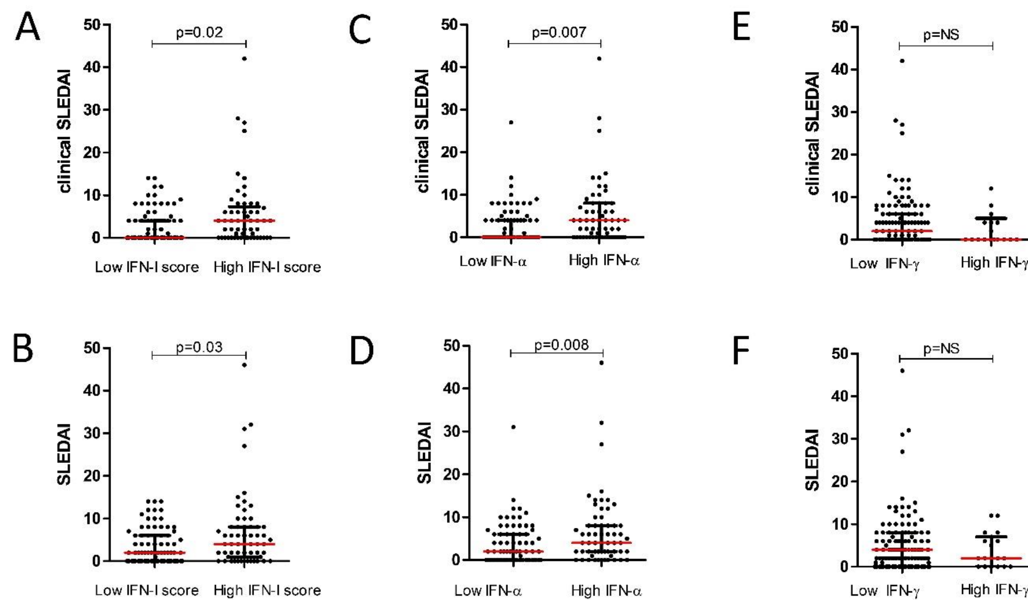
Supplementary Figure s1. Association between IFN-I gene score, serum IFN-α and serum IFN-γ with SLE activity. Active SLE was defined by clinical SLEDAI >0. Applied statistics were the Chi-square test in **A**, **C** and **E**, and the Mann-Whitney U test in **B, D** and **F.** Similar results were observed after the exclusion of patients with African descent



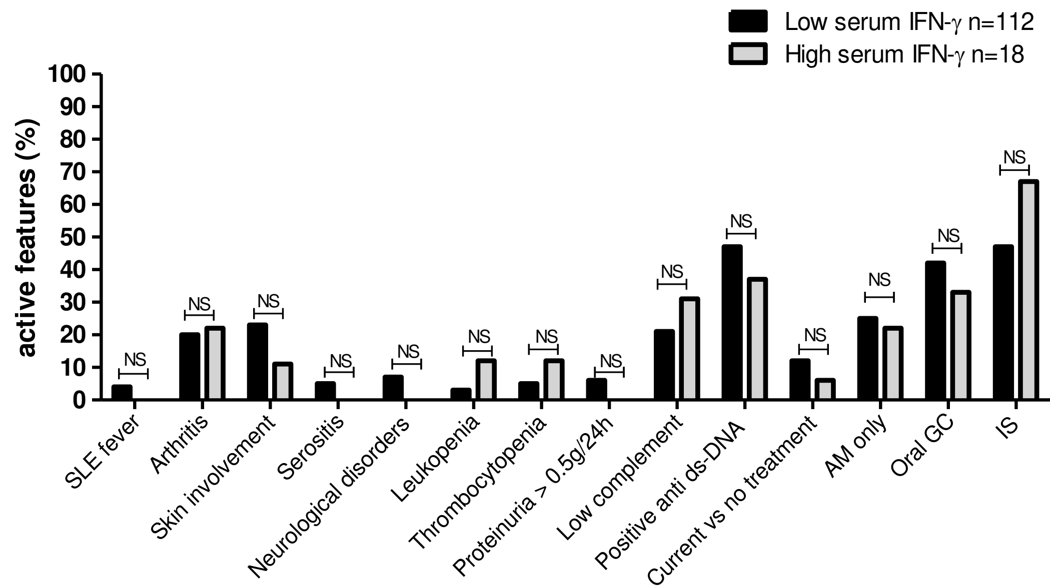
Supplementary Figure s2. Association between IFN-I gene score, serum IFN-α and serum IFN-γ with SLE activity. Active SLE was defined by SLEDAI ≥4. Applied statistics were the Chi-square test in **A**, **C** and **E**, and the Mann-Whitney U test in **B, D** and **F.**



Supplementary Figure s3. Comparison of clinical SLEDAI and SLEDAI among patients with high or low IFN-α, IFN-γ levels or IFN-I score. A and B. High IFN-I score was defined by a score > 17.5 UI as previously described[13]. **C and D.** A threshold value of 136 fg/mL was used to define high IFN-α levels based on 3 SD above the mean value from 68 HC as previously described [15] E and F. A threshold value of 2558 fg/mL was used to define high IFN-γ based on 3 SD above the mean value from 74 HC. Applied statistics the Mann-Whitney U test in all cases.



Supplementary Figure s4. Association between serum IFN-γ levels with SLE activity and active phenotypes. Chi-square and Fisher's exact tests were used to compare categorical variables as appropriate. NS: Not significant



Supplementary Figure s3. **Receiver operative characteristic curve analysis of IFN-I gene scores, serum IFN-α and IFN-γ, C3 and anti-dsDNA levels discriminating active and inactive SLE**. SLE activity was defined according to clinical SLEDAI, inactive SLE (clinical SLEDAI=0) and active SLE (SLEDAI >0). AUC: area under the receiver operating characteristic curve.