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# Hypersensitive IFN Responses in Lupus Keratinocytes Reveal Key Mechanistic Determinants in Cutaneous Lupus

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Systemic lupus erythematosus (SLE) is a complex autoimmune disease in which 70% of patients experience disfiguring skin inflammation (grouped under the rubric of cutaneous lupus erythematosus [CLE]). There are limited treatment options for SLE and no Food and Drug Administration–approved therapies for CLE. Studies have revealed that IFNs are important mediators for SLE and CLE, but the mechanisms by which IFNs lead to disease are still poorly understood. We aimed to investigate how IFN responses in SLE keratinocytes contribute to development of CLE. A cohort of 72 RNA sequencing samples from 14 individuals (seven SLE and seven healthy controls) were analyzed to study the transcriptomic effects of type I and type II IFNs on SLE versus control keratinocytes. In-depth analysis of the IFN responses was conducted. Bioinformatics and functional assays were conducted to provide implications for the change of IFN response. A significant hypersensitive response to IFNs was identified in lupus keratinocytes, including genes (*IFIH1*, *STAT1*, and *IRF7*) encompassed in SLE susceptibility loci. Binding sites for the transcription factor PITX1 were enriched in genes that exhibit IFN-sensitive responses. PITX1 expression was increased in CLE lesions based on immunohistochemistry, and by using small interfering RNA knockdown, we illustrated that *PITX1* was required for upregulation of IFN-regulated genes in vitro. SLE patients exhibit increased IFN signatures in their skin secondary to increased production and a robust, skewed IFN response that is regulated by PITX1. Targeting these exaggerated pathways may prove to be beneficial to prevent and treat hyperinflammatory responses in SLE skin. *The Journal of Immunology*, 2019, 202: 2121–2130.

**S**ystemic lupus erythematosus (SLE) is a chronic autoimmune disease that results in inflammatory organ damage. SLE has a complex genetic architecture, and it affects up to 0.4% of the population worldwide (1). Significantly, around 70% of SLE patients experience cutaneous manifestations, referred to as cutaneous lupus erythematosus (CLE) (2), and the flares of lesions can lead to significant loss of productivity and quality of life (3, 4). Despite a few commonly used, yet usually insufficient, treatment options for SLE, there is no Food and Drug Administration–approved therapy for CLE.

Previous studies have revealed the importance of even small amounts of IFNs for priming cells to maintain homeostasis and respond to immune stimuli (5, 6). Importantly, type I IFNs are mediators in the pathogenesis of SLE and CLE, and recent trials blocking

type I IFN signaling show efficacy for skin lesions (7). Previous studies have illustrated that type I IFNs are highly upregulated in CLE lesions (8) and are induced in keratinocytes by UV light (9), an important trigger for CLE flares (10–12). Recently, we identified IFN- $\kappa$ , a keratinocyte-produced type I IFN, to be overexpressed in CLE lesions and in nonlesional SLE keratinocytes (13). In addition, IFN- $\alpha$ , another type I IFN, is released from plasmacytoid dendritic cells recruited to the dermal–epidermal junction and is detected in CLE lesions after UVB-promoted chemotaxis of plasmacytoid dendritic cells (14). The consequences of chronic type I IFN signaling in the skin are important for immune activation: they can induce chemokine and cytokine production from keratinocytes (9, 15, 16), promote UVB-mediated apoptosis (13), activate the adaptive immune system (17, 18), and promote inflammasome activation

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L.C.T., G.A.H., J.E.G., and J.M.K. wrote the manuscript. All authors edited and approved the manuscript. L.C.T., G.A.H., C.C.B., M.K.S., T.J.R., R.U., M.P., K.R., X.X., J.L., E.X., and K.H. collected data. L.C.T., G.A.H., C.C.B., M.K.S., J.E.G., and J.M.K. analyzed the data.

The sequences presented in this article have been submitted to the Gene Expression Omnibus (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE124939>) under accession number GSE124939.

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Abbreviations used in this article: CLE, cutaneous lupus erythematosus; DE, differentially expressed; DEG, DE gene; DLE, discoid lupus erythematosus; FC, fold change; FDR, false discovery rate; LN, lupus nephritis; LSI, lupus-sensitive IFN; O/E, observed/expected; PC, principal component; RNA-seq, RNA sequencing; SCLE, subacute CLE; siRNA, small interfering RNA; SLE, systemic lupus erythematosus.

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in monocytes (19). They also have important functions to promote autoreactive B cell activation and class switching (20, 21). Although all type I IFNs signal through the type I IFN receptor, not all type I IFNs have identical effects on cells; for instance, IFN- $\beta$  has been reported to have both pro-survival and anti-infective effects when compared with other type I IFNs (22, 23). Differential responses to type I IFNs have variable effects on cells, but how this relates to changes in keratinocyte function and inflammation in SLE and/or CLE is unknown.

Given the high frequency of skin disease among SLE patients, the prominence of type I IFN responses in lesional skin of CLE, and the elevated IFN production in SLE keratinocytes, this study aims to understand pathological mechanisms of CLE through profiling the IFN responses in keratinocytes. Specifically, we evaluated and compared the responses of keratinocytes from normal versus lupus patients upon IFN stimulation by setting up a cohort of 72 RNA sequencing (RNA-seq) samples from 14 individuals to study the transcriptomic effects of different cytokine stimulations in keratinocytes derived from normal or lupus patients. Notably, we identified a significant hypersensitive response to IFNs in lupus keratinocytes. We also provided biological implications and evaluated potential regulatory mechanisms for the IFN-responding genes and highlighted *STAT1*, *IRF7*, and *IFIH1*, which are within lupus-associated loci (24), to be key lupus-sensitive components participating in the pathology of the disease. Importantly, we revealed an enrichment of binding sites for the transcription factor PITX1 in genes that exhibit

IFN-sensitive responses, and we validated *PITX1* expression and its regulator effect on IFN-regulated gene expression. The identification of lupus-sensitive IFN (LSI) responses in our study provides implications for understanding the differentiated nature of IFN responses in keratinocytes between normal and SLE patients, and it will facilitate development of appropriately targeted, novel, and specific therapies of downstream global IFN inhibition for CLE.

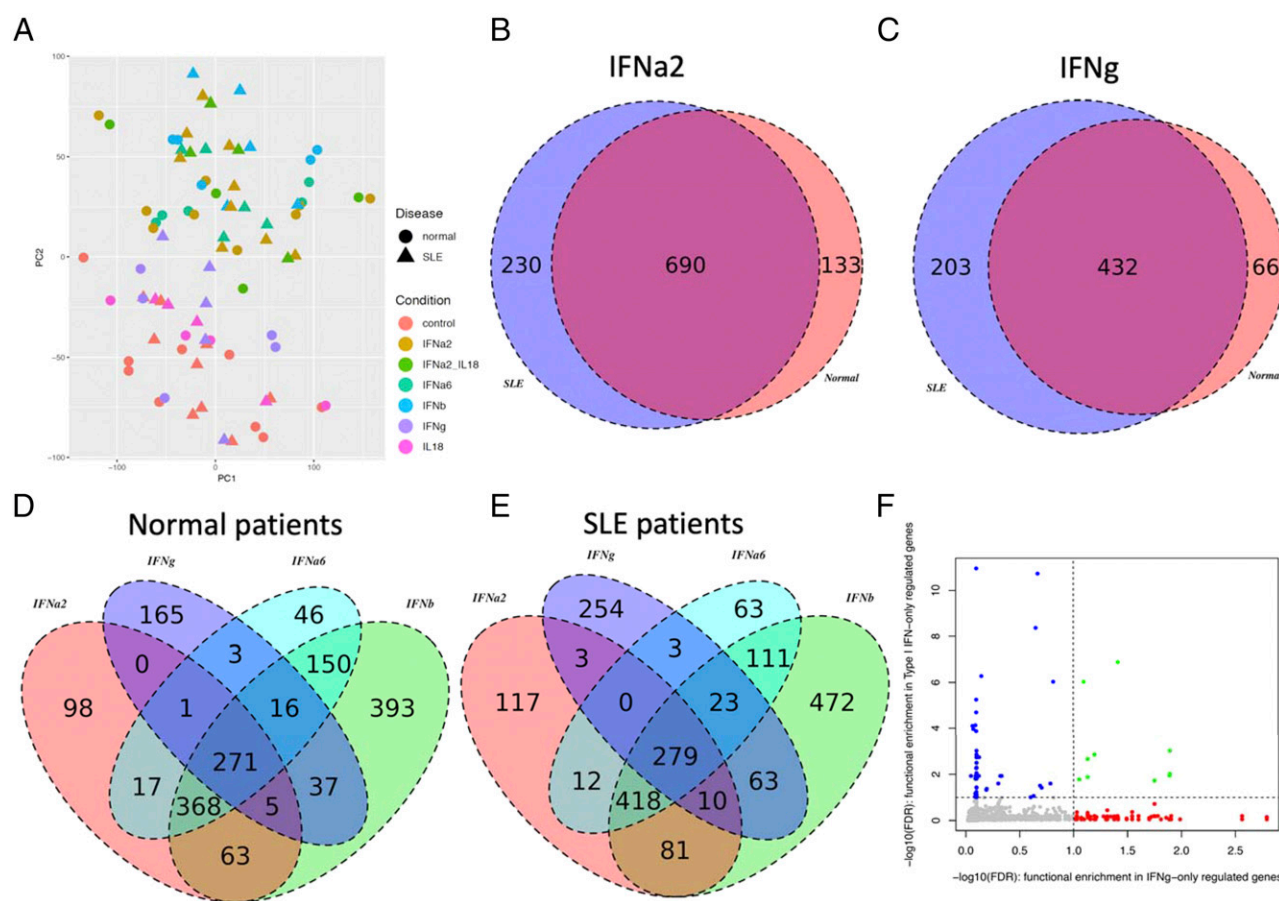
## Materials and Methods

### Patient population

SLE patient keratinocytes were isolated from skin biopsy samples from participants of the Michigan Lupus Cohort under Institutional Review Board no. 00066116. All subjects were treated according to the declaration of Helsinki; gave written, informed consent; and fulfilled four or more of the American College of Rheumatology criteria for SLE (25). SLE patients were required to have a history of cutaneous disease to be included. Sex- and age-matched control subjects were identified via advertisement. All fresh biopsies were obtained from nonlesional, non-sun-exposed skin on the upper thigh. Cases of discoid lupus erythematosus (DLE) and subacute CLE (SCLE) biopsies for microarray studies and histopathology were identified and acquired from the University of Michigan Pathology Database under Institutional Review Board no. HUM72843.

### Keratinocyte isolation

Two 6-mm punch skin biopsy samples were taken from each subject and placed in HBSS overnight. Keratinocytes were liberated via incubation in basal media (100 mmol/l NaCl, 22.5 mmol/l HEPES, 7.6 mmol/l



**FIGURE 1.** Transcriptomic analysis on keratinocytes upon IFN stimulations. **(A)** PC analysis (top two PCs are shown) illustrating the pronounced transcriptomic changes during type I IFN stimulations when comparing against other cytokine stimulations. **(B and C)** Large overlap between genes being DE by IFN- $\alpha$ 2 (B) and IFN- $\gamma$  (C) stimulations in normal and lupus keratinocytes. **(D and E)** Venn diagrams illustrating the overlap between DEGs in different IFN stimulations in keratinocytes derived from normal (D) and lupus (E) patients. **(F)** Functional enrichment test results (in  $-\log_{10}(\text{FDR})$ ) for genes DE only in IFN- $\gamma$  (x-axis) or only in type I IFNs (y-axis). Significant functions are colored in red (only significant in IFN- $\gamma$ ), blue (only significant in type I IFNs), or green (significant in both type I and II IFNs).

glucose, 2.25 mmol/l KCl, 0.75 mmol/l  $\text{Na}_2\text{HPO}_4$ ) with 0.17% trypsin for 2 h at 37°C. Keratinocytes were cultured in Epilife (Life Technologies, Thermo Fisher Scientific, Waltham, MA) with keratinocyte growth supplement and passaged at 60% confluency to avoid differentiation. Media were replaced every 2–3 d. Keratinocyte culture purity was confirmed by morphology. For all experiments, cells were used at passage 3, and patient samples were chosen that had robust growth during the experimental phase.

#### Keratinocyte treatment, RNA extraction, and RNA-seq

Keratinocytes from biopsy samples were treated with IFN- $\alpha$ 2 (1000 U/ml), IFN- $\beta$  (1000 U/ml), IFN- $\gamma$  (5 ng/ml), or IFN- $\alpha$ 6 (1000 U/ml) for 6 h at passage 3. Cells were harvested in Tripure (Sigma-Aldrich, St. Louis, MO). RNA was isolated from cell cultures using QIAGEN RNeasy kit (catalog no. 74136). Libraries for RNA-seq were generated from polyadenylated RNA and sequenced at six libraries per lane on an Illumina HiSeq 4000 with the assistance of the University of Michigan DNA sequencing core using 50 bp single-end reads.

#### RNA-seq transcriptome profiling

We generated on average 48 million single-end reads per sample. For each RNA-seq sample, we performed quality control (FastQC version 0.11.7) (26) and adapter trimming (Trimmomatic-0.36) (27). We used STAR-2.5.2 (28) to align the reads to the human reference genome (build 37), with genes annotated in GENCODE version 24. We then used HTSeq-0.6.1 (29) to count the number of uniquely mapped reads for each gene (–a 50 –t exon –m union), and on average >80% of reads were uniquely mapped to gene regions. The gene expression level was modeled using a negative binomial distribution and normalized in DESeq2 (30). Only genes with on average  $\geq 1$  read per sample were used in the subsequent analysis. Principal component (PC) analysis was performed on DESeq2 normalized expression matrix with additional inverse normalization; COMBAT was used to remove any batch effect (31). The coefficients of biological variation for each condition are illustrated in Supplemental Table I. We conducted differential expression analysis for each cytokine stimulation, conditioning on the patient-specific effect, for keratinocytes derived from

normal and lupus patients separately. The significant genes were declared as having a false discovery rate (FDR)  $\leq 10\%$  and  $|\log_2(\text{fold change [FC]})| \geq 1$ . The RNA-seq datasets are available at <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE124939>.

#### Downstream analysis of expression data

We conducted functional enrichment analysis using the hypergeometric test, and we examined the enrichment for pathways/functions annotated from Gene Ontology (32), Reactome (33), Kyoto Encyclopedia of Genes and Genomes (34), and Biocarta (35), compiled from the Gene Set Enrichment Analysis (36). We investigated the 5218 functions/pathways with  $\geq 10$  and  $\leq 300$  annotated genes that are expressed in our dataset. Significant functions were declared as having FDR  $\leq 10\%$  and observed/expected (O/E) ratio  $\geq 1.5$ .

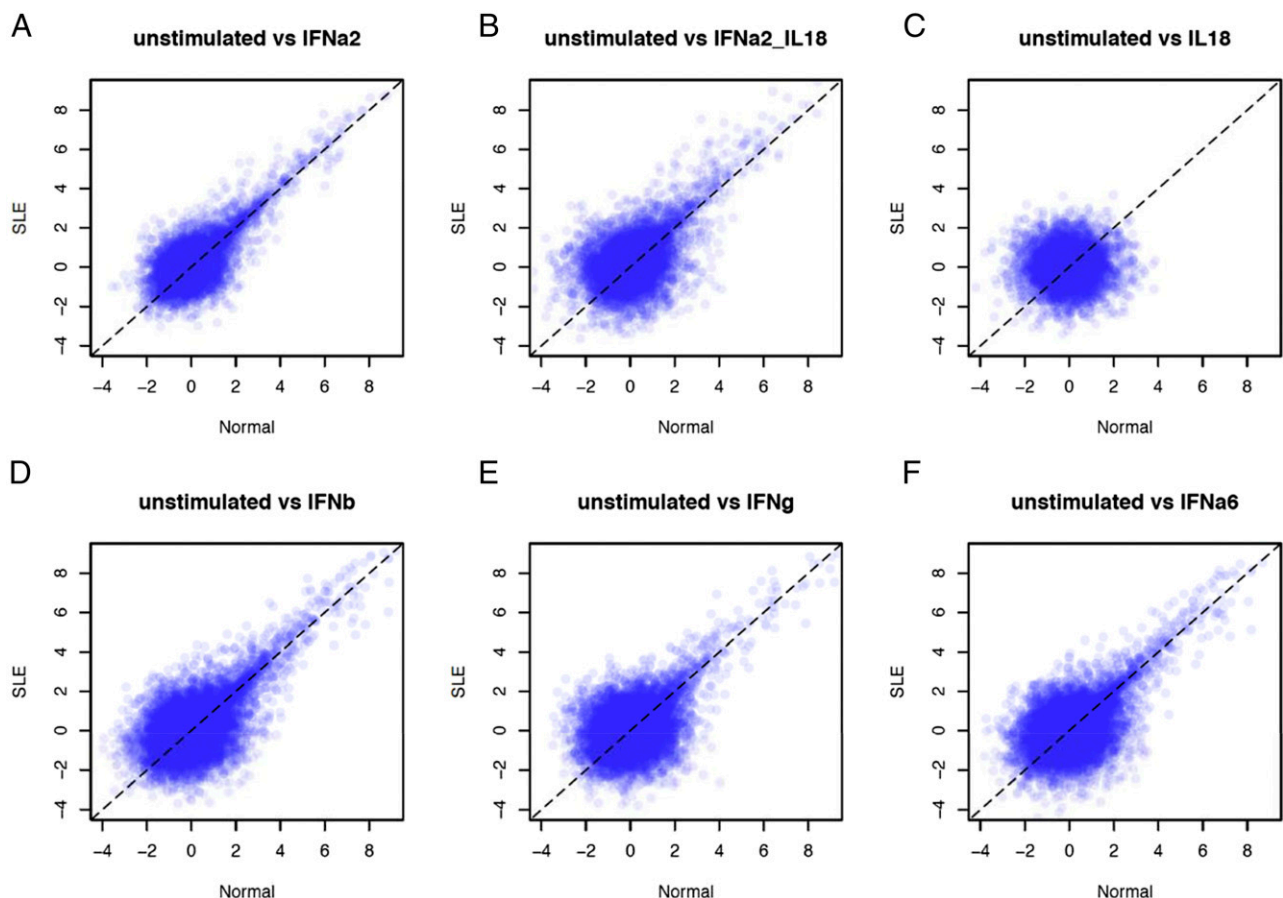
To compute the statistical significance for observing  $X$  IFN-responding genes with consistently higher response in lupus keratinocytes upon different IFN stimulation, we first computed the probability  $d_i$  that an IFN-responding gene would have a higher effect in lupus keratinocytes under the stimulation of IFN  $i$ . The probability that an IFN-responding gene would have a higher effect in lupus keratinocytes for all the different IFNs being considered would be:

$$a = \prod_i d_i$$

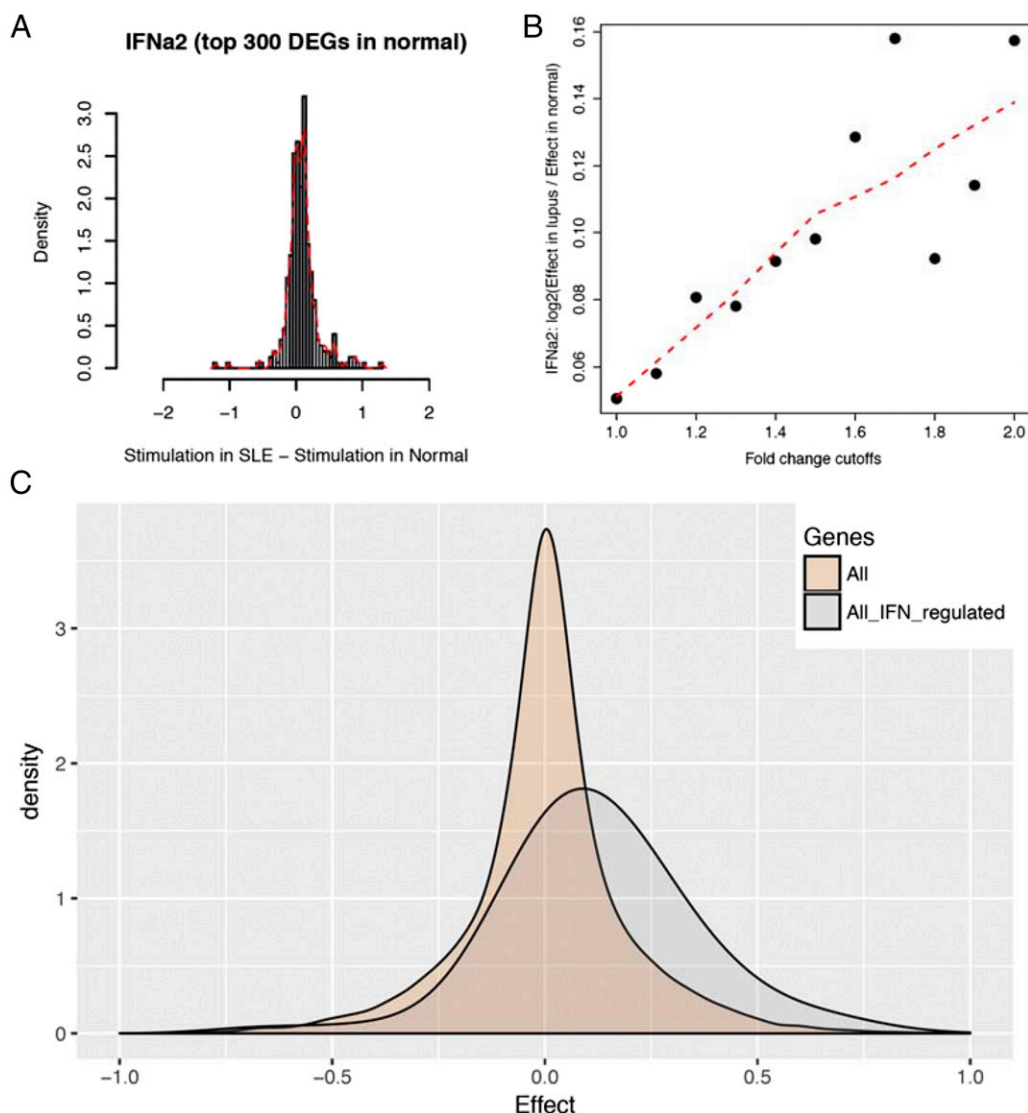
We could then compute the probability of observing  $X$  among all  $Y$  IFN-responding genes by:

$$p = \sum_{x=X}^Y \binom{Y}{x} a^x (1-a)^{Y-x}$$

We then used mixed-effect regression to model the effect size of IFN response:  $y = (SLE)\beta_1 + X\beta_2 + Pu + \epsilon$ , where  $\beta$  represents the fixed-effects regression coefficient (for lupus or different IFNs),  $u$  is the random effect for patient, and  $\epsilon$  is a vector of random errors. The R library “lmer” was used for the modeling (37). The Wilcoxon rank sum test was used to compare the lupus effect values estimated for the IFN-responding genes



**FIGURE 2.** (A–F) The effect sizes (i.e.,  $\log_2[\text{FC}]$ ) under indicated cytokine stimulations in normal (x-axis) and lupus (y-axis) keratinocytes. Diagonal lines represent what would be expected if the effect sizes are correlated between the two keratinocyte types.



**FIGURE 3.** The identification of genes with LSI responses. **(A)** The changes in effect sizes between normal and lupus keratinocytes upon IFN- $\alpha$ 2 stimulation. **(B)** Associations between different effect size cutoffs upon IFN- $\alpha$ 2 stimulation in normal keratinocytes and the magnitude of the change in effect size in lupus keratinocytes. **(C)** The changes in effect sizes upon all (type I and type II) IFN stimulation in lupus keratinocytes (when comparing against normal keratinocytes) for all genes and cytokine-regulated genes.

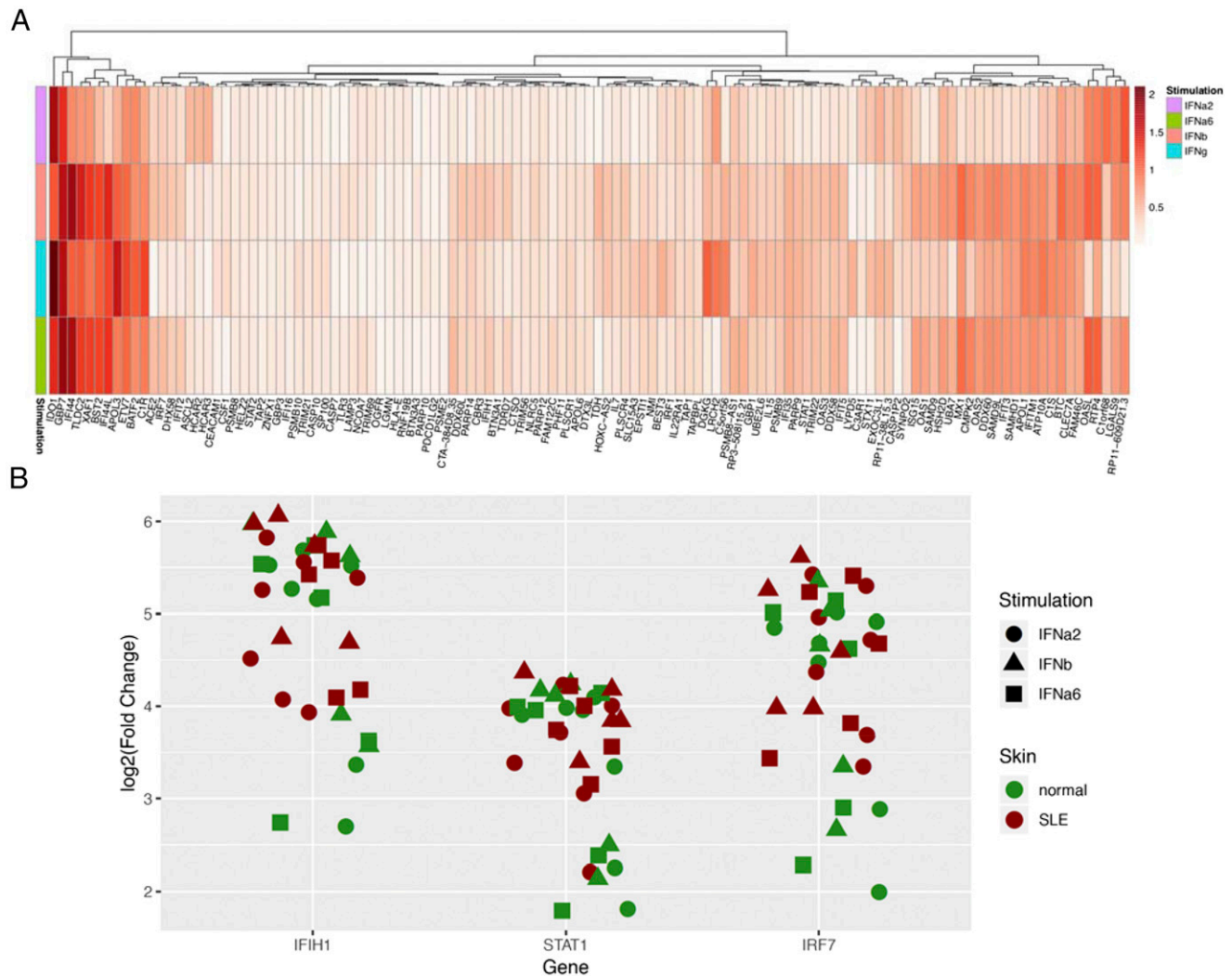
versus those for the nonresponding genes. We applied Minimum Distance-Based Enrichment Analysis for Genetic Association, which uses a graphical algorithm to integrate interaction information, to evaluate functional (i.e., LSI response in this case) enrichment among the established lupus loci (38). We used the interaction data from the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) (39) for generating the interactome and the genetic variants from 1000 genomes as background (40).

We compared the cytokine-stimulated effects measured in our gene expression experiments with a previously published, independent microarray dataset measuring global transcriptomic differences in skin biopsies for 47 DLE and 43 SCLE (Gene Expression Omnibus GSE81071). We performed the motif enrichment by screening the motif of every transcription factor across the 2000 bp upstream region from the transcription start site (41). We then evaluated the enrichment of each motif in the LSI-responding genes by setting the IFN-responding genes as background.

#### *PITX1* immunohistochemistry, knockdown, quantitative RT-PCR, and Western blot

We obtained 5- $\mu$ m thick slides from formalin-fixed, paraffin-embedded skin tissue specimens. *PITX1* (no. LS-C100923/55637; Lifespan Biosciences) staining was performed at 1:50 dilution and at pH 6 along with isotype controls. N/TERTs (42), an immortalized keratinocyte line, were plated in a 96-well plate (30,000 cells/well) and incubated at 37°C with

5% CO<sub>2</sub> overnight. One hundred micromolar Accell small interfering RNA (siRNA; no. E-017246-00-0005, *PITX1*; Dharmacon) was prepared in 1 $\times$  siRNA buffer (no. B-002000-UB-100; Dharmacon). One microliter of 100  $\mu$ M siRNA or control siRNA was diluted with 100  $\mu$ l of Accell delivery medium (no. B-005000; Dharmacon) for each well of the 96-well plate. Growth medium was removed from the cells, 100  $\mu$ l of the appropriate delivery mix with siRNA was added to each well, and the plate was incubated at 37°C with 5% CO<sub>2</sub>. Accell nontargeting control siRNA (no. D-001910-01-05; Dharmacon) was used as a negative control. After 48 h, cells were stimulated with 5 ng/ml of each cytokine (no. 11100-1, IFN- $\alpha$ 2; no. 8499-IF, IFN- $\beta$ ; no. 11165-1, IFN- $\alpha$ 6; all from R&D Systems) separately for 24 h and then harvested for RNA preparation. RNA was isolated from cell cultures using QIAGEN RNeasy Plus Kit (catalog no. 74136). Reverse transcription was performed using the High Capacity cDNA Transcription Kit (no. 4368813; Thermo Fisher). Quantitative PCR was performed on a 7900HT Fast Real-Time PCR System (Thermo Fisher) with TaqMan Universal PCR Master Mix (no. 4304437; Thermo Fisher) using TaqMan primers (*PITX1*: Hs00267528\_m1, *MX1*: Hs00895608\_m1; Thermo Fisher Scientific). *RPLP0* (no. Hs99999902\_m1; Thermo Fisher) was used as a loading control, as it is not regulated by IFN stimulation. For Western blot, protein was harvested 72 h after siRNA treatment in RIPA buffer with protease inhibitors. Total protein was measured using BCA assay. Protein from each sample was separated on 10% acrylamide gel and transferred to the Amersham Protran 0.2  $\mu$ M NC nitrocellulose membrane (GE Healthcare). The membranes were blocked with 5% milk and the



**FIGURE 4.** LSI genes having higher IFN responses in lupus patients. **(A)** Heatmap illustrates the increase in  $\log_2(\text{FC})$  (the darker the color, the higher the increase in FC) upon different IFN stimulations in keratinocytes from lupus patients compared with those from healthy individuals for the 119 LSI genes. **(B)** The  $\log_2(\text{FC})$  values for three LSI genes from lupus susceptibility regions illustrate that keratinocytes from lupus patients have modestly elevated values.

incubated with rabbit anti-human PITX1 (1:2500 dilution in PBS plus 5% BSA) at 4°C overnight, followed by HRP-conjugated anti-rabbit IgG (both of the Abs were from Abcam, Cambridge, MA). The same membrane was also blotted with anti-human  $\beta$ -actin (1:1000 dilution, purchased from Cell Signaling Technology, Danvers, MA) following the same procedure. Protein expression bands were detected by chemiluminescence using WesternBright Quantum Western blot detection reagent (Advanta, Menlo Park, CA), and the protein bands were imaged by Omega Lum C (Gel Company, San Francisco, CA).

## Results

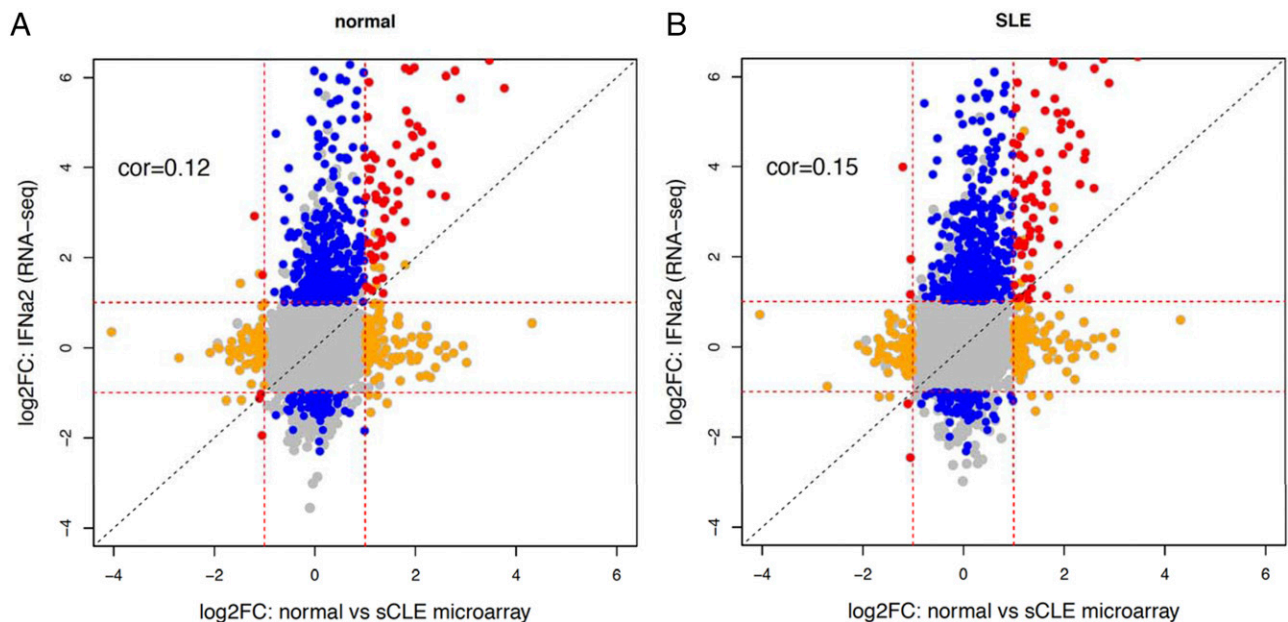
### Shared and distinct responses to different IFNs in keratinocytes

We obtained and cultured keratinocytes derived from skin biopsies of seven normal and seven lupus patients from nonlesional skin (Supplemental Table II). We stimulated the keratinocytes using different type I (i.e., IFN- $\alpha$ 2, IFN- $\beta$ , IFN- $\alpha$ 6) and type II (IFN- $\gamma$ ) IFNs and/or IL-18, a non-Jak-STAT signaling cytokine with implications in CLE (43). RNA-seq was conducted to profile the expression of unstimulated and cytokine-stimulated conditions, and in total we assayed 72 distinct transcriptomes (Supplemental Table III) and detected 27,574 genes being expressed. We first investigated the transcriptomic differences between the various conditions and skin origin by PC analysis. Interestingly, we did not observe systematic differences between the keratinocytes derived

from normal versus lupus skin under the unstimulated (or stimulated) condition when using only the top two PCs; however, the analysis illustrated a strong contrast between the type I IFN stimulations and unstimulated or other, including IFN- $\gamma$ -stimulated, conditions (Fig. 1A). Notably, the response under IL-18 and IFN- $\alpha$  costimulation is more similar to the other type I IFN responses than IFN- $\gamma$  stimulation.

Next, we conducted differential analysis to compare the expression profiles between the unstimulated condition and each of the cytokine-stimulated conditions in normal or lupus keratinocytes separately (Supplemental Table IV). We observed >400 differentially expressed (DE) genes (DEGs;  $\text{FDR} \leq 10\%$  and  $|\log_2[\text{FC}]| \geq 1$ ) in each of the IFN stimulations for both control and lupus keratinocytes; in contrast, the IL-18 stimulation, which served as a non-IFN control, did not result in any significant transcriptional changes. In concordance with the PC analysis, the genes DE upon IFN stimulations in normal keratinocytes also tended to be DE in lupus keratinocytes (Fig. 1B, 1C, Supplemental Fig. 1). Whereas there was substantial overlap between the DEGs under different IFN stimulations either in normal (Fig. 1D) or lupus (Fig. 1E) keratinocytes (271 and 279 genes DE under all four IFNs in normal and lupus keratinocytes, respectively), we consistently observed a large number of genes DE only by type I IFN but not





**FIGURE 5.** Comparison of keratinocyte IFN response with dysregulated gene expression in lupus skin. The changes in effect sizes upon IFN stimulation (y-axis) in normal (A) and lupus (B) keratinocytes were plotted against dysregulated genes in SCLE (x-axis). Red color represents genes DE in both conditions; orange color represents genes DE only in lupus skin; blue color represents genes DE only under IFN response.

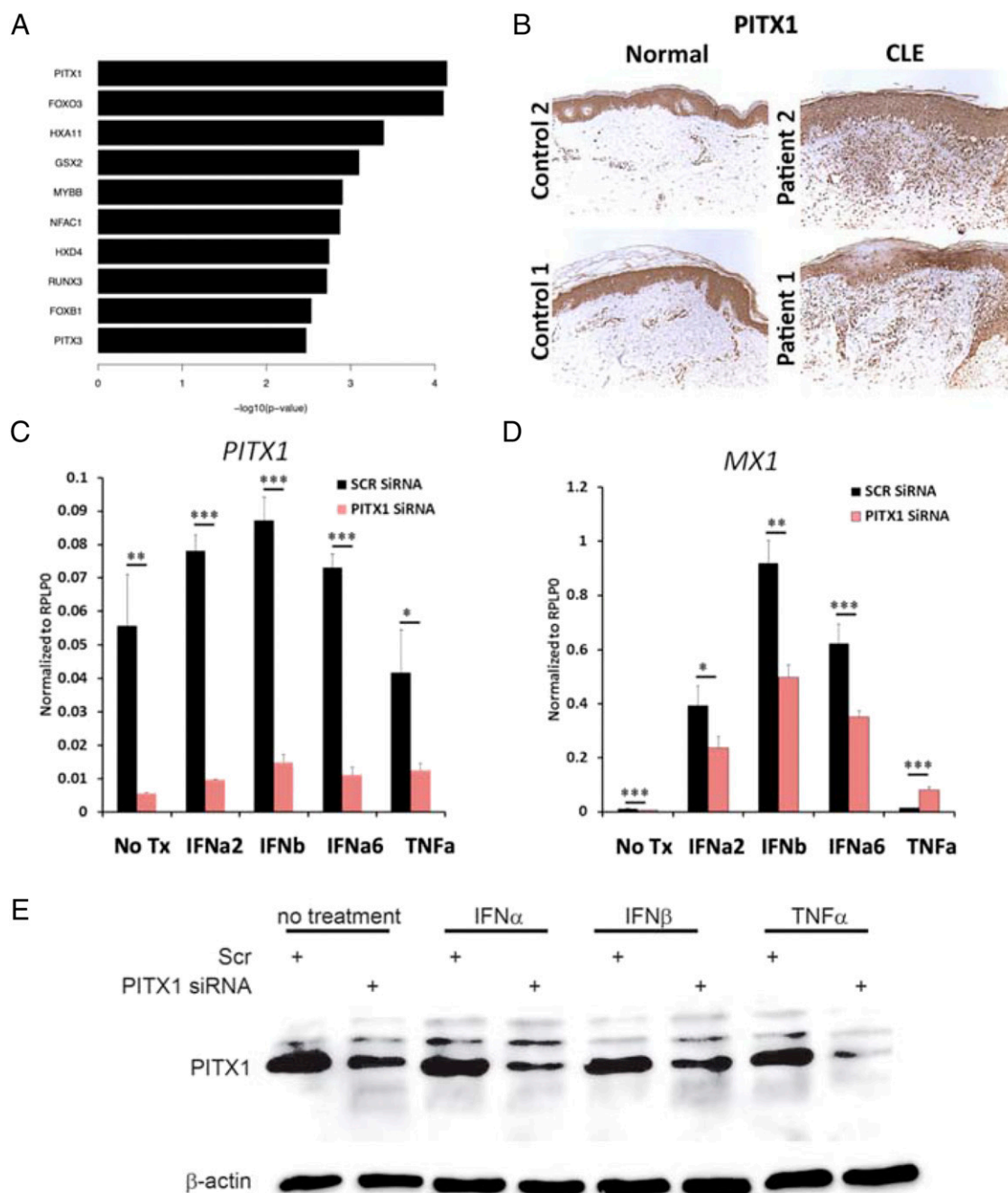
respectively; Fig. 3C; see *Materials and Methods*), concordant with the above results. These results illustrate the hypersensitive IFN responses in keratinocytes from lupus patients. We thus deemed these 119 IFN response genes with higher effects in SLE versus normal keratinocytes (from both the random effect model and differential expression analysis for every IFN stimulation) as LSI responses (Fig. 4, Supplemental Table VI, examples in Table I). There are 259 LSI response genes if we only consider type I IFNs using the same definition.

#### Pathologic implications for LSI responses

We then aimed to characterize the LSI responses and attempted to provide pathologic implications. We first investigated the functions that are enriched among these genes. Although it is expected that the LSI-responding genes are enriched among functions/pathways for IFN signaling (Supplemental Table V), we identified functions related to the regulation of DDX58/IFIH1 signaling that were significant (e.g., negative regulation of DDX58/IFIH1 signaling,  $p < 2.3 \times 10^{-8}$ ) only among the LSI genes, but NS in the functional analysis for genes only regulated by type I or type II IFNs from the above analysis. Because *IFIH1* is within one of the lupus-associated loci (24), we next investigated the overlap between the genes with LSI responses and the lupus susceptibility regions using a systems biology approach, Minimum Distance-Based Enrichment Analysis for Genetic Association (38). By considering the functional enrichment and the coherence in gene–gene interactions, we found that genes within the  $\pm 100$ -kb intervals of the lupus-associated regions were enriched in LSI response ( $p = 3.7 \times 10^{-2}$ ). Specifically, *IFIH1*, *STAT1*, and *IRF7* from three lupus susceptibility loci exhibited LSI responses (Fig. 4), and they also interacted with other LSI-responding genes, including *IRF9*, *DDX58*, and *ISG15*. These results provide prioritization for the candidate genes from the lupus-associated loci. In addition, genes exhibiting the LSI responses for functional analysis provide insight on the possible relevance of the *IFIH1/DDX58* signaling cascade, which may contribute to the pathology of CLE. To validate the relevance of LSI genes in disease, we

then performed a direct comparison against an independent transcriptomic cohort of cutaneous lupus, comparing healthy control versus CLE lesional tissue. Fig. 5 illustrates that the genes regulated by our IFN experiments overlap with genes that are dysregulated in the SCLE tissue, and we also observed a modest correlation between the effect sizes in the lesional skin and the IFN stimulation in lupus versus normal keratinocytes, thus concurring with our above findings (Supplemental Fig. 6 shows similar results for DLE). Notably, we found significant enrichment of our LSI-responding genes among the dysregulated genes in SCLE tissue, where 51 ( $p = 1.5 \times 10^{-42}$ ) and 40 ( $p = 9.1 \times 10^{-43}$ ) out of 258 SCLE dysregulated genes exhibit LSI response for type I IFNs and all IFNs, respectively, including *IFIH1* and *STAT1*. This analysis provided translational implications for our in vitro and in silico findings.

To further understand the regulatory mechanism of these LSI responses, we screened the promoter regions of LSI genes to reveal transcription factor binding sites that were enriched, using all the IFN-regulated genes' promoter regions as background. Notably, we identified the binding motif of PITX1, a homeoprotein transcription repressor, to be significantly enriched ( $p = 7.2 \times 10^{-5}$ ; Fig. 6A). PITX1 has been shown to physically interact with *IRF3* and *IRF7* on *IFNA* promoters (44), but its function in regulation of IFN response genes is unknown. Immunohistochemical analysis of normal and CLE skin identified PITX1 as expressed in the epidermis and globally elevated in CLE lesions (Fig. 6B). To determine whether PITX1 has a regulatory role in type I IFN-mediated transcriptional changes, we evaluated whether downregulation of PITX1 expression was sufficient to interrupt type I IFN-induced expression of *MX1*. As shown in Fig. 6C–E, baseline and type I IFN-mediated expression of *MX1*, a known type I IFN-regulated gene (and importantly, also an LSI gene), was significantly repressed when expression of *PITX1* was inhibited via siRNA. Interestingly, the inhibition of *PITX1* also has an inhibiting effect on *IRF3/IRF7/OASL* expression (Supplemental Fig. 7). These results implicate PITX1 as an important regulator of IFN responses, including LSI genes, and a potential target for further investigation.



**FIGURE 6.** PITX1 is a regulator for LSI response. **(A)** The  $p$  value enrichment for transcription factor binding sites among LSI-responding genes. **(B)** Immunohistochemical analysis of normal and CLE skin for PITX1. **(C–E)** Knockdown of *PITX1* was achieved through siRNA in N/TERT keratinocytes (C), and it significantly repressed expression of *MX1* upon type I IFN stimulation (D). **(E)** Western blot showing efficient knockdown of PITX1 protein expression. \* $p < 0.05$ , \*\* $p < 0.005$ , \*\*\* $p < 0.0005$ .

## Discussion

In this work we have identified, to our knowledge, a novel mechanism by which skin from lupus patients is prone to inflammation. Not only does lupus skin, even in nonlesional tissue, produce increased type I IFNs [likely a mix of IFN- $\alpha$  and IFN- $\kappa$  (9, 45)], we now also reveal that lupus keratinocytes are primed for hypersensitive responses to both type I and type II IFNs and that the LSI genes link to genetic risk for SLE and are identified as overexpressed in cutaneous lupus lesions, giving credence to their pathologic relevance. Further, we have identified upstream regulators, including PITX1, that may serve as important targets for downregulation of LSI responses.

Lupus is a complex autoimmune disease, and previous genetic studies (24, 46) have revealed multiple susceptibility regions associated with the disease. However, similar to other complex conditions, most of these signals reside within noncoding regions and affect the regulation of nearby genes in a context-specific pattern (47). Although the most promising candidate cell types for the complex autoimmune conditions are immune cells (47), we and others have identified a strong IFN signature in lesional and nonlesional lupus keratinocytes (9, 45, 48). We thus hypothesized that the keratinocytes in lupus patients would exhibit an enhanced response to IFNs. Previous SLE genome-wide association studies also revealed that most signals have a modest effect size, and therefore it

is not too surprising that the subtly elevated lupus-specific IFN response would not be revealed for each individual gene in our dataset. However, using IFN-responding genes as a unit, we identified a significant lupus-specific effect ( $p < 5 \times 10^{-12}$ ).

After revealing lupus-specific IFN responses, we integrated a computational approach and in vitro validation to identify PITX1 as an intriguing candidate in the IFN response. PITX1 is able to bind to IRF3 and IRF7, which results in negative regulation of various IFN genes (44). Importantly, interactions with IRF3 and IRF7 can overcome the repressive activity of PITX1 (44), suggesting that, in chronic IFN high states in which IRF3 and IRF7 levels are elevated, such as cutaneous lupus, PITX1 may be an important switch to skew increased transcription of IFN-regulated genes. Intriguingly, PITX1 is required for IFN-driven expression of IRF3 and IRF7, which suggests that it may also be important for enhanced IFN priming in SLE keratinocytes. Importantly, the role of PITX1 in regulating key upstream signaling and transcriptional mediators involved in type I IFN pathways, including IFN- $\kappa$  production in keratinocytes, should also be investigated. Among other transcription factors with their binding sites enriched among the promoters of the LSI genes (Fig. 6A), it is also noteworthy that FOXO3 can modulate proinflammatory cytokine production and serve as an IKK- $\epsilon$ -regulated checkpoint of IFN regulatory factor (49), and GSX2 is within a genomic region outside MHC showing the strongest association with lupus nephritis (LN) among SLE patients (50).

We also identified unique keratinocyte responses for each type I and type II IFN stimulation in normal and SLE keratinocytes. All type I IFNs, including IFN- $\alpha$  and IFN- $\beta$ , signal through a common type I IFN receptor (IFNAR), yet it remains unknown why the molecular responses of these unique IFNs differ. IFN- $\beta$  has been identified to have a higher affinity for IFNAR1, which may promote prolonged signaling despite negative regulatory mechanisms (51). Other explanations, such as degradation versus recycling of the receptor, have also been explored (52). However, the contribution of downstream signaling molecules in differential IFN responses is unknown. Certainly, it will be of interest to identify whether regulators of LSI responses participate in differential IFN responses, as well.

Previous studies have illustrated the importance of low levels of type I IFNs for maintaining immune responses (6), and increased levels can drive chronic IFN priming effects in lupus patients (13). In this study, we have also conducted differential expression analysis to compare the untreated keratinocytes from healthy individuals with the keratinocytes from the lupus patients (Supplemental Table VII). Despite fewer significantly DEGs in the normal versus SLE keratinocytes comparison, we did observe significant positive correlations (Supplemental Fig. 8) against the effect sizes upon IFN stimulation, indicating a modest priming effect in the keratinocytes among SLE patients. In contrast, a previous study using in vivo data (comparing 240 LN patients versus 89 controls) illustrates that keratinocytes (not cultured) from skin biopsies in LN patients have elevated IFN response signatures (45), and this result can be contributed by both intrinsic effects or previous exposure to IFNs. As we cultured the keratinocytes from the skin biopsies of normal and lupus patients for our study prior to RNA-seq, this may explain the reason for more subtle priming effects in our study.

In summary, cutaneous manifestations of SLE affect a majority of lupus patients, and a better understanding of its pathology is essential to achieve effective treatment of patients. Using RNA-seq and bioinformatics approaches, we have confirmed a skewed IFN response in keratinocytes from lupus patients and have identified PITX1 as a potential targetable regulator of this response. Continued

work to understand the LSI responses in the skin will lead to novel and better therapies for SLE and potentially other skin diseases characterized by dysregulated IFN responses.

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## Disclosures

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## References

- Somers, E. C., W. Marder, P. Cagnoli, E. E. Lewis, P. DeGuire, C. Gordon, C. G. Helmick, L. Wang, J. J. Wing, J. P. Dhar, et al. 2014. Population-based incidence and prevalence of systemic lupus erythematosus: the michigan lupus epidemiology and surveillance program. *Arthritis Rheumatol.* 66: 369–378.
- Mikita, N., T. Ikeda, M. Ishiguro, and F. Furukawa. 2011. Recent advances in cytokines in cutaneous and systemic lupus erythematosus. *J. Dermatol.* 38: 839–849.
- Panopalis, P., A. E. Clarke, and E. Yelin. 2012. The economic burden of systemic lupus erythematosus. *Best Pract. Res. Clin. Rheumatol.* 26: 695–704.
- Klein, R., S. Moghadam-Kia, L. Taylor, C. Coley, J. Okawa, J. LoMonico, M. M. Chren, and V. P. Werth. 2011. Quality of life in cutaneous lupus erythematosus. *J. Am. Acad. Dermatol.* 64: 849–858.
- Taniguchi, T., and A. Takaoka. 2001. A weak signal for strong responses: interferon- $\alpha/\beta$  revisited. *Nat. Rev. Mol. Cell Biol.* 2: 378–386.
- Gough, D. J., N. L. Messina, C. J. Clarke, R. W. Johnstone, and D. E. Levy. 2012. Constitutive type I interferon modulates homeostatic balance through tonic signaling. *Immunity* 36: 166–174.
- Furie, R., M. Khamashta, J. T. Merrill, V. P. Werth, K. Kalunian, P. Brohawn, G. G. Illei, J. Drappa, L. Wang, and S. Yoo. CD1013 Study Investigators. 2017. Anifrolumab, an anti-interferon- $\alpha$  receptor monoclonal antibody, in moderate-to-severe systemic lupus erythematosus. *Arthritis Rheumatol.* 69: 376–386.
- Wenzel, J., E. Wörenkämper, S. Freutel, S. Henze, O. Haller, T. Bieber, and T. Tüting. 2005. Enhanced type I interferon signalling promotes Th1-biased inflammation in cutaneous lupus erythematosus. *J. Pathol.* 205: 435–442.
- Stannard, J. N., T. J. Reed, E. Myers, L. Lowe, M. K. Sarkar, X. Xing, J. E. Gudjonsson, and J. M. Kahlenberg. 2017. Lupus skin is primed for IL-6 inflammatory responses through a keratinocyte-mediated autocrine Type I interferon loop. *J. Invest. Dermatol.* 137: 115–122.
- Foering, K., A. Y. Chang, E. W. Piette, A. Cucchiara, J. Okawa, and V. P. Werth. 2013. Characterization of clinical photosensitivity in cutaneous lupus erythematosus. *J. Am. Acad. Dermatol.* 69: 205–213.
- Furukawa, F. 2003. Photosensitivity in cutaneous lupus erythematosus: lessons from mice and men. *J. Dermatol. Sci.* 33: 81–89.
- Sanders, C. J., H. Van Weelden, G. A. Kazzaz, V. Sigurdsson, J. Toonstra, and C. A. Bruijnzeel-Koomen. 2003. Photosensitivity in patients with lupus erythematosus: a clinical and photobiological study of 100 patients using a prolonged phototest protocol. *Br. J. Dermatol.* 149: 131–137.
- Sarkar, M. K., G. A. Hile, L. C. Tsoi, X. Xing, J. Liu, Y. Liang, C. C. Berthier, W. R. Swindell, M. T. Patrick, S. Shao, et al. 2018. Photosensitivity and type I IFN responses in cutaneous lupus are driven by epidermal-derived interferon kappa. *Ann. Rheum. Dis.* 77: 1653–1664.
- Zahn, S., M. Graef, N. Patsinakidis, A. Landmann, C. Surber, J. Wenzel, and A. Kuhn. 2014. Ultraviolet light protection by a sunscreen prevents interferon-driven skin inflammation in cutaneous lupus erythematosus. *Exp. Dermatol.* 23: 516–518.
- Meller, S., F. Winterberg, M. Gilliet, A. Müller, I. Lauceviciute, J. Rieker, N. J. Neumann, R. Kubitz, M. Gombert, E. Bünemann, et al. 2005. Ultraviolet radiation-induced injury, chemokines, and leukocyte recruitment: an amplification cycle triggering cutaneous lupus erythematosus. *Arthritis Rheum.* 52: 1504–1516.
- Reefman, E., H. Kuiper, P. C. Limburg, C. G. M. Kallenberg, and M. Bijl. 2008. Type I interferons are involved in the development of ultraviolet B-induced inflammatory skin lesions in systemic lupus erythematosus patients. *Ann. Rheum. Dis.* 67: 11–18.
- Kovats, S., N. Jacob, H. Agrawal, E. Carreras-Margalef, S. Bajana-Mirand, and C. O. Jacob. 2007. Altered dendritic cell differentiation and activation in lupus prone NZM 2328 mice lacking the type I interferon receptor. *Arthritis Rheum. (Suppl.)*: 399.
- Ramanujam, M., P. Kahn, W. Huang, H. Tao, M. P. Madaio, S. M. Factor, and A. Davidson. 2009. Interferon- $\alpha$  treatment of female (NZW x BXSB)F(1) mice mimics some but not all features associated with the Yaa mutation. *Arthritis Rheum.* 60: 1096–1101.
- Liu, J., C. C. Berthier, and J. M. Kahlenberg. 2017. Enhanced inflammasome activity in systemic lupus erythematosus is mediated via type I interferon-induced up-regulation of interferon regulatory factor 1. *Arthritis Rheumatol.* 69: 1840–1849.

20. Das, A., B. A. Heesters, A. Bialas, J. O'Flynn, I. R. Rifkin, J. Ochando, N. Mittereder, G. Carlesso, R. Herbst, and M. C. Carroll. 2017. Follicular dendritic cell activation by TLR ligands promotes autoreactive B cell responses. *Immunity* 46: 106–119.
21. Hamilton, J. A., Q. Wu, P. Yang, B. Luo, S. Liu, H. Hong, J. Li, M. R. Walter, E. N. Fish, H. C. Hsu, and J. D. Mountz. 2017. Cutting edge: endogenous IFN- $\beta$  regulates survival and development of transitional B cells. *J. Immunol.* 199: 2618–2623.
22. Shephardson, K. M., K. Larson, R. V. Morton, J. R. Prigge, E. E. Schmidt, V. C. Huber, and A. Rynda-Apple. 2016. Differential type I interferon signaling is a master regulator of susceptibility to postinfluenza bacterial superinfection. *MBio* 7: e00506-16.
23. van Boxel-Dezaire, A. H., J. A. Zula, Y. Xu, R. M. Ransohoff, J. W. Jacobberger, and G. R. Stark. 2010. Major differences in the responses of primary human leukocyte subsets to IFN- $\beta$ . *J. Immunol.* 185: 5888–5899.
24. Morris, D. L., Y. Sheng, Y. Zhang, Y. F. Wang, Z. Zhu, P. Tomblinson, L. Chen, D. S. Cunningham-Graham, J. Benthams, A. L. Roberts, et al. 2016. Genome-wide association meta-analysis in Chinese and European individuals identifies ten new loci associated with systemic lupus erythematosus. *Nat. Genet.* 48: 940–946.
25. Hochberg, M. C. 1997. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* 40: 1725.
26. Andrews, S. 2010. FastQC: a quality control tool for high throughput sequence data, v0.11.7. Babraham Bioinformatics, Babraham Institute, Babraham, U.K. Available at: <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>.
27. Bolger, A. M., M. Lohse, and B. Usadel. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30: 2114–2120.
28. Dobin, A., C. A. Davis, F. Schlesinger, J. Drenkow, C. Zaleski, S. Jha, P. Batut, M. Chaisson, and T. R. Gingeras. 2013. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29: 15–21.
29. Anders, S., P. T. Pyl, and W. Huber. 2015. HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics* 31: 166–169.
30. Love, M. I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15: 550.
31. Johnson, W. E., C. Li, and A. Rabinovic. 2007. Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics* 8: 118–127.
32. Ashburner, M., C. A. Ball, J. A. Blake, D. Botstein, H. Butler, J. M. Cherry, A. P. Davis, K. Dolinski, S. S. Dwight, J. T. Eppig, et al. The Gene Ontology Consortium. 2000. Gene ontology: tool for the unification of biology. *Nat. Genet.* 25: 25–29.
33. Fabregat, A., S. Jupe, L. Matthews, K. Sidiropoulos, M. Gillespie, P. Garapati, R. Haw, B. Jassal, F. Korninger, B. May, et al. 2018. The reactome pathway knowledgebase. *Nucleic Acids Res.* 46(D1): D649–D655.
34. Kanehisa, M., S. Goto, Y. Sato, M. Furumichi, and M. Tanabe. 2012. KEGG for integration and interpretation of large-scale molecular data sets. *Nucleic Acids Res.* 40(Database issue): D109–D114.
35. Nishimura, D. 2001. Biocarta. *Biotech Softw. Internet Rep.* 2: 117–120.
36. Subramanian, A., P. Tamayo, V. K. Mootha, S. Mukherjee, B. L. Ebert, M. A. Gillette, A. Paulovich, S. L. Pomeroy, T. R. Golub, E. S. Lander, and J. P. Mesirov. 2005. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl. Acad. Sci. USA* 102: 15545–15550.
37. Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67: 1–48.
38. Tsoi, L. C., J. T. Elder, and G. R. Abecasis. 2015. Graphical algorithm for integration of genetic and biological data: proof of principle using psoriasis as a model. *Bioinformatics* 31: 1243–1249.
39. Szklarczyk, D., J. H. Morris, H. Cook, M. Kuhn, S. Wyder, M. Simonovic, A. Santos, N. T. Doncheva, A. Roth, P. Bork, et al. 2017. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. *Nucleic Acids Res.* 45(D1): D362–D368.
40. 1000 Genomes Project Consortium, G. R. Abecasis, A. Auton, L. D. Brooks, M. A. DePristo, R. M. Durbin, R. E. Handsaker, H. M. Kang, G. T. Marth, and G. A. McVean. 2012. An integrated map of genetic variation from 1,092 human genomes. *Nature* 491: 56–65.
41. Bailey, T. L., M. Boden, F. A. Buske, M. Frith, C. E. Grant, L. Clementi, J. Ren, W. W. Li, and W. S. Noble. 2009. MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Res.* 37(Web Server issue): W202–W208.
42. Dickson, M. A., W. C. Hahn, Y. Ino, V. Ronfard, J. Y. Wu, R. A. Weinberg, D. N. Louis, F. P. Li, and J. G. Rheinwald. 2000. Human keratinocytes that express hTERT and also bypass a p16(INK4a)-enforced mechanism that limits life span become immortal yet retain normal growth and differentiation characteristics. *Mol. Cell. Biol.* 20: 1436–1447.
43. Wang, D., M. Drenker, B. Eiz-Vesper, T. Werfel, and M. Wittmann. 2008. Evidence for a pathogenetic role of interleukin-18 in cutaneous lupus erythematosus. *Arthritis Rheum.* 58: 3205–3215.
44. Island, M. L., T. Mesplede, N. Darraq, M. T. Bandu, N. Christeff, P. Djan, J. Drouin, and S. Navarro. 2002. Repression by homeoprotein pitx1 of virus-induced interferon  $\alpha$  promoters is mediated by physical interaction and trans repression of IRF3 and IRF7. *Mol. Cell. Biol.* 22: 7120–7133.
45. Der, E., S. Ranabothu, H. Suryawanshi, K. M. Akat, R. Clancy, P. Morozov, M. Kustagi, M. Czuppa, P. Izmirly, H. M. Belmont, et al. 2017. Single cell RNA sequencing to dissect the molecular heterogeneity in lupus nephritis. *JCI Insight* 2: e93009.
46. Li, Y., H. Cheng, X. B. Zuo, Y. J. Sheng, F. S. Zhou, X. F. Tang, H. Y. Tang, J. P. Gao, Z. Zhang, S. M. He, et al. 2013. Association analyses identifying two common susceptibility loci shared by psoriasis and systemic lupus erythematosus in the Chinese Han population. *J. Med. Genet.* 50: 812–818.
47. Farh, K. K., A. Marson, J. Zhu, M. Kleinewietfeld, W. J. Housley, S. Beik, N. Shores, H. Whittom, R. J. Ryan, A. A. Shishkin, et al. 2015. Genetic and epigenetic fine mapping of causal autoimmune disease variants. *Nature* 518: 337–343.
48. Zahn, S., C. Rehkämper, B. M. Kümmerer, S. Ferring-Schmidt, T. Bieber, T. Tüting, and J. Wenzel. 2011. Evidence for a pathophysiological role of keratinocyte-derived type III interferon (IFN $\lambda$ ) in cutaneous lupus erythematosus. *J. Invest. Dermatol.* 131: 133–140.
49. Luron, L., D. Saliba, K. Blazek, A. Lanfrancotti, and I. A. Udalova. 2012. FOXO3 as a new IKK- $\epsilon$ -controlled check-point of regulation of IFN- $\beta$  expression. *Eur. J. Immunol.* 42: 1030–1037.
50. Chung, S. A., E. E. Brown, A. H. Williams, P. S. Ramos, C. C. Berthier, T. Bhargale, M. E. Alarcon-Riquelme, T. W. Behrens, L. A. Criswell, D. C. Graham, et al. International Consortium for Systemic Lupus Erythematosus Genetics. 2014. Lupus nephritis susceptibility loci in women with systemic lupus erythematosus. *J. Am. Soc. Nephrol.* 25: 2859–2870.
51. Wilmes, S., O. Beutel, Z. Li, V. Francois-Newton, C. P. Richter, D. Janning, C. Kroll, P. Hanhart, K. Hötte, C. You, et al. 2015. Receptor dimerization dynamics as a regulatory valve for plasticity of type I interferon signaling. *J. Cell Biol.* 209: 579–593.
52. Marijanovic, Z., J. Ragimbeau, J. van der Heyden, G. Uzé, and S. Pellegrini. 2007. Comparable potency of IFN $\alpha$ 2 and IFN $\beta$ 2 on immediate JAK/STAT activation but differential down-regulation of IFNAR2. *Biochem. J.* 407: 141–151.