Acta Biochim Biophys Sin 2024, 56(11): 1659–1672
https://doi.org/10.3724/abbs.2024132
Advance Access Publication Date: 2 August 2024
Original Article

Original Article

SPATS2L is a positive feedback regulator of the type I interferon signaling pathway and plays a vital role in lupus

Mengke Chen[1,†], Yutong Zhang[1,†], Weiwen Shi[1,†], Xuejiao Song[6,†], Yue Yang[6,7], Guojun Hou[1], Huihua Ding[1], Sheng Chen[1], Wanling Yang[2], Nan Shen[1,3,4,5], Yong Cui[6], Xianbo Zuo[6,7,*], and Yuanjia Tang[1,*]

[1]Shanghai Institute of Rheumatology, Renji Hospital, Shanghai Jiao Tong University School of Medicine (SJTUSM), Shanghai 200001, China, [2]Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Hong Kong 999077, China, [3]State Key Laboratory of Oncogenes and Related Genes, Shanghai Cancer Institute, Renji Hospital, Shanghai Jiao Tong University School of Medicine (SJTUSM), Shanghai 200032, China, [4]Center for Autoimmune Genomics and Etiology, Cincinnati Children's Hospital Medical Center, Cincinnati OH 45229, USA, [5]Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati OH 45229, USA, [6]Department of Dermatology, China-Japan Friendship Hospital, Beijing 100029, China, and [7]Department of Pharmacy, China-Japan Friendship Hospital, Beijing 100029, China
[†]These authors contributed equally to this work.
*Correspondence address. Tel: +86-18919681889; E-mail: zuoxianbo@qq.com (X.Z.) / Tel: +86-21-53882259; E-mail: yjtang@sibs.ac.cn (Y.T.)

Received 4 February 2024 Accepted 3 April 2024

Abstract

Through genome-wide association studies (GWAS) and integrated expression quantitative trait locus (eQTL) analyses, numerous susceptibility genes ("eGenes", whose expressions are significantly associated with common variants) associated with systemic lupus erythematosus (SLE) have been identified. Notably, a subset of these eGenes is correlated with disease activity. However, the precise mechanisms through which these genes contribute to the initiation and progression of the disease remain to be fully elucidated. In this investigation, we initially identify SPATS2L as an SLE eGene correlated with disease activity. eSignaling and transcriptomic analyses suggest its involvement in the type I interferon (IFN) pathway. We observe a significant increase in SPATS2L expression following type I IFN stimulation, and the expression levels are dependent on both the concentration and duration of stimulation. Furthermore, through dual-luciferase reporter assays, western blot analysis, and imaging flow cytometry, we confirm that SPATS2L positively modulates the type I IFN pathway, acting as a positive feedback regulator. Notably, siRNA-mediated intervention targeting *SPATS2L*, an interferon-inducible gene, in peripheral blood mononuclear cells (PBMCs) from patients with SLE reverses the activation of the interferon pathway. In conclusion, our research highlights the pivotal role of SPATS2L as a positive-feedback regulatory molecule within the type I IFN pathway. Our findings suggest that SPATS2L plays a critical role in the onset and progression of SLE and may serve as a promising target for disease activity assessment and intervention strategies.

Key words systemic lupus erythematosus, SPATS2L, quantitative trait loci, type I interferon

Introduction

Systemic lupus erythematosus (SLE) is a typical chronic autoimmune disorder characterized by a notable genetic predisposition [1]. This condition manifests as multi-organ involvement, affecting diverse bodily systems, including the integumentary, musculoskeletal, renal, pulmonary, and cardiovascular systems. Despite extensive research endeavors, the exact etiology of lupus remains incompletely understood. Nonetheless, numerous investigations have revealed potential associations with genetic, environmental, and hormonal factors [2].

Genetic variations play a crucial role in both the etiology and pathogenesis of SLE [3–5]. In recent years, many studies, predominantly genome-wide association studies (GWAS), have made significant advancements in elucidating the underlying pathogenic mechanisms of SLE [6–12]. Through the analysis of large-scale genotype data, current investigations have identified

numerous genetic variations linked to susceptibility to SLE. Notably, a considerable proportion of these variations are associated with the dysregulated expression of genes, such as *IRF5*, *TYK2*, and *STAT4*, within the interferon (IFN) pathway [13–16], underscoring the significant involvement of the IFN signaling network in the pathogenesis of SLE.

Previous studies have demonstrated that the majority of individuals with SLE exhibit dysregulated expression of IFN-stimulated genes (ISGs), commonly referred to as the "IFN signature" [17–19]. Consequently, elevated level of type I IFN in serum serves as a notable marker for SLE patients [20–22]. Excessive IFN stimulates the generation of autoantibodies and instigates the upregulation of genes associated with immune responses, including cytokines, chemokines, and other inflammatory mediators, thereby promoting inflammation and contributing to the progression of SLE [23–25]. The aberrant activation of type I IFN is a major pathogenic factor in SLE and plays a pivotal role in the initiation and progression of lupus. Consequently, targeting this pathway has emerged as a promising therapeutic approach for managing this disease. Currently, numerous therapeutic strategies targeting interferons or their receptors are extensively employed in the treatment of SLE. These include, an approved monoclonal antibody targeting the type I interferon receptor (IFNAR), as well as investigational therapeutics such as interferon alpha (IFN-α) kinoid, and those which specifically target IFN-α [26–31].

SLE is a complex disease characterized by recurrent exacerbations, underscoring the importance of assessing disease activity to inform clinical management. A previous study delineated comprehensive gene signatures of SLE, encompassing both disease-state and disease-activity signatures. This inquiry underscores the clinical significance of disease activity signatures, as they are closely linked to organ involvement and therapeutic responses [32]. Nevertheless, evaluating SLE activity in clinical settings presents challenges due to its heterogeneous clinical manifestations and potential fluctuations over time. Consequently, identifying dependable biomarkers for measuring disease activity has become a prominent focus of contemporary research. Previous studies have demonstrated that individuals exhibiting elevated level of type I IFN often present with more pronounced clinical manifestations and are at high risk for nephritis and other severe sequelae [33,34]. Investigating these genes associated with SLE flare can provide valuable insights into the pathogenic mechanisms of SLE, identify new therapeutic targets, and pave the way for precision medicine. However, despite the observed correlation between the dysregulated expressions of ISGs and SLE flares, elucidating their precise role in disease progression remains a topic for further investigation.

Research into the genetic factors underlying SLE enhances our understanding of its etiology. Although several large-scale genome-wide association studies have identified numerous susceptibility loci for SLE [35,36], the majority (approximately 93%) are located within non-coding regions. However, their precise contributions to disease pathogenesis remain largely elusive [37,38]. Genetic susceptibility loci located in non-coding regions often influence disease occurrence by modulating gene expression. Analysis of expression quantitative trait loci (eQTL) reveals the relationship between genetic variations and gene expression changes, providing essential clues to link variants to phenotypes [16,39–41]. An eQTL comprises a variant (referred to as an "eSNP") and a gene (referred to as an "eGene") [42]. Some eSNPs exhibit both cis-eGenes (genes located near the eSNP) and trans-eGenes (genes located distant from the eSNP). Notably, more than 60% of the trans-eGenes associated with these eSNPs are regulated by the cis-eGene [42–47]. Additionally, trans-eGenes of a specific eSNP may tend to cluster within a particular signaling pathway, termed "eSignaling" herein. Analyzing the eSignaling of eSNPs can provide insights into the functional roles of cis-eGenes and valuable clues for unraveling the signaling pathways involving cis-eGenes. This approach facilitates the discovery of new biomarkers through further exploration of their functions and regulatory networks, enabling more precise and individualized approaches to the diagnosis and treatment of SLE. However, further experimental validation and functional studies are necessary to confirm these hypotheses and establish the functional and clinical significance of eGenes in SLE.

In this investigation, we utilized large-scale data mining to elucidate the relationship between the lupus eGene SPATS2L and SLE flares. By integrating eQTL analysis, transcriptomic, and epigenomic data alongside experimental approaches such as lentivirus-mediated gene knockdown and overexpression, dual luciferase reporter assay, western blot analysis, and imaging flow cytometry, we revealed that SPATS2L acts as a positive feedback regulator within the type I IFN signaling pathway. Suppression of SPATS2L expression facilitates the restoration of the dysregulated type I IFN signaling pathway observed in individuals with SLE. Our findings underscore the significant role of SPATS2L in the etiology and progression of SLE.

Materials and Methods

Transcriptome data acquisition and processing

The Affymetrix microarray (GSE121239 [48] and GSE185047 [49]) and single-cell RNA sequencing (scRNA-seq) (GSE135779 [50] and GSE142637 [51]) datasets were retrieved from the publicly available GEO database maintained by the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/geo/). First, the GSE121239 dataset was normalized by the Limma package of R software (normalizeBetweenArrays function). Gene symbols were converted from probes and then prepared into matrix files. Single-cell data were filtered using the R package Seurat (nFeature_RNA>200 & nFeature_RNA<6000 & percent.mt<5), followed by integration, standardization, and transformation for downstream analysis. Batch effects were removed by Harmony. Principal component analysis (PCA) was performed, and *t*-distributed stochastic neighbor embedding (*t*SNE) was used for dimensionality reduction. Cell clusters were annotated using the SingleR package supplemented with manual curation. Visualization of the data was carried out using the R package ggplot2.

Identification of differentially expressed genes

Differential analysis of the normalized microarray data was conducted using the Limma package, applying a significance threshold of adjusted *P*-value (padj)<0.05 and $|\log_2$-fold change (FC)$|$>1 to identify DEGs associated with lupus. A DEG heatmap was created using the pheatmap package.

Correlation analysis and gene enrichment analyses

Correlation analysis between the expression of SPATS2L and that of other genes was performed using the R package Hmisc to calculate the *P* values and correlation coefficients.

Enrichr (https://maayanlab.cloud/Enrichr/) was used for func-

tional and pathway enrichment analyses. Enrichr is an online annotation tool that contains 180,184 annotated gene sets from 102 gene set libraries. Finally, the data were visualized using ggplot2.

Regional visualization of genome-wide association study

A regional association plot of genome-wide significant loci was created with LocusZoom. LD information from the 1000 Genomes Project East Asian data was used to calculate the r^2 between regional variants (colored according to r^2).

Vector construction

The SPATS2L overexpression plasmid was purchased from GENECHEM (Shanghai, China). To construct the shRNA expression vector, the plasmid pKLV-U6gRNA (BbsI)-PGKpuro2ABFP (50946, Addgene, Watertown, USA) was linearized with BbsI (R3059L, NEB, Ipswich, USA) and BamHI (R0136S, NEB) and then gel purified. Two sets of shSPATS2L oligos were synthesized by Tsingke (Shanghai, China). The sense and antisense strands of shSPATS2L were annealed to form complementary double-stranded DNA fragments to ligate linear 50946 plasmids. The ligated plasmids were then transformed into *E. coli* Stbl3 receptor cells. Colonies containing the shSPATS2L plasmid were inoculated in LB media supplemented with 100 μg/mL ampicillin. The plasmids were isolated using the NucleoBond Xtra Midi kit (740410.50, MACHEREY-NAGEL, Düren, Germany). All recombinant plasmids were verified by sequencing at Tsingke. The shRNA oligos used are listed in Supplementary Table S1.

Cell culture

All cell lines were purchased from the Chinese Academy of Science Cell Bank (Shanghai, China). Jurkat (TCHu123) and Ramos (TCHu250) cells were cultured in 10% (v/v) fetal bovine serum (FBS) and 90% RPMI-1640 medium. HEK-293T (GNHu17) cells were cultured with 10% (v/v) FBS and 90% Dulbecco's modified Eagle's medium. PBMCs were freshly isolated from human peripheral blood using Ficoll-Paque (GE Healthcare, Pittsburgh, USA) and cultured in RPMI 1640 (Gibco, Carlsbad, USA) supplemented with 10% FBS. All cells were maintained at 37°C in a 5% CO_2 atmosphere.

Lentivirus production

HEK-293T cells were seeded in a 6-well plate at 5×10^5 cells per well and incubated overnight at 37°C with 5% CO_2. Then, the cells were transfected with 1 μg of the target plasmid, 250 ng of pMD2. G (12259; Addgene), and 750 ng of psPAX2 (12260; Addgene) using 3 μL of Lipofectamine 2000 (11668-019; Thermo Fisher Scientific, Waltham, USA). The plasmids and transfection reagents were diluted with Opti-MEMI (31985062; Gibco) and incubated at room temperature for 20 min after gentle mixing, after which the transfection mixture was added to the prepared HEK-293T cells. Fresh media were exchanged 6 h after transfection. Seventy-two hours after transfection, the viral supernatant was collected and centrifuged at 1000 *g* and 4°C for 10 min to remove debris. The viral supernatant was aliquoted and stored at −80°C.

Cell stimulation, transduction, and siRNA transfection

Type I IFN (PBL) was added separately to 3×10^5 Jurkat cells and PBMCs at final concentrations of 50/200/1000 units/mL at different time points. The lentivirus encoding shSPATS2L described above was transduced into Jurkat and Ramos cells. A lentivirus overexpressing SPATS2L was similarly transduced into Jurkat cells. The media were replaced by fresh media 24 h after transduction. After transduction, the cells were selected with 1 μg/mL puromycin for 72 h. The siRNAs targeting SPATS2L were synthesized by Tsingke. Before transfection, 2×10^5 cells were seeded into a 24-well plate and incubated at 37°C and 5% CO_2 overnight. The siRNA (200 nM) was transfected into primary cells using the NEON Transfection System (Thermo Fisher Scientific). The siRNA oligos used are listed in Supplementary Table S1.

RNA library preparation, sequencing, and gene expression analysis

Total RNA was extracted from the Jurkat cells in the KD-SPATS2L and NC groups using TRIzol reagent (15596026; Thermo Fisher Scientific). The Illumina NEBNext® Ultra™ RNA Library Prep Kit (NEB) was used to prepare the libraries. The library preparations were sequenced on an Illumina HiSeq platform, and 2×150 paired-end reads were generated. The paired-end reads were processed and analyzed using Fastp, HISAT2, and featureCounts software. Statistical normalization and differential analysis were conducted in R using the DESeq2 package. Gene set enrichment analysis (GSEA) was conducted using GSEA (version 4.1.0) [52,53]. To assess the enrichment magnitude and statistical significance of our results, the normalized enrichment score (NES) and false discovery rate (FDR) were utilized, respectively.

PBMC isolation

Individuals with SLE and healthy individuals were recruited and signed informed consent according to the internal review and ethics boards of Renji Hospital, Shanghai Jiao Tong University (SJTUSM). The experiments were approved by the internal review and ethics boards of Renji Hospital, Shanghai Jiao Tong University School of Medicine (SJTUSM). Peripheral blood was mixed at a 1:2 ratio with phosphate-buffered saline (PBS) containing 2% FBS and 2 mM EDTA and gently placed on Ficoll-Paque density gradient solution (density=1.077 g/mL; GE Healthcare) to isolate PBMCs. After density gradient centrifugation (400×*g*, 35 min, no brakes), the PBMC layer was carefully removed, and the cell pellets were washed twice with PBS for subsequent experiments.

RNA extraction and real-time PCR (RT-qPCR)

Total RNA was extracted using TRIzol reagent (Thermo Fisher Scientific), and cDNA was subsequently synthesized via reverse transcription using the PrimeScript RT Reagent Kit (Takara, Kyoto, Japan), followed by amplification and quantification via RT-qPCR with Taq Pro Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China) on a QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems, Foster City, USA). The relative expression levels were calculated using the $2^{-\Delta\Delta CT}$ method. *GAPDH* expression was used as an internal reference. The primers used are listed in Supplementary Table S2.

Luciferase reporter assay

A total of 1000 ng of the ISRE-luciferase reporter vector (Clontech, Mountain View, USA) and 100 ng of the Renilla vector (Promega, Madison, USA) were transfected into each well of 3×10^5 cells seeded in a 24-well plate. Twenty-four hours after transfection, the cell lysates were added to a 96-well black flat bottom microplate

(Greiner Bio-one, Kremsmuster, Austria), and luciferase activity was measured on a CENTRO XS3 LB 960 luminometer (Berthold, Oak Ridge, USA) using a Dual-Luciferase Reporter Assay System (Promega). The ratio of firefly to Renilla luciferase activity in each well was calculated and analyzed. All experiments were performed in triplicate or quadruplicate.

Western blot analysis

Jurkat cells harboring shSPATS2L oligos were stimulated with type I IFN (1000 units/mL) for 15 min or 1 h. Subsequently, the cells were lysed using RIPA buffer (Thermo Fisher Scientific) mixed with a protease inhibitor cocktail (Thermo Fisher Scientific). The cell proteins were subjected to 10% SDS-PAGE and blotted with the appropriate antibodies. The following antibodies (Supplementary Table S3) were used: anti-GAPDH rabbit mAb (HRP conjugate), anti-SPATS2L rabbit mAb, anti-STAT1 rabbit mAb, anti-phospho-STAT1 rabbit mAb, and HRP-linked anti-rabbit IgG. All the antibodies were obtained from Cell Signaling Technology (Danvers, USA). The primary antibodies were diluted at 1:1000, except for SPATS2L, which was diluted at 1:500. The secondary antibody was diluted at 1:3000.

Imaging flow cytometry

Jurkat cells transfected with either negative control or shSPATS2L were stimulated with type I IFN (1000 units/mL) for 60 min. After stimulation, the cells were fixed and permeabilized using the eBioscience Transcription Factor Staining Buffer Set (Invitrogen, Carlsbad, USA) according to the manufacturer's protocol. The cells were then resuspended in 100 μL of FACS buffer and incubated with antibodies (diluted 1:50) at room temperature in the dark for at least 1 h. DAPI staining was performed for less than 5 min to visualize the cell nuclei. Subsequently, the cells were washed and resuspended in FACS buffer (volume ranging from 20 to 200 μL) at a proper cell concentration of 1-2×10^7 cells/mL. The cell samples were loaded and analyzed using Amnis ImageStream Mark II (Merck, San Diego, USA). The similarity between STAT1 and nuclear staining patterns was calculated. The antibody (Supplementary Table S3) used in the imaging flow cytometry experiment was a rabbit monoclonal antibody against STAT1 (PE Conjugate; Cell Signaling Technology).

Statistical analysis

All the statistical analyses were performed using R (version 4.2.2) and GraphPad Prism 9 software. Data are shown as the mean±SEM. Details of the statistical analysis for each experiment can be found in the relevant figure legends. All the statistical analyses were performed using paired or unpaired two-tailed Student's *t*-test as indicated in the figure legends, unless otherwise mentioned.

Results

Increased expression of the lupus eGene SPATS2L in SLE patients is correlated significantly with disease progression

We conducted a comprehensive search in the Gene Expression Omnibus (GEO) database to gather whole blood expression profiles of adult individuals with lupus, ensuring the availability of relevant clinical information for subsequent analyses. We incorporated a dataset with a considerable sample size (GSE121239) [48] and conducted an analysis of gene transcription expression levels between individuals with SLE and their healthy counterparts. Subsequently, we identified a set of 110 genes exhibiting significant differential expression (padj<0.05; |logFC|>1). Among these 63 genes, *IFI27*, *IFI44L*, *ISG15*, *RSAD2*, and *SPATS2L* were significantly upregulated in patients with SLE compared to healthy controls.

To further elucidate the genes implicated in the progression of SLE, we stratified the patient cohort into distinct subsets based on their SLE Disease Activity Index (SELENA-SLEDAI) scores [54]. Patients with SLEDAI scores exceeding 4 were considered to be in an active state of SLE, while those with scores ranging from 0 to 4 were classified as being in a stable state. Subsequently, we performed differential analysis between these delineated groups, resulting in the identification of the top 10 SLE-related genes exhibiting the most significant differential expression changes as disease activity increased (Figure 1A). Subsequently, receiver operating characteristic (ROC) curve analysis was conducted for the set of 10 genes associated with disease progression. It was observed that, except lincRNA-FLJ42418, SPATS2L displayed superior diagnostic efficacy regarding SLE disease progression compared to other genes exhibiting differential expression (e.g., *IFI44L*, *USP18*, etc.). Furthermore, compared to FLJ42418, SPATS2L demonstrated a more robust ability to predict SLE (Figure 1B, Table 1). Moreover, a correlation analysis focusing on the mRNA expression levels of the SPATS2L gene in the peripheral blood of individuals with SLE and their SLEDAI scores was performed. Spearman rank correlation analysis revealed a significant positive correlation between the SPATS2L level and the SLEDAI score (R= 0.4524, P<0.0001; Figure 1C). Additionally, the expression level of SPATS2L was significantly greater in patients with stable SLE than in patients with stable SLE, indicating a close association between the expression of SPATS2L and disease progression (Figure 1D).

To further substantiate our finding that SPATS2L expression is correlated with SLE progression, we revisited our genome-wide association study on lupus [36]. In this study, a genome-wide association meta-analysis of 1,3377 SLE patients and 19,4993 controls from East Asians was conducted, which was the largest genetic association study of SLE to date. The lead missense variants are shown in Figure 1E and lie within the region of SPATS2L. We further conducted an eQTL analysis using SNPs located within SPATS2L, screened eQTLs Gene affected by SNPs using the whole blood sample database from GTEx (dbGaP Accession phs000424.v8.p2), and found that SPATS2L is an eQTL target of SNPs associated with lupus progression in East Asians (Supplementary Table S4). A previous study reported that an SNP (rs3769433) located in an intron of SPATS2L is associated with SLE in individuals of European ancestry (EA) [46]. SPATS2L, an eQTL target of rs3769433, acts as a cis-eGene for SLE (Figure 1F,G). Taken together, these data strongly suggest a role for elevated expression of SPATS2L in disease progression.

SPATS2L regulates the expression of IFN pathway-related genes

eQTL analysis offers important insights into gene function and regulation by revealing the relationships between genotypes and gene expression. Initially, we conducted an analysis to identify eSNPs that possess cis-QTL associations with SPATS2L while also exhibiting trans-eQTL associations with other genes. Subsequently, we performed an eSignaling analysis on the enriched pathways of trans-eGenes associated with these eSNPs. We identified 690 eQTLs

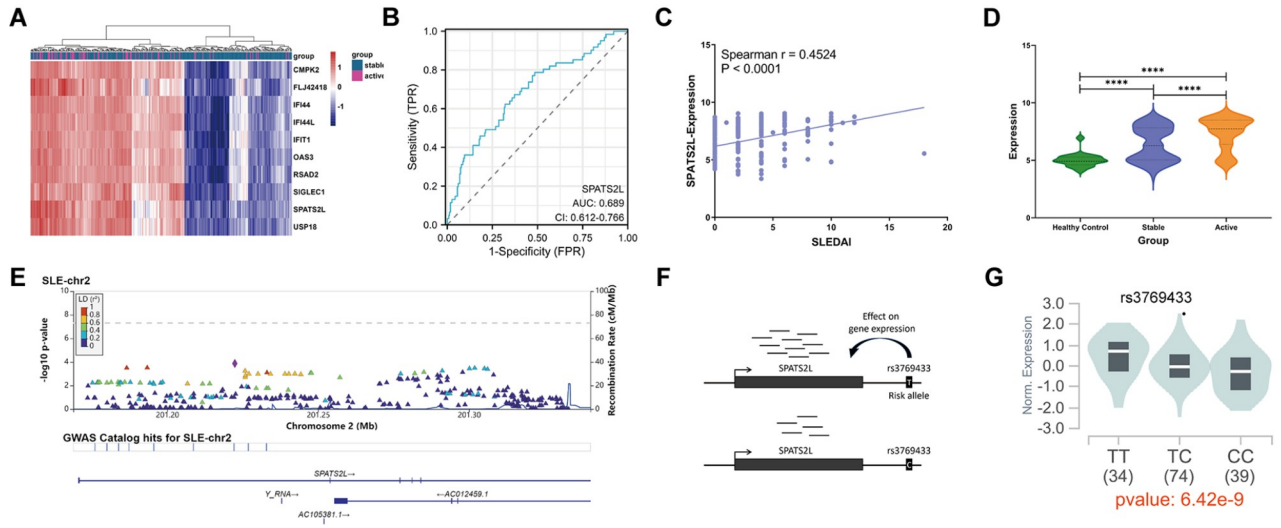

Figure 1. High expression of the eGene SPATS2L in SLE is strongly correlated with disease progression (A) The heatmap shows the top 10 SLE-related genes with the most significant differential expression changes as disease activity increased. (B) Diagnostic ROC analysis of the ability of SPATS2L to predict disease progression. (C) Correlation analysis between *SPATS2L* gene mRNA expression levels in whole blood of SLE patients and SLEDAI scores. SPATS2L was significantly positively correlated with the SLEDAI score (R=0.4524, P<0.0001). (D) SPATS2L is significantly upregulated in patients with SLE compared to patients in the stable phase of SLE. (E) Lead variants identified on chromosome 2 or near the eGene SPATS2L. The region plot was created via LocusZoom [55]. The lead SNP is labeled with a purple diamond, and recombination rates in the region are visualized as blue curves. LD, linkage disequilibrium; Mb, megabases. (F,G) SPATS2L acts as a cis-gene for SLE. SPATS2L is significantly influenced by the SLE-associated SNP rs3769433, which serves as an eGene for SLE. The Genotype-Tissue Expression (GTEx) project revealed differential expression of SPATS2L based on the allelic variation of rs3769433.

Table 1. ROC analysis for diagnosing SLE and predicting disease progression

Gene symbol	Area under the curve	Confidence interval
ROC analysis for diagnosing SLE		
IFI44L	0.817	0.761–0.872
SIGLEC1	0.808	0.740–0.875
SPATS2L	0.793	0.728–0.859
IFIT1	0.789	0.729–0.850
RSAD2	0.786	0.725–0.848
OAS3	0.779	0.724–0.834
CMPK2	0.761	0.702–0.820
IFI44	0.761	0.704–0.818
USP18	0.760	0.700–0.820
FLJ42418	0.759	0.704–0.814
ROC analysis for predicting disease progression		
FLJ42418	0.705	0.633–0.776
SPATS2L	0.689	0.612–0.766
RSAD2	0.675	0.597–0.753
IFI44L	0.674	0.597–0.751
IFI44	0.674	0.599–0.749
CMPK2	0.669	0.594–0.744
IFIT1	0.669	0.591–0.746
USP18	0.665	0.587–0.743
OAS3	0.658	0.583–0.733
SIGLEC1	0.654	0.579–0.730

with significant regulatory cis effects on SPATS2L by examining the expression of all genes associated with genetic loci (Bonferroni corrected P<0.05) on the eQTLGen website [16] (https://www.eqtlgen.org/). Among these loci, we further identified trans-eQTLs (P<0.005) and found that rs295140 and rs1367858 potentially impact SPATS2L function (Supplementary Table S5). Enrichment analysis of trans-eGenes revealed that rs1367858 may regulate the IFN signaling pathway, which involves OAS1, IRF4, and other ISGs, by modulating SPATS2L (Figure 2A and Supplementary Figure S1A). Moreover, on the GeneNetwork website [56] (https://www.genenetwork.nl/), a platform used for predicting gene function and identifying potential disease-related genes, we observed that SPATS2L is primarily associated with IFN signaling (Supplementary Figure S1B and Supplementary Table S5).

In addition, we conducted a correlation analysis in patients with SLE and identified 58 genes with a correlation coefficient greater than 0.8 with SPATS2L (P<0.05) (Supplementary Table S5). These genes were primarily enriched in the IFN signaling pathway (Figure 2B). Moreover, SPATS2L was strongly correlated with the trans-eGene OAS1 (R=0.8981, P<0.0001) (Figure 2C). *OAS1* is a well-established IFN-inducible gene [55].

To further investigate the function of SPATS2L, we generated Jurkat cell lines with *SPATS2L* knockdown and performed RNA-seq on two independent knockdown (KD) clones and two corresponding negative control (NC) clones. Gene set enrichment analysis (GSEA) revealed that in addition to being associated with cell cycle-related pathways, SPATS2L exhibited strong correlations with IFN signaling pathways, including the INTERFERON_ALPHA_RESPONSE, IL6_JAK_STAT3_SIGNALING, and IL2_STAT5_SIGNALING pathways (Supplementary Figure S1C). Moreover, through analysis of RNA sequencing data, we confirmed the anticipated downregulation of the expression levels of ISGs such as *OAS1* upon

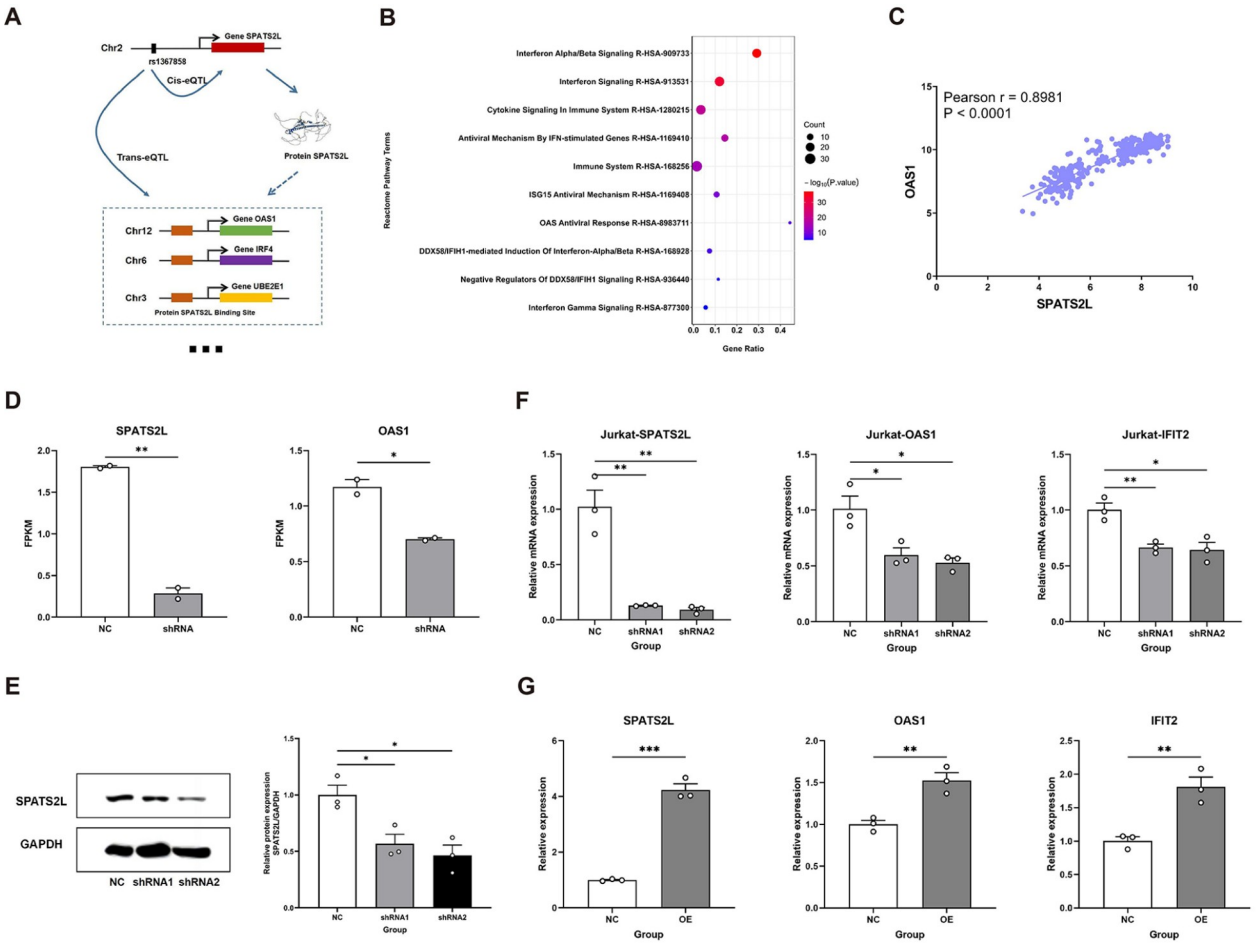

Figure 2. SPATS2L is closely associated with the IFN signaling pathway (A) The eQTL analysis suggested that SPATS2L is associated with the IFN signaling pathway. Rs1367858 may regulate the eSignaling IFN signaling pathway including trans-eGenes such as *OAS1*, *IRF4*, and other ISGs, by regulating the cis-eGene *SPATS2L*. (B) Pathways enriched with SPATS2L-related genes. Enrichment analysis of genes with a correlation coefficient greater than 0.8 with SPATS2L in patients with SLE (P<0.05) was performed using the Enrichr website, and the results indicated that SPATS2L is primarily associated with the IFN signaling pathway. (C) Correlation analysis revealed a strong correlation between SPATS2L and the trans gene *OAS1* in patients with SLE (R=0.8981, P<0.0001). (D) We confirmed through RNA sequencing data that the expression levels of ISGs, such as *OAS1*, were significantly decreased in Jurkat cells with *SPATS2L* knockdown. (E) Western blot analysis confirmed the knockdown of *SPATS2L* at the protein level. (F) The RT-qPCR results indicate that knocking down *SPATS2L* in Jurkat cells affects the expression of downstream genes in the type I IFN signaling pathway, such as *OAS1* and *IFIT2*. (G) In Jurkat cell lines overexpressing SPATS2L, we observed a significant increase in the expression levels of OAS1 and IFIT2 as SPATS2L expression increased. Data are presented as the mean±SEM of two independent samples, and the P values were analyzed using a two-tailed unpaired t test. *P<0.05, **P<0.01, ***P<0.001.

SPATS2L knockdown (Figure 2D). The depletion of SPATS2L at the protein level was confirmed through western blot analysis experiments (Figure 2E). Our findings were further validated via RT-qPCR. The RT-qPCR results also suggested that *SPATS2L* knockdown had an impact on downstream genes in the type I IFN signaling pathway, including *OAS1* and *IFIT2*, at the mRNA level (Figure 2F). Similarly, in Jurkat cells overexpressing SPATS2L, a significant increase in the expressions of OAS1 and IFIT2 were observed concurrently with the increase in the expression of SPATS2L (Figure 2G).

Extending our analysis to Ramos cells, we employed identical methodologies to evaluate the consistency of the effects of *SPATS2L* knockdown on ISGs such as *OAS1* and *IFIT2*. Consistent with our findings in Jurkat cells, the knockdown group demonstrated a notable downward trend in the expressions of OAS1 and IFIT2 (Supplementary Figure S1D).

SPATS2L influences the magnitude and phosphorylation status of STAT1 within the type I IFN signaling pathway

Type I interferons exert their effects by binding to their receptors, thereby activating the JAK/STAT pathway. This activation subsequently regulates the transcriptional activity of interferon-stimulated response elements (ISREs) on ISGs, thereby influencing ISGs expression [57–59]. To further elucidate the role of SPATS2L in the IFN signaling cascade, we initially employed an ISRE-luciferase reporter gene assay. The results demonstrated that knockdown of *SPATS2L* significantly reduced the activity of ISRE (Figure 3A).

The identification of the impact of SPATS2L on the expression levels of key molecules, STAT1 and JAK1, within the IFN signaling pathway is more convincing. *SPATS2L* knockdown resulted in a significant reduction in the mRNA levels of both *STAT1* and *JAK1*, regardless of the activation status of the IFN signaling pathway (Figure 3B). Similarly, upon SPATS2L overexpression, the expression

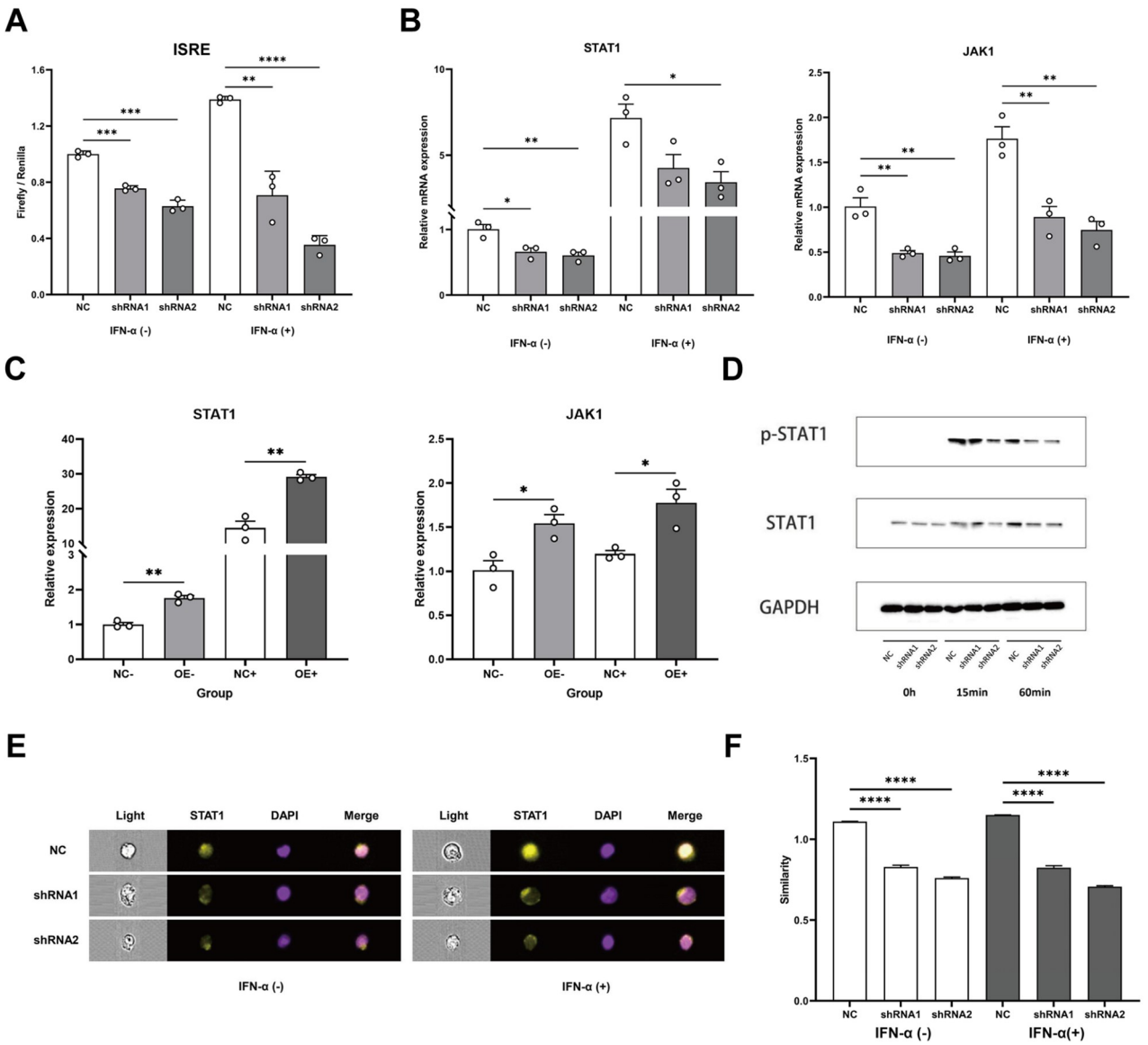

Figure 3. SPATS2L influences the expression level and phosphorylation of STAT1 within the type I IFN signaling pathway (A) The dual-luciferase reporter assay suggested that SPATS2L may regulate the IFN signaling pathway by enhancing the activity of ISRE. Attenuation of SPATS2L expression results in a decrease in ISRE activity. Notably, this reduction was particularly conspicuous after the induction of the IFN signaling pathway (IFN-α, 1000 U/mL, 6 h). (B) SPATS2L affects the expression level of the key molecule STAT1 in the IFN signaling pathway. RT-qPCR revealed that the mRNA levels of *STAT1* and *JAK1* were significantly attenuated after *SPATS2L* knockdown, regardless of whether the IFN signaling pathway was activated. (C) The RT-qPCR results also indicated that overexpression of SPATS2L led to a significant increase in the expression of both STAT1 and JAK1. Data are presented as the mean±SEM of three independent experiments, and the *P* values were analyzed using a two-tailed unpaired *t*-test. (D) *SPATS2L* knockdown significantly reduced the phosphorylation of STAT1. The Jurkat cells in the KD and NC groups were stimulated with IFN-α (1000 U/mL) for 15 and 60 min, respectively. Subsequently, we extracted proteins for western blot analysis. (E,F) Imaging flow cytometry revealed that the downregulation of SPATS2L expression had an impact on the nuclear translocation efficiency of STAT1. After *SPATS2L* was knocked down in Jurkat cells and stimulated with IFN-α (1000 U/mL) for 60 min, the nuclear translocation efficiency was significantly reduced compared to that in the NC group. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

levels of STAT1 and JAK1 were significantly increased (Figure 3C).

We further explored the involvement of SPATS2L in the IFN signaling pathway by assessing the phosphorylation status of STAT1. Western blot analysis of Jurkat cells transfected with shRNA targeting SPATS2L revealed a significant reduction in STAT1 phosphorylation following *SPATS2L* knockdown (Figure 3D and Supplementary Figure S2A). Furthermore, as *STAT1* is also an interferon-inducible gene, the experimental results revealed a marginal reduction in the total expression level of STAT1 after *SPATS2L* knockdown.

To corroborate these findings, we used an Amnis® ImageStream®X Mk II imaging flow cytometer. Specifically, Jurkat cells from both the NC and KD groups were stimulated with type I IFN (1000 U/mL) for 60 min, followed by membrane fixation. Post-fixation, the cells were subjected to staining with a STAT1 antibody and DAPI, and the results were analyzed using imaging flow cytometry. Using flow imaging technology, we detected a noticeable impact of diminished SPATS2L expression on the efficiency of

STAT1 nuclear translocation (Figure 3E,F).

Type I IFN regulates the expression of SPATS2L

Based on the above findings, we confirmed the regulatory role of SPATS2L in the IFN signaling pathway and its close correlation with disease progression. Nonetheless, the factors underlying the abnormal expression of SPATS2L in individuals affected by SLE remain unclear. In addition to the aforementioned genetic factors that can influence the expression of SPATS2L, our subsequent investigation focused on whether the promoter region of SPATS2L is regulated by lupus-associated transcription factors. By analyzing ENCODE ChIP-seq data [60,61], we identified binding signals for IRF9, STAT1, and STAT2 in proximity to the transcription start site of SPATS2L, which coincided with the presence of ISRE motifs (Figure 4A). This observation implies that upon activation of the IFN signaling pathway, the ISRE motif segment interacts with transcription factors such as STAT1, thereby contributing to the induction of SPATS2L expression.

To corroborate our findings, we recruited a cohort of four healthy individuals and isolated peripheral blood mononuclear cells (PBMCs) from their blood samples. Subsequently, the cells were exposed to various concentrations (0, 50, 200, or 1000 ng) of IFN-α for different stimulation time intervals (0, 3, 6, or 12 h). We observed a significant increase in SPATS2L expression following IFN signaling pathway activation, which was dependent on the dosage of IFN-α (Figure 4B). Furthermore, as the duration of stimulation increased, there was a corresponding increase in SPATS2L expression (Figure 4C). Similar IFN-α-triggered and dose-dependent effects involving SPATS2L were also observed in the Jurkat cell line (Figure 4D).

Furthermore, analysis of peripheral blood single-cell sequencing data obtained from healthy volunteers after 4 h of stimulation with 100 ng/mL IFN-α (GSE142637) [62] revealed a notable increase in SPATS2L expression across diverse cellular subpopulations, includ-

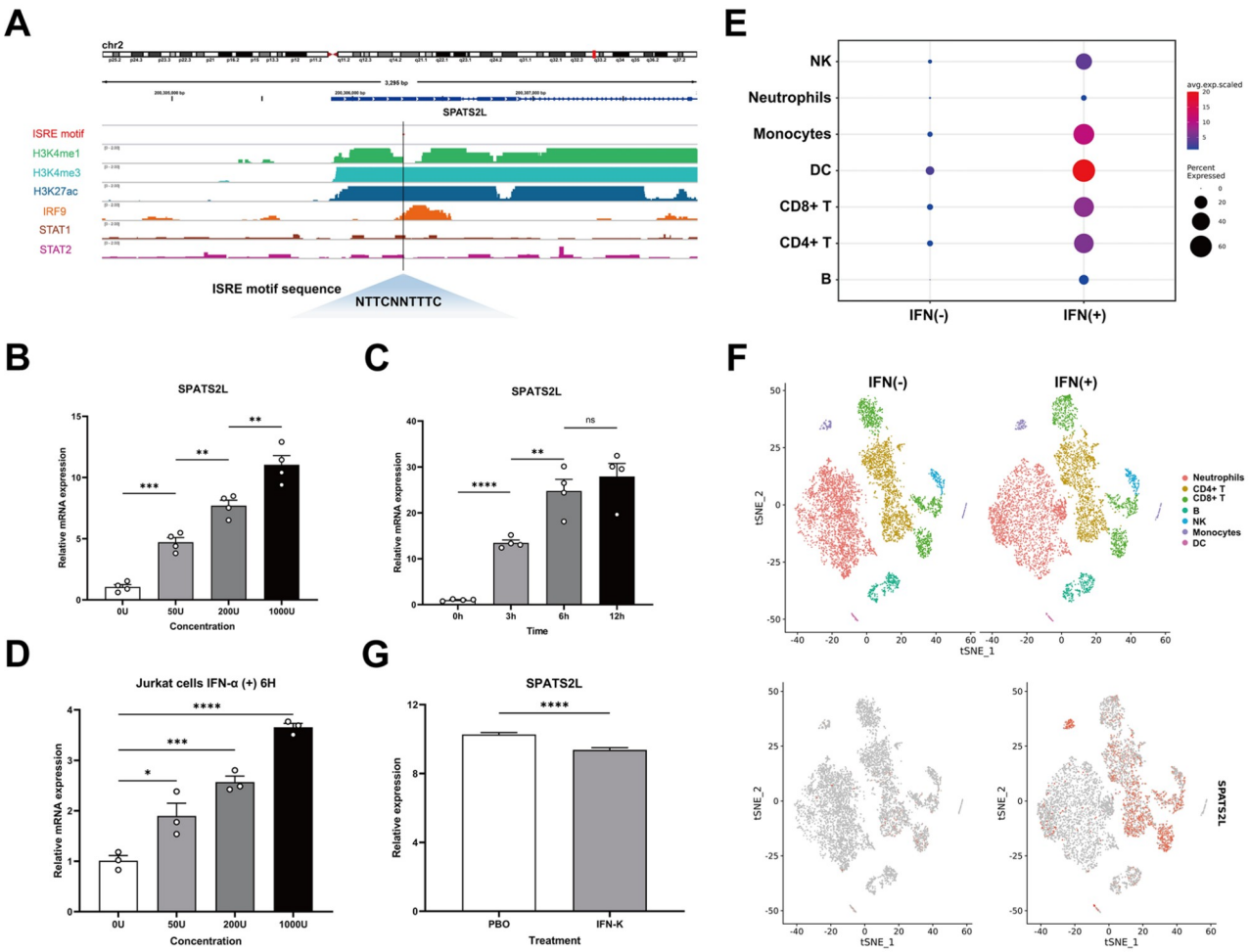

Figure 4. Type I IFNs regulate the expression of SPATS2L (A) ChIP-seq data from the K562 cell line obtained from the ENCODE database indicated the presence of binding peaks for STAT1, STAT2, and IRF9 in the promoter region of *SPATS2L*. Additionally, an ISRE motif sequence was found within the promoter region of *SPATS2L*. (B,C) PBMCs from four healthy individuals were treated with different concentrations (0, 50, 200, or 1000 ng) of IFN-α and divided into various time groups (0, 3, 6, or 12 h) of stimulation. SPATS2L was induced by IFN-α in a concentration-dependent manner. (D) IFN-α induces the expression of SAST2L in Jurkat cells in a concentration-dependent manner. Data are presented as the mean±SEM of four independent experiments, and the *P* values were analyzed using a two-tailed unpaired *t* test. (E,F) In the PBMCs of healthy volunteers, after 4 h of stimulation with 100 ng/mL IFN-α, the expression level of SPATS2L was significantly increased in various cell subpopulations, including DCs, CD8+ T cells, CD4+ T cells, monocytes, and NK cells. (G) Compared with that in patients receiving placebo treatment, the expression of SPATS2L in patients with SLE was significantly lower after 36 weeks of IFN-K treatment. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

ing DCs (dendritic cells), CD8+ T cells, CD4+ T cells, monocytes, and NK cells (natural killer cells), etc. (Figure 4E,F).

IFN-α kinoid (IFN-K) functions by inducing specific antibody production against IFN-α in patients, thereby significantly reducing the IFN gene signature, leading to the attainment of the Lupus low disease activity state (LLDAS) and gradual corticosteroid tapering. Serving as a therapeutic vaccine for SLE, IFN-K is presently in the clinical trial phase [29,51,63,49]. Through differential expression analysis of SLE patients following 36 weeks of treatment with either IFN-K or a placebo (GSE185047) [49], we observed significant downregulation of SPATS2L at the transcriptomic level subsequent to IFN-K treatment (Figure 4G), surpassing the responsiveness to IFN-K exhibited by ISG15 and other ISGs (Supplementary Table S6).

Targeting SPATS2L in individuals with SLE is beneficial for restoring aberrant activation of the IFN signaling pathway

SPATS2L has emerged as a potential biomarker for SLE and is significantly upregulated in affected individuals. However, its expression within cell subsets in the peripheral blood of SLE patients, as well as its specificity, remain unknown. Thus, we analyzed single-cell sequencing data obtained from the GSE135779 dataset [64]. Our investigation revealed distinct expression patterns of SPATS2L across various cell subpopulations within peripheral blood mononuclear cells (PBMCs) from both patients with SLE and healthy controls. High expression levels of SPATS2L were also detected in conventional dendritic cells (cDCs) and monocytes. Furthermore, a distinct increase in SPATS2L expression was observed across multiple cell subpopulations subsequent to SLE onset, including CD14+ and CD16+ monocytes, CD4+ and CD8+ T cells, and B cells (Figure 5A,B).

Considering that SPATS2L functions as a positive feedback regulator within the type I IFN pathway, we explored the possibility of targeting SPATS2L to potentially mitigate the excessive activation of the IFN pathway in patients with SLE. To this end, we utilized chemically synthesized siRNAs to selectively inhibit SPATS2L expression in patients with SLE and subsequently observed alterations in the expressions of key molecules in the IFN signaling pathway.

We enrolled a cohort of 4 individuals with SLE to investigate the correlation between the expression of SPATS2L and the IFN signaling pathway in PBMCs. Consistent with previous observations in cell lines, silencing of *SPATS2L* led to the inhibition of the IFN signaling pathway in lupus patients. Noteworthy reductions were observed in the expression levels of pivotal molecules within the type I IFN pathway, such as STAT1, along with significant repression of ISGs, including *OAS1* and *IFIT2* (Figure 5C,D). These findings suggest dysregulated activation of the IFN signaling pathway in patients with SLE, highlighting the potential for targeted intervention involving SPATS2L to reverse this aberrant IFN signaling pathway activation, thereby contributing to disease management and prognosis improvement.

Through our investigations, we demonstrated that the upregulation of SPATS2L is induced by type I IFN, positioning it as a positive regulator of the IFN signaling pathway (Figure 5E). By promoting further activation of the IFN pathway, SPATS2L exacerbates disease severity and promotes disease progression in patients with SLE.

Discussion

SLE is a complex autoimmune disease characterized by significant clinical heterogeneity. Increasing evidence suggests that genetic susceptibility contributes to the pathogenesis of SLE [3,4]. Extensive research has investigated the intricate clinical phenotypes and traits of SLE from a genetic perspective. Our study highlights the role of the lupus eGene SPATS2L in the aberrant activation of the interferon pathway in SLE patients and reveals the involvement of SPATS2L as a disease activity-related gene, revealing its mechanistic contribution to disease progression.

SPATS2L was initially shown to regulate cell growth and proliferation via ribosomal processes and translational control in myoblasts under oxidative stress [50]. Genome-wide association studies indicate its association with asthma occurrence and the molecular genetic mechanisms of schizophrenia [65,66]. With the widespread adoption of transcriptome sequencing, SPATS2L has been increasingly implicated in conditions such as hypertension [67], skeletal muscle differentiation [68], psoriasis [69], diabetes [70], chronic abdominal pain [71], and acute myeloid leukemia [72]. A recent machine learning-based model for SLE risk prediction suggested that SPATS2L may serve as a diagnostic biomarker for SLE [73]. However, extensive experimental validation of the function of SPATS2L is still lacking.

Researchers have undertaken extensive genetic investigations related to SLE to explore gene expression regulatory mechanisms associated with this disease [46,47]. The aforementioned findings collectively validate that SPATS2L functions as an eGene in the context of SLE and plays a significant role in the pathogenic mechanisms underlying SLE. A previous study revealed that the expression level of SPATS2L is regulated by the lupus risk locus rs3769433, indicating that it is an SLE eGene [46]. Through the LDlink database, we identified eight loci strongly linked (r^2>0.8) to rs3769433 in the European population, albeit all of which were located within the intronic regions of the SPATS2L gene (Supplementary Table S7) [74]. However, research has shown that risk loci within intronic regions also exhibit promoter and enhancer activity, thereby modulating gene expression and function [75]. Notably, ChIP-seq data from the GM12878 cell line derived from the ENCODE project revealed enhancer signals associated with epigenetic modifications within a region proximal to the linked locus rs1318721 [61,62]. This observation potentially signifies its involvement in the regulatory control of *SPATS2L* gene expression. Based on Hi-C data, we identified chromatin interactions between the genomic region encompassing the rs1318721 locus and the *SPATS2L* promoter region, revealing potential gene regulatory networks and chromosomal folding patterns (Supplementary Figure S3A [76,77]. Beyond rs3769433, we identified 8 more lupus risk loci within the *SPATS2L* gene through the PhenoScanner database (sourced from UK BioBank data) [78]. These loci exhibit significant correlations with disease phenotypes, and the LDlink database revealed robust linkage disequilibrium (LD) among them in the European population (Supplementary Figure S3B and Supplementary Table S7) [74]. Leveraging ENCODE ChIP-seq data, we have gained insights into allelic associations with genotypes at these risk loci. Among these risk loci, the risk region around rs182410721 displays distinct epigenetic modification signals, including enhancer and promoter marks. Furthermore, Hi-C data suggested interactions between this locus and the *SPATS2L* gene region (Supplementary Figure S3B). However, due to the exceedingly low frequencies of mutations at these loci, resulting in the limited occurrence of mutations within the sample set, the statistical power

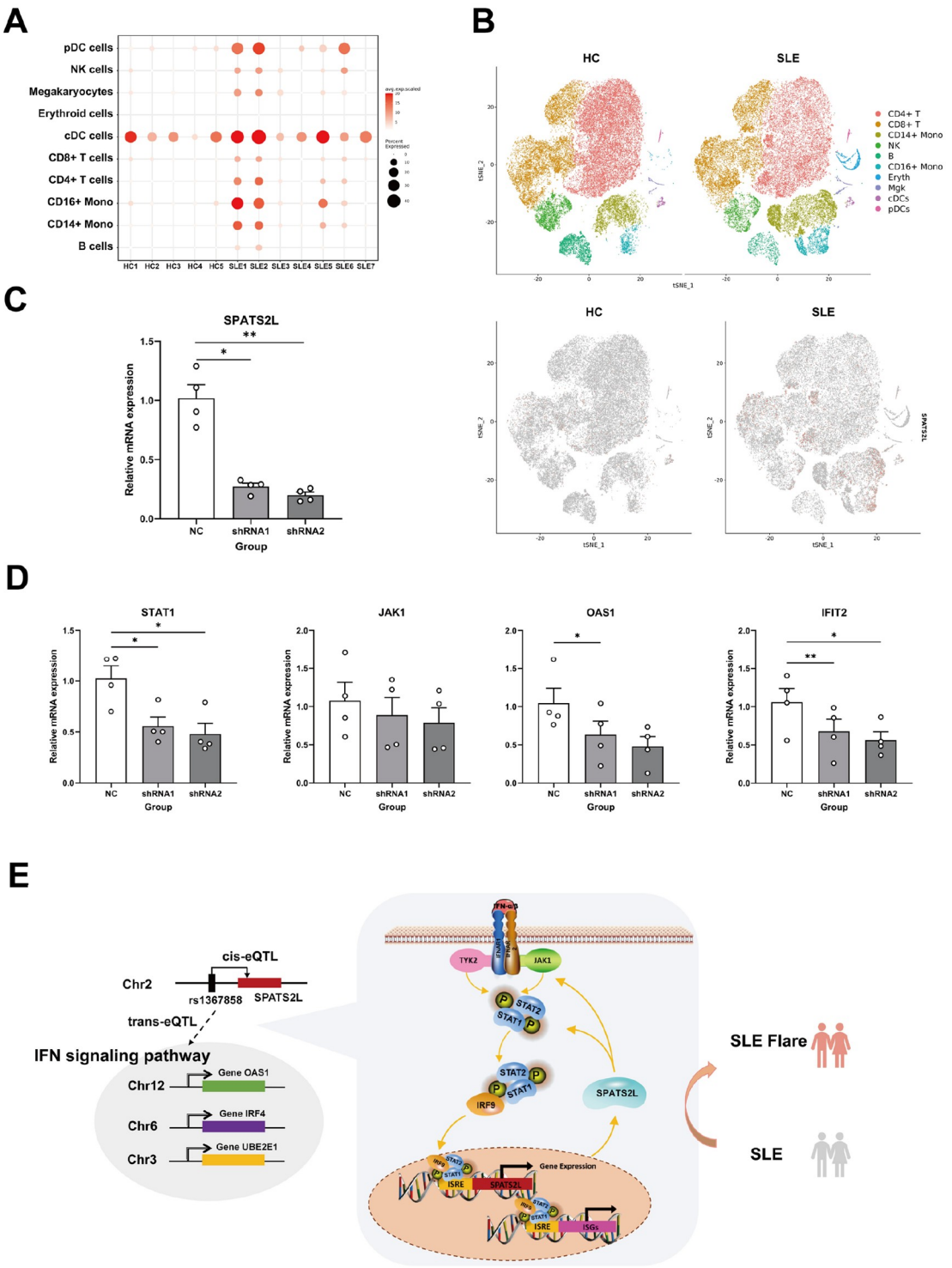

Figure 5. Targeted intervention targeting the positive regulator SPATS2L within the IFN signaling pathway is beneficial for restoring the abnormally activated IFN signaling pathway in SLE patients (A) The scRNA-seq indicated that SPATS2L is highly expressed in cDCs and monocytes. Additionally, compared to that in healthy individuals, SPATS2L expression is significantly upregulated in various cell subsets of SLE patients, including CD14+ and CD16+ monocytes, CD4+ and CD8+ T cells, and B cells. (B) SPATS2L expression in single cells. (C,D) siRNA-mediated inhibition of SPATS2L gene expression in SLE patients using siRNA. After *SPATS2L* was knocked down, the IFN signaling pathway in patients with SLE was suppressed, leading to the significant downregulation of ISGs such as *STAT1*, *OAS1*, and *IFIT2*. Data are presented as the mean±SEM of four independent experiments, and the *P* values were analyzed using a two-tailed paired *t*-test. **P*<0.05, ***P*<0.01, ****P*<0.001. (E) The working model of SPATS2L-mediated positive regulation of the type I IFN signaling pathway triggering SLE flare-up. *SPATS2L* is an IFN-inducible gene that is highly expressed in patients with SLE, and even more so in patients with SLE. SPATS2L exerts its regulatory effects on ISGs mainly by positively modulating type I IFN signaling, thereby further promoting the progression of SLE.

of eQTL analysis has been constrained, preventing the confirmation of an association between the risk loci and SPATS2L expression. We used Phenoscanner database to explore the correlations between SNPs found in our study and the associated phenotype [48] (Supplementary Table S8) and found that these SNPs are associated with schizophrenia [79,80], bipolar disorders [81] or atrial fibrillation [82,83], all of which are comorbidities of lupus. Among the multitude of potential eGenes that could play pivotal roles in SLE onset and disease progression, our study provides a strategy for identifying critical eGenes and annotating their functions. Furthermore, considering the potential influence of LD, the identification of genetic variants through GWAS does not necessarily imply causality [5]. The direct correlation between risk genetic variants and eGene expression levels, as well as their specific functionalities, warrants further investigation and validation.

Through eQTL analysis, we investigated the functional characteristics of the cis-eGene *SPATS2L*. This investigation revealed the existence of a SNP that has the potential to exert a trans-regulatory effect on the *OAS1* gene as well as other ISGs. Moreover, the enrichment of trans-eGenes in the eSignaling pathway is predominantly associated with the IFN pathway. Concurrently, transcriptomic sequencing and RT-qPCR confirmed the suppression of OAS1 and other ISGs upon *SPATS2L* knockdown in Jurkat cells, while overexpression of SPATS2L increased OAS1 and IFIT2 expression levels. Although we also observed a similar regulatory effect of SPATS2L on OAS1 and other ISGs in Ramos cells, the universality of the regulatory role of SPATS2L in the IFN signaling pathway across diverse cell types remains to be further elucidated. In addition, a dual luciferase reporter assay indicated a significant decrease in ISRE activity following *SPATS2L* knockdown. Both western blot analysis and imaging flow cytometry confirmed that SPATS2L regulates the IFN signaling pathway by modulating the phosphorylation level and nuclear translocation efficiency of the pivotal transcription factor STAT1. Our study revealed that SPATS2L positively regulates the type I interferon pathway. However, the precise molecular mechanisms underlying the functional roles of SPATS2L remain elusive.

The molecular mechanism underlying the action of a gene often involves interactions with other molecules, such as proteins, RNAs, miRNAs, and DNA. By querying the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [84], we obtained clues indicating that SPATS2L can interact with proteins such as CUX1, RAPGEF1, SET, PLXNA3, USMG5, and SGOL2. A prior study highlighted the critical functions of SET (TAF-I) and histone H1 in negatively regulating the transcriptional activation of IFN-responsive genes by modulating chromatin architecture, thus repressing transcription [85]. We hypothesize that this may be one of the mechanisms through which SPATS2L regulates the JAK-STAT pathway, but further research is needed to validate and comprehensively elucidate these hypotheses.

Moreover, both the data from the GeneNetwork website [56] and the correlation analysis of *SPATS2L* gene expression in individuals with SLE support the notion that the function of SPATS2L is primarily related to the IFN signaling pathway. In a recent study, increased expression of SPATS2L was demonstrated to potentially activate the JAK/STAT pathway in acute myeloid leukemia (AML) cell lines [72]. Conversely, the knockdown of *SPATS2L* hindered cell proliferation, induced apoptosis, and suppressed critical proteins of the JAK/STAT pathway, including JAK2, STAT3, and STAT5, in AML cells. This evidence suggests that SPATS2L may promote disease progression by enhancing the IFN signaling pathway, which is a key factor in the pathogenesis of SLE. However, considering the conservation of SPATS2L across humans and mice, additional efforts are needed to explore the expression profile and underlying regulatory mechanisms of SPATS2L in murine models of lupus, shedding light on its specific roles *in vivo*.

Furthermore, we established a strong correlation between SPATS2L and SLE flares by analyzing whole-blood expression profiles in patients with SLE and by combining the ROC analysis and SLEDAI correlation assessment. A previously newly developed SLE disease risk prediction model suggested that SPATS2L, a key genetic biomarker of lupus, can be used to effectively identify SLE, which was also validated in the GSE185047 dataset [73]. Single-cell data also revealed widespread overexpression of SPATS2L across various cell types in patients with SLE. SPATS2L has emerged as a notable signature of SLE disease activity, with significant clinical implications linked to organ involvement and treatment responses [32]. This study further confirmed that SPATS2L, an interferon-induced gene, is a positive regulator of the IFN signaling pathway. By influencing STAT1 expression and phosphorylation, SPATS2L exacerbates disease conditions and facilitates disease progression. Targeting the excessive activation of IFN or other molecules associated with SLE holds promise for therapeutic intervention to alleviate inflammatory responses, modulate the immune system, and improve the quality of life and prognosis of patients with SLE [24,25]. Notably, the use of small interference RNA to attenuate SPATS2L expression in the PBMCs of individuals with active SLE individuals shows potential to mitigate the dysregulation of the IFN signaling pathway. SPATS2L, as a prospective biomarker and therapeutic target, plays a critical role in the investigation and management of disease activity in patients with SLE and could serve as a target for screening small molecule drugs to offer innovative strategies for both the treatment and diagnosis of SLE.

In conclusion, our study revealed a significant correlation between the eGene *SPATS2L* and SLE flare-up, indicating that this gene is a positive regulator of the type I IFN signaling pathway, thereby facilitating the progression of SLE (Figure 5E). Consequently, SPATS2L has potential as a biomarker for disease diagnosis, an indicator of disease activity, and a promising therapeutic target.

Supplementary Data

Supplementary data is available at *Acta Biochimica et Biophysica Sinica* online.

Funding

This work was supported by the grants from the National Natural Science Foundation of China (Nos. 82271830, 32141004, and 82273523), the Innovative Research Team of High-level Local Universities in Shanghai (No. SHSMU-ZDCX20210600), the National High-Level Hospital Clinical Research Funding (Nos. 2022-NHLHCRF-LX-03 and 2023-NHLHCRF-YXHZ-ZRZD-06), and the Capital's Funds for Health Improvement and Research (No. CFH 2020-1-2031).

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Deng Y, Tsao BP. Genetic susceptibility to systemic lupus erythematosus in the genomic era. *Nat Rev Rheumatol* 2010, 6: 683–692
2. Kaul A, Gordon C, Crow MK, Touma Z, Urowitz MB, van Vollenhoven R, Ruiz-Irastorza G, *et al.* Systemic lupus erythematosus. *Nat Rev Dis Primers* 2016, 2: 16039
3. Bentham J, Morris DL, Cunninghame Graham DS, Pinder CL, Tombleson P, Behrens TW, Martín J, *et al.* Genetic association analyses implicate aberrant regulation of innate and adaptive immunity genes in the pathogenesis of systemic lupus erythematosus. *Nat Genet* 2015, 47: 1457–1464
4. Langefeld CD, Ainsworth HC, Graham DSC, Kelly JA, Comeau ME, Marion MC, Howard TD, *et al.* Transancestral mapping and genetic load in systemic lupus erythematosus. *Nat Commun* 2017, 8: 16021
5. Farh KKH, Marson A, Zhu J, Kleinewietfeld M, Housley WJ, Beik S, Shoresh N, *et al.* Genetic and epigenetic fine mapping of causal autoimmune disease variants. *Nature* 2015, 518: 337–343
6. Lessard CJ, Adrianto I, Ice JA, Wiley GB, Kelly JA, Glenn SB, Adler AJ, *et al.* Identification of IRF8, TMEM39A, and IKZF3-ZPBP2 as susceptibility loci for systemic lupus erythematosus in a large-scale multiracial replication study. *Am J Hum Genet* 2012, 90: 648–660
7. Cui Y, Sheng Y, Zhang X. Genetic susceptibility to SLE: recent progress from GWAS. *J Autoimmunity* 2013, 41: 25–33
8. Harley JB, Alarcón-Riquelme ME, Criswell LA, Jacob CO, Kimberly RP, Moser KL, Tsao BP, *et al.* Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci. *Nat Genet* 2008, 40: 204–210
9. Alarcón-Segovia D, Alarcón-Riquelme ME, Cardiel MH, Caeiro F, Massardo L, Villa AR, Pons-Estel BA. Familial aggregation of systemic lupus erythematosus, rheumatoid arthritis, and other autoimmune diseases in 1,177 lupus patients from the GLADEL cohort. *Arthritis Rheum* 2005, 52: 1138–1147
10. Han JW, Zheng HF, Cui Y, Sun LD, Ye DQ, Hu Z, Xu JH, *et al.* Genome-wide association study in a Chinese Han population identifies nine new susceptibility loci for systemic lupus erythematosus. *Nat Genet* 2009, 41: 1234–1237
11. Gateva V, Sandling JK, Hom G, Taylor KE, Chung SA, Sun X, Ortmann W, *et al.* A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus. *Nat Genet* 2009, 41: 1228–1233
12. Wang YF, Zhang Y, Lin Z, Zhang H, Wang TY, Cao Y, Morris DL, *et al.* Identification of 38 novel loci for systemic lupus erythematosus and genetic heterogeneity between ancestral groups. *Nat Commun* 2021, 12: 772
13. Sigurdsson S, Nordmark G, Göring HHH, Lindroos K, Wiman AC, Sturfelt G, Jönsen A, *et al.* Polymorphisms in the tyrosine kinase 2 and interferon regulatory factor 5 genes are associated with systemic lupus erythematosus. *Am J Hum Genet* 2005, 76: 528–537
14. Graham RR, Kozyrev SV, Baechler EC, Reddy MVPL, Plenge RM, Bauer JW, Ortmann WA, *et al.* A common haplotype of interferon regulatory factor 5 (IRF5) regulates splicing and expression and is associated with increased risk of systemic lupus erythematosus. *Nat Genet* 2006, 38: 550–555
15. Remmers EF, Plenge RM, Lee AT, Graham RR, Hom G, Behrens TW, de Bakker PIW, *et al.* *STAT4* and the risk of rheumatoid arthritis and systemic lupus erythematosus. *N Engl J Med* 2007, 357: 977–986
16. Võsa U, Claringbould A, Westra HJ, Bonder MJ, Deelen P, Zeng B, Kirsten H, *et al.* Large-scale cis- and trans-eQTL analyses identify thousands of genetic loci and polygenic scores that regulate blood gene expression. *Nat Genet* 2021, 53: 1300–1310
17. Baechler EC, Batliwalla FM, Karypis G, Gaffney PM, Ortmann WA, Espe KJ, Shark KB, *et al.* Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc Natl Acad Sci USA* 2003, 100: 2610–2615
18. Rönnblom L, Leonard D. Interferon pathway in SLE: one key to unlocking the mystery of the disease. *Lupus Sci Med* 2019, 6: e000270
19. Banchereau R, Hong S, Cantarel B, Baldwin N, Baisch J, Edens M, Cepika AM, *et al.* Personalized immunomonitoring uncovers molecular networks that stratify lupus patients. *Cell* 2016, 165: 551–565
20. Ytterberg SR, Schnitzer TJ. Serum interferon levels in patients with systemic lupus erythematosus. *Arthritis Rheum* 1982, 25: 401–406
21. Preble OT, Black RJ, Friedman RM, Klippel JH, Vilček J. Systemic lupus erythematosus: presence in human serum of an unusual acid-labile leukocyte interferon. *Science* 1982, 216: 429–431
22. Caielli S, Cardenas J, de Jesus AA, Baisch J, Walters L, Blanck JP, Balasubramanian P, *et al.* Erythroid mitochondrial retention triggers myeloid-dependent type I interferon in human SLE. *Cell* 2021, 184: 4464–4479.e19
23. Caielli S, Wan Z, Pascual V. Systemic lupus erythematosus pathogenesis: interferon and beyond. *Annu Rev Immunol* 2023, 41: 533–560
24. Postal M, Vivaldo JF, Fernandez-Ruiz R, Paredes JL, Appenzeller S, Niewold TB. Type I interferon in the pathogenesis of systemic lupus erythematosus. *Curr Opin Immunol* 2020, 67: 87–94
25. Banchereau J, Pascual V. Type I interferon in systemic lupus erythematosus and other autoimmune diseases. *Immunity* 2006, 25: 383–392
26. Lazar S, Kahlenberg JM. Systemic lupus erythematosus: new diagnostic and therapeutic approaches. *Annu Rev Med* 2023, 74: 339–352
27. Riggs JM, Hanna RN, Rajan B, Zerrouki K, Karnell JL, Sagar D, Vainshtein I, *et al.* Characterisation of anifrolumab, a fully human anti-interferon receptor antagonist antibody for the treatment of systemic lupus erythematosus. *Lupus Sci Med* 2018, 5: e000261
28. Peng L, Oganesyan V, Wu H, Dall'Acqua WF, Damschroder MM. Molecular basis for antagonistic activity of anifrolumab, an anti-interferon–α receptor 1 antibody. *mAbs* 2015, 7: 428–439
29. Ducreux J, Houssiau FA, Vandepapelière P, Jorgensen C, Lazaro E, Spertini F, Colaone F, *et al.* Interferon α kinoid induces neutralizing anti-interferon α antibodies that decrease the expression of interferon-induced and B cell activation associated transcripts: analysis of extended follow-up data from the interferon α kinoid phase I/II study. *Rheumatology* 2016, 55: 1901–1905
30. Khamashta M, Merrill JT, Werth VP, Furie R, Kalunian K, Illei GG, Drappa J, *et al.* Sifalimumab, an anti-interferon-α monoclonal antibody, in moderate to severe systemic lupus erythematosus: a randomised, double-blind, placebo-controlled study. *Ann Rheum Dis* 2016, 75: 1909–1916
31. Kalunian KC, Merrill JT, Maciuca R, McBride JM, Townsend MJ, Wei X, Davis Jr JC, *et al.* A Phase II study of the efficacy and safety of rontalizumab (rhuMAb interferon-α) in patients with systemic lupus erythematosus (ROSE). *Ann Rheum Dis* 2016, 75: 196–202
32. Nakano M, Ota M, Takeshima Y, Iwasaki Y, Hatano H, Nagafuchi Y, Itamiya T, *et al.* Distinct transcriptome architectures underlying lupus establishment and exacerbation. *Cell* 2022, 185: 3375–3389
33. Feng X, Wu H, Grossman JM, Hanvivadhanakul P, FitzGerald JD, Park GS, Dong X, *et al.* Association of increased interferon-inducible gene expression with disease activity and lupus nephritis in patients with systemic lupus erythematosus. *Arthritis Rheum* 2006, 54: 2951–2962
34. Mai L, Asaduzzaman A, Noamani B, Fortin PR, Gladman DD, Touma Z, Urowitz MB, *et al.* The baseline interferon signature predicts disease

severity over the subsequent 5 years in systemic lupus erythematosus. *Arthritis Res Ther* 2021, 23: 29
35. Morris DL, Sheng Y, Zhang Y, Wang YF, Zhu Z, Tombleson P, Chen L, *et al.* Genome-wide association meta-analysis in Chinese and European individuals identifies ten new loci associated with systemic lupus erythematosus. *Nat Genet* 2016, 48: 940–946
36. Wen L, Zhu C, Zhu Z, Yang C, Zheng X, Liu L, Zuo X, *et al.* Exome-wide association study identifies four novel loci for systemic lupus erythematosus in Han Chinese population. *Ann Rheum Dis* 2018, 77: 417
37. Maurano MT, Humbert R, Rynes E, Thurman RE, Haugen E, Wang H, Reynolds AP, *et al.* Systematic localization of common disease-associated variation in regulatory DNA. *Science* 2012, 337: 1190–1195
38. Hou G, Harley ITW, Lu X, Zhou T, Xu N, Yao C, Qin Y, *et al.* SLE non-coding genetic risk variant determines the epigenetic dysfunction of an immune cell specific enhancer that controls disease-critical microRNA expression. *Nat Commun* 2021, 12: 135
39. Gilad Y, Rifkin SA, Pritchard JK. Revealing the architecture of gene regulation: the promise of eQTL studies. *Trends Genet* 2008, 24: 408–415
40. Cookson W, Liang L, Abecasis G, Moffatt M, Lathrop M. Mapping complex disease traits with global gene expression. *Nat Rev Genet* 2009, 10: 184–194
41. Majewski J, Pastinen T. The study of eQTL variations by RNA-seq: from SNPs to phenotypes. *Trends Genet* 2011, 27: 72–79
42. Liu S, Won H, Clarke D, Matoba N, Khullar S, Mu Y, Wang D, *et al.* Illuminating links between cis-regulators and trans-acting variants in the human prefrontal cortex. *Genome Med* 2022, 14: 133
43. Westra HJ, Peters MJ, Esko T, Yaghootkar H, Schurmann C, Kettunen J, Christiansen MW, *et al.* Systematic identification of trans eQTLs as putative drivers of known disease associations. *Nat Genet* 2013, 45: 1238–1243
44. Pierce BL, Tong L, Chen LS, Rahaman R, Argos M, Jasmine F, Roy S, *et al.* Mediation analysis demonstrates that trans-eQTLs are often explained by cis-mediation: a genome-wide analysis among 1,800 south asians. *PLoS Genet* 2014, 10: e1004818
45. Shan N, Wang Z, Hou L. Identification of trans-eQTLs using mediation analysis with multiple mediators. *BMC Bioinformatics* 2019, 20: 126
46. Owen KA, Bell KA, Price A, Bachali P, Ainsworth H, Marion MC, Howard TD, *et al.* Molecular pathways identified from single nucleotide polymorphisms demonstrate mechanistic differences in systemic lupus erythematosus patients of Asian and European ancestry. *Sci Rep* 2023, 13: 5339
47. Ota M, Nagafuchi Y, Hatano H, Ishigaki K, Terao C, Takeshima Y, Yanaoka H, *et al.* Dynamic landscape of immune cell-specific gene regulation in immune-mediated diseases. *Cell* 2021, 184: 3006–3021.e17
48. Yin X, Kim K, Suetsugu H, Bang SY, Wen L, Koido M, Ha E, *et al.* Meta-analysis of 208370 East Asians identifies 113 susceptibility loci for systemic lupus erythematosus. *Ann Rheum Dis* 2021, 80: 632–640
49. Houssiau FA, Thanou A, Mazur M, Ramiterre E, Gomez Mora DA, Misterska-Skora M, Perich-Campos RA, *et al.* IFN-α kinoid in systemic lupus erythematosus: results from a phase IIb, randomised, placebo-controlled study. *Ann Rheum Dis* 2020, 79: 347–355
50. Zhu CH, Kim J, Shay JW, Wright WE, Preiss T. SGNP: an essential stress granule/nucleolar protein potentially involved in 5.8s rRNA processing/transport. *PLoS One* 2008, 3: e3716
51. Zagury D, Le Buanec H, Mathian A, Larcier P, Burnett R, Amoura Z, Emilie D, *et al.* IFNα kinoid vaccine-induced neutralizing antibodies prevent clinical manifestations in a lupus flare murine model. *Proc Natl Acad Sci USA* 2009, 106: 5294–5299
52. Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, *et al.* PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet* 2003, 34: 267–273
53. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, *et al.* Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA* 2005, 102: 15545–15550
54. Toro-Domínguez D, Martorell-Marugán J, Goldman D, Petri M, Carmona-Sáez P, Alarcón-Riquelme ME. Stratification of systemic lupus erythematosus patients into three groups of disease activity progression according to longitudinal gene expression. *Arthritis Rheum* 2018, 70: 2025–2035
55. Li Y, Banerjee S, Wang Y, Goldstein SA, Dong B, Gaughan C, Silverman RH, *et al.* Activation of RNase L is dependent on OAS3 expression during infection with diverse human viruses. *Proc Natl Acad Sci USA* 2016, 113: 2241–2246
56. Deelen P, van Dam S, Herkert JC, Karjalainen JM, Brugge H, Abbott KM, van Diemen CC, *et al.* Improving the diagnostic yield of exome-sequencing by predicting gene–phenotype associations using large-scale gene expression analysis. *Nat Commun* 2019, 10: 2837
57. Platanias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nat Rev Immunol* 2005, 5: 375–386
58. Ivashkiv LB, Donlin LT. Regulation of type I interferon responses. *Nat Rev Immunol* 2014, 14: 36–49
59. Schneider WM, Chevillotte MD, Rice CM. Interferon-stimulated genes: a complex web of host defenses. *Annu Rev Immunol* 2014, 32: 513–545
60. Consortium, E.P. An integrated encyclopedia of DNA elements in the human genome. *Nature*, 2012, 489: 57–74
61. Luo Y, Hitz BC, Gabdank I, Hilton JA, Kagda MS, Lam B, Myers Z, *et al.* New developments on the Encyclopedia of DNA Elements (ENCODE) data portal. *Nucleic Acids Res* 2020, 48: D882–D889
62. Goel RR, Wang X, O'Neil LJ, Nakabo S, Hasneen K, Gupta S, Wigerblad G, *et al.* Interferon lambda promotes immune dysregulation and tissue inflammation in TLR7-induced lupus. *Proc Natl Acad Sci USA* 2020, 117: 5409–5419
63. Mathian A, Amoura Z, Adam E, Colaone F, Hoekman MFM, Dhellin O, Vandepapelière P, *et al.* Active immunisation of human interferon α transgenic mice with a human interferon α Kinoid induces antibodies that neutralise interferon α in sera from patients with systemic lupus erythematosus. *Ann Rheum Dis* 2011, 70: 1138–1143
64. Franklyn K, Lau CS, Navarra SV, Louthrenoo W, Lateef A, Hamijoyo L, Wahono CS, *et al.* Definition and initial validation of a lupus low disease activity state (LLDAS). *Ann Rheum Dis* 2016, 75: 1615–1621
65. Himes BE, Jiang X, Hu R, Wu AC, Lasky-Su JA, Klanderman BJ, Ziniti J, *et al.* Genome-wide association analysis in asthma subjects identifies SPATS2L as a novel bronchodilator response gene. *PLoS Genet* 2012, 8: e1002824
66. Smeland OB, Wang Y, Frei O, Li W, Hibar DP, Franke B, Bettella F, *et al.* Genetic overlap between schizophrenia and volumes of hippocampus, putamen, and intracranial volume indicates shared molecular genetic mechanisms. *Schizophr Bull* 2018, 44: 854–864
67. de Las Fuentes L, Sung YJ, Schwander KL, Kalathiveetil S, Hunt SC, Arnett DK, Rao DC. The role of SNP-loop diuretic interactions in hypertension across ethnic groups in HyperGEN. *Front Genet* 2013, 4: 304
68. Meyer SU, Krebs S, Thirion C, Blum H, Krause S, Pfaffl MW, Asakura A. Tumor necrosis factor alpha and insulin-like growth factor 1 induced modifications of the gene expression kinetics of differentiating skeletal muscle cells. *PLoS One* 2015, 10: e0139520
69. Palau N, Julià A, Ferrándiz C, Puig L, Fonseca E, Fernández E, López-Lasanta M, *et al.* Genome-wide transcriptional analysis of T cell activation

reveals differential gene expression associated with psoriasis. *BMC Genomics* 2013, 14: 825

70. Lou Y, Lu Y, Ye X, Wang Y, Ma Y, Fan C, Jiang H, *et al.* PML-RARA monitoring in newly diagnosed acute promyelocytic leukemia treated with an entirely oral chemotherapy-free postremission approach: a multiple institution experience. *Hematol Oncol* 2020, 38: 618–621
71. Reddy SY, Rasmussen NA, Fourie NH, Berger RS, Martino AC, Gill J, Longchamps R, *et al.* Sleep quality, BDNF genotype and gene expression in individuals with chronic abdominal pain. *BMC Med Genomics* 2014, 7: 61
72. Li F, Ye W, Yao Y, Wei W, Lin X, Zhuang H, Li C, *et al.* Spermatogenesis associated serine rich 2 like plays a prognostic factor and therapeutic target in acute myeloid leukemia by regulating the JAK2/STAT3/STAT5 axis. *J Transl Med* 2023, 21: 115
73. Chen H, Huang L, Jiang X, Wang Y, Bian Y, Ma S, Liu X. Establishment and analysis of a disease risk prediction model for the systemic lupus erythematosus with random forest. *Front Immunol* 2022, 13: 1025688
74. Machiela MJ, Chanock SJ. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. *Bioinformatics* 2015, 31: 3555–3557
75. Hua JT, Ahmed M, Guo H, Zhang Y, Chen S, Soares F, Lu J, *et al.* Risk SNP-mediated promoter-enhancer switching drives prostate cancer through lncRNA PCAT19. *Cell* 2018, 174: 564–575.e18
76. Rao SSP, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, Sanborn AL, *et al.* A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* 2014, 159: 1665–1680
77. Mifsud B, Tavares-Cadete F, Young AN, Sugar R, Schoenfelder S, Ferreira L, Wingett SW, *et al.* Mapping long-range promoter contacts in human cells with high-resolution capture Hi-C. *Nat Genet* 2015, 47: 598–606
78. Kamat MA, Blackshaw JA, Young R, Surendran P, Burgess S, Danesh J, Butterworth AS, *et al.* PhenoScanner V2: an expanded tool for searching human genotype–phenotype associations. *Bioinformatics* 2019, 35: 4851–4853
79. Chen YM, Chen HK, Wu BJ, Chen IC, Chen JP, Lin CH, Hsiao TH. Systemic lupus erythematosus and autoimmune features in chronic hospitalized patients with schizophrenia. *Schizophr Res* 2021, 237: 166–173
80. Ikeda M, Takahashi A, Kamatani Y, Momozawa Y, Saito T, Kondo K, Shimasaki A, *et al.* Genome-wide association study detected novel susceptibility genes for schizophrenia and shared trans-populations/diseases genetic effect. *Schizophr Bull* 2019, 45: 824–834
81. Bigdeli TB, Fanous AH, Li Y, Rajeevan N, Sayward F, Genovese G, Gupta R, *et al.* Genome-wide association studies of schizophrenia and bipolar disorder in a diverse cohort of US veterans. *Schizophr Bull* 2021, 47: 517–529
82. Chen Y, Fu L, Pu S, Xue Y. Systemic lupus erythematosus increases risk of incident atrial fibrillation: a systematic review andmeta-analysis. *Int J Rheum Dis* 2022, 25: 1097–1106
83. Roselli C, Chaffin MD, Weng LC, Aeschbacher S, Ahlberg G, Albert CM, Almgren P, *et al.* Multi-ethnic genome-wide association study for atrial fibrillation. *Nat Genet* 2018, 50: 1225–1233
84. Hein MY, Hubner NC, Poser I, Cox J, Nagaraj N, Toyoda Y, Gak IA, *et al.* A human interactome in three quantitative dimensions organized by stoichiometries and abundances. *Cell* 2015, 163: 712–723
85. Kadota S, Nagata K. Silencing of IFN-stimulated gene transcription is regulated by histone H1 and its chaperone TAF-I. *Nucleic Acids Res* 2014, 42: 7642–7653