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Serine and arginine rich splicing factor 1 deficiency alters pathways involved in IL-17A expression and is implicated in human psoriasis

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Abstract

Serine and Arginine Rich Splicing Factor 1 (SRSF1) is a splicing factor that binds to exonic enhancers and stimulates splicing and is previously implicated with autoimmunity. Herein, we investigate the role of SRSF1 in regulating innate immune functions that are pertinent in the pathogenesis of auto-inflammatory diseases. Specifically, we show that conditional deletion of SRSF1 in mature lymphocytes resulted in higher expression of *il-17a* and *il-17 f* and an expansion of $IL17A^+$ CD8 T cells. Mechanistically, the aberrant expression of IL-17A in SRSF1 cKO mice could not be attributed to alternative splicing of *il-17a or il-17 f* genes but possibly to defective $CD11B^+LY6C^+$ myeloid derived suppressor function in the spleen. Finally, meta-analysis of RNA-Seq collected from psoriasis patients demonstrate a clear correlation between SRSF1 and psoriasis that suggests a putative role of SRSF1 in IL-17A-induced psoriasis.

Keywords

SRSF1; Il-17; Psoriasis; Autoimmunity; Alternative splicing

1. Introduction

Psoriasis is a chronically inflammatory skin disease the manifestation of which includes epidermal hyperplasia, leukocyte infiltration, and memory T cell accumulation [1]. It

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has been increasingly appreciated that psoriasis is an immune mediated disease that is fueled by the dynamic interplay between T cells, dermal dendritic cells and keratinocytes. Therapeutics against the IL-23/IL-17A axis has shown excellent efficacy in psoriasis however the pathogenic mechanisms remain elusive [2,3].

IL-17A is elevated in multiple autoimmune diseases, including psoriasis [2]. Many factors regulate the expression and signaling of IL-17, including RORγt, RUNX1, STAT3, NF-κB, as well as certain microRNAs [4]. IL-17A expression is also regulated by alternative splicing, a regulatory process enabling the generation of protein isoforms from a single gene [5–8]. Alternative splicing is a naturally occurring process that increases protein diversity [9]. However, dysregulated splicing has been linked with many diseases [10]. Specifically, the Serine and Arginine Rich Splicing Factor 1 (SRSF1), a splicing factor that binds to exonic enhancers and stimulates splicing, has been shown to influence Th17 differentiation [7,11]. SRSF1 is a multi-functional serine arginine protein that not only controls pre-mRNA splicing, but also regulates many other splicing independent activities such as nonsense-mediated mRNA decay, nuclear export of mRNA, and mTOR activation [12–15]. Recent studies have suggested that SRSF1 also plays an important role in autoimmunity [7,8,16]. Particularly, increased mRNA expression levels of *Il-17a* and *Il-17 f* have been observed in regulatory T cells of a mouse model with T cell specific conditional knockout of Srsf1 [8]. On the other hand, IL-17 has been shown to enhance the stability of CXCL1 mRNA by inhibiting the interaction between SRSF1 and CXCL1 in the TRAF cascade, which mediates IL-17 signaling [17,18]. These studies collectively suggest that SRSF1 is involved in multiple steps in the IL-17 signaling pathway.

Myeloid-derived suppressor cells (MDSCs) are a specific population of cells that can expand and suppress T cell responses during inflammation and infection. In mice, there are two types of MDSCs characterized by the co-expression of CD11B as well as either Ly6G ($Ly6G^+Ly6C^-$ granulocytes) or LY6C ($Ly6G^-Ly6C^+$ monocytes) [19]. Monocytic MDSCs are more suppressive than granulocytic MDSCs and in murine viral infection models, monocytic MDSCs have been found to suppress $CD8^+$ T cells in the spleen [20]. MDSCs suppress T-cell function by down-regulating the expression of CD3 ζ chain in T cells [21]. Interestingly, SRSF1 also regulates mRNA expression of CD3 ζ chain in T cells [22]. In this study, we propose that SRSF1 might be involved in IL-17 signaling through MDSCs, which might support a putative role of SRSF1 in innate immune responses and autoinflammatory diseases such as psoriasis.

2. Methods

2.1. Animals

$Srsf1^{fl/fl}$ (JAX: 018020) and $dLck^{cre}$ (JAX: 012837) mice were purchased from Jackson Laboratory. $Srsf1^{fl/fl}$ and $dLck^{cre}$ mice were crossbred to generate $Srsf1^{fl/fl}.dLck^{cre}$ conditional knockout mice, referred to as $Srsf1^{fl/fl}.dLck^{cre}$. Eight to twelve-week-old male and female mice were used in this study. All mice were bred and housed under specific pathogen-free conditions and exposed to a constant 12-h light-dark cycle in the animal facilities at Beth Israel Deaconess Medical Center (BIDMC). All experiments were

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conducted in accordance with the guidelines and with the approval of the Institutional Animal Care and Use Committee of BIDMC.

2.2. Antibodies and reagents

CD3 (145-2C11, BioLegend), CD4 (GK1.5, Invitrogen), CD8a (53–6.7, BioLegend), CD25 (PC61, BioLegend), CD127 (A7R34, BioLegend), IL-17A (TC11-18H10.1, BioLegend), IL-17F (9D3.1C8, BioLegend), FOXP3 (MF-14, BioLegend), RORγt (B2D, BioLegend), Ly6C (HK1.4, BioLegend), Ly6G (1A8, BioLegend), CD11B (M1/70, BioLegend).

2.3. Acute lymphocytic choriomeningitis virus (LCMV) infection

Eight to twelve-week-old WT and $Srsf1^{fl/f}$.$dLck^{cre}$ mice were infected with 2×10^5 PFU of Armstrong strain LCMV by *i.p.* injection. Age- and sex-matched mice injected with PBS served as controls. Bone marrow and splenocytes were harvested at 24 h post infection.

2.4. Flow cytometry

Flow cytometry was performed using a CytoFLEX LX Flow Cytometer (Beckman Coulter) and analyzed using FlowJo. Single cell suspension of mouse bone marrow cells or splenocytes were collected for flow cytometric analyses. ZombieNIR Viability Kit (BioLegend) staining was performed to eliminate dead cells. Surface staining was performed in PBS with Fc block at 4 °C for 30 min. For intracellular cytokine staining, cells were stimulated for 4 h in culture medium with PMA (1 mM, Sigma-Aldrich), Ionomycin (1 μM, Sigma-Aldrich) and monensin (1 μL/mL, BD Biosciences). Cytofix/Cytoperm and Perm/Wash buffer (BD Biosciences) were used for fixation and permeabilization. Appropriate antibodies were used for intracellular staining for cytokines or transcription factors according to manufacturer's instructions.

2.5. RNA-Seq analysis

RNA-Seq data of natural regulatory T cells (nTregs) of WT and $Srsf1^{fl/f}$.$dLck^{cre}$ mice were acquired from the NCBI Gene Expression Omnibus (GEO) from the series under accession number: GSE173268. RNA-Seq data from human normal and psoriatic lesional skin samples were acquired from GEO from the series under accession numbers: GSE63980 and GSE121212. Four total datasets were used: three independent datasets (SRP165679, SRP026042, SRP057087) and one dataset comprised of two combined experimental datasets published by the same research group (SRP035988, SRP050971) [23–25]. The latter included 99 lesional psoriatic and 90 normal skin biopsies from subjects of European descent enrolled in the Southeast Michigan area. Psoriasis patients who volunteered for a skin biopsy underwent a wash-out period of all immunosuppressive medications prior to the procedure.

2.6. Bioinformatic analyses

Raw sequencing data were acquired in FASTQ format. Data quality was evaluated using FastQC [26] and data were pre-processed with Cutadapt [27] for adapter removal following best practices [28]. Alignment of sequencing reads to the reference genome (GRCh38 and GRCm38 for human and mouse, respectively) was performed using STAR [29], and the

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quantification of reads was carried out with the SubRead package [30]. Gene expression level normalization and differential expression analysis was carried out using the DESeq2 Bioconductor R package (version 1.6.3) [31], while ClusterProfiler [32] was utilized for downstream functional investigations and pathway enrichment analyses. Differential expression *p*-values were corrected for multiple testing using the false discovery rate (FDR) and Storey's q-value [33]. Genes with adjusted $p < 0.05$ were considered as differentially expressed. Differential exon usage analysis was performed with DEXSEq. (v1.34.1) [34,35]. First, the exon annotation was created based on the GRCm38 Ensembl v98 gene annotation. Differential exon usage *p*-values were corrected for multiple testing using FDR method. Plots were generated in R using ggplot2 (v3.3.3) [36] ComplexHeatmap (v2.6.2) [37], and ggsashimi (v1.1.0) [38].

2.7. Statistical analyses

Comparisons between two groups were done by unpaired two-tailed Student's *t*-test. Non-parametric tests were applied to the datasets with non-Gaussian distribution. Statistical analyses were performed with GraphPad Prism 9.0 software (GraphPad Software). Data are shown as mean ± standard error (SEM). *P* values <0.05 were considered significant.

3. Results

3.1. Increased gene and protein expression of IL-17A in Srsf1$^{fl/fl}$.dLck^{cre} mice

In order to elucidate the role of SRSF1 in mature T cells, we crossed the Srsf1$^{fl/fl}$ mice [39] with dLck^{Cre} mice [40], which express the Cre recombinase under the control of the *lck* distal promoter. Using this system, we deleted the floxed gene fragment selectively at the late stage in thymocyte development to conditionally inactivate SRSF1 in mature T cells (Fig. 1A). Analysis of the RNA-Seq data (GSE173268) showed that T cells from Srsf1$^{fl/fl}$.dLck^{cre} mice exhibited higher expression of *Il-17a* and *Il-17 f* (Fig. 1B). In order to investigate whether the elevated *Il-17a* and *Il-17 f* transcripts also reflect on the protein level in naïve state, we performed flow cytometric analysis on the splenocytes of Srsf1$^{fl/fl}$.dLck^{cre}. We confirmed the RNAseq observations by performing intracellular staining of IL-17A in various T cell subsets. We detected a significant elevation of IL-17A^{+}CD8^{+} T cells in the Srsf1$^{fl/fl}$.dLck^{cre} mice (Fig. 1C) and a non-significant trend of elevated IL-17A and IL-17F protein in CD4^{+}CD25^{high}CD127^{low} Tregs (data not shown).

3.2. Alternative splicing is not involved in the expression of Il-17a or Il-17 f in Srsf1$^{fl/fl}$.dLck^{cre} mice

The observed difference in RNAseq and the protein levels of IL-17A in Srsf1$^{fl/fl}$.dLck^{cre} mice led us to speculate whether the elevated gene expression might be due to aberrant alternative splicing caused by SRSF1 deficiency. In order to directly capture the effect of SRSF1 deficiency in alternative splicing we compared differentially spliced genes between Srsf1$^{fl/fl}$dLck^{cre} and control mice. Differential exon usage analysis identified 578 genes (667 exons) with potential differential usage of exons in Srsf1$^{fl/fl}$dLck^{cre}, under a permissive FDR < 0.1 (Fig 2A). However, there were only 5 genes falling within the overlap between differentially expressed and differentially spliced genes identified. Further analysis detected that among the 578 genes with potential differential usage of exons,

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43% had increased exon usage and 57% had decreased exon usage (Fig. 2B). Both *Il-17a* and *Il-17 f*, although differentially expressed, were not differentially spliced (Fig. 2C, D). Following further manual evaluation, 2 out of 3 samples showed RNA processing patterns practically indistinguishable from the control group, while one sample showed some potential differential exon usage. Even under permissive statistical significance thresholds, investigations of differential exon usage did not reveal any statistically significant difference, pointing to differential expression events not directly affected by SRSF1 splicing activity. Subsequently, we analyzed transcription factors RORγt, STAT3, Stat5a, Stat5b, TBX21 and GATA3 that affect *Il-17a* and Il-17 f expression, but did not detect any significant differential expression or alternatively splicing in these genes [41–43]. Next, we looked at differentially expressed genes (DEGs) that showed differential exon usage that could affect the secretion of IL-17A or IL-17F, such as Rab23, a molecule involved in vesicular trafficking, and Pikfyve, a molecule that affects a number of trafficking pathways. Further visual inspection suggested that the observed effect is limited to a small fraction of the exons (Fig. 2E, F). Taken together in these experiments we did not detect any significant changes that suggest SRSF1 mediated alternative splicing in the expression of *Il-17a* and *Il-17 f*.

3.3. Srsf1$^{fl/fl}$.dLck^{cre} Tregs possess distinct gene signatures associated with innate immunity

In order to explore the potential pathways that the altered gene expression of *Il-17a* and *Il-17 f* in Tregs of Srsf1$^{fl/fl}$.dLck^{cre} mice might play a role in, we performed pathway enrichment and over-representation analysis. Our data showed that the differentially expressed genes are highly enriched in innate immune response pathways, as well as neutrophil and monocyte chemotaxis (Fig. 3A). Specifically, heatmap of the RNA-seq data showed that, among the differentially expressed genes involved in innate immunity, (Fig. 3B), the T cells of Srsf1$^{fl/fl}$.dLck^{cre} mice showed elevated chemokine expression related to monocytes and neutrophils, such as *Ccl3, Cel1, Ccl17, Ccl20*, and *Ccl22* (Fig. 3C). These data suggested that Srsf1 deficiency might regulate innate immunity.

3.4. Srsf1$^{fl/fl}$.dLck^{cre} mice have reduced innate immune responses upon acute viral infection

In order to demonstrate that Srsf1 deficiency in T cells affects innate immunity, we performed flow cytometry analysis of bone marrow and spleen myeloid cells in WT and Srsf1$^{fl/fl}$dLck^{cre} mice in their naive state or 24 h after LCMV infection. We found that Srsf1$^{fl/fl}$dLck^{cre} mice exhibited lower frequency of total CD11B^{+} myeloid cells in bone marrow (Fig. 4A). In the spleen, however, Srsf1$^{fl/fl}$dLck^{cre} mice exhibited significantly higher CD11B^{+} frequencies at naive state compared to their WT counterparts (Fig. 4B). The difference of CD11B^{+} cells in the bone marrow between Srsf1$^{fl/fl}$dLck^{cre} and WT was not altered 24 h after acute LCMV infection (Fig. 4A). The difference of splenic CD11B^{+} myeloid cells between Srsf1$^{fl/fl}$dLck^{cre} and WT was diminished 24 h after acute LCMV infection, as the frequency of CD11B^{+} myeloid cells doubled in WT mice but remained the same in Srsf1$^{fl/fl}$dLck^{cre} mice (Fig. 4B).

We then investigated the effect of SRSF1 deficient T cells in MDSCs by measuring the frequencies of CD11B^{+}Ly6G^{+}Ly6C^{lo} polymorphonuclear MDSCs (PMN-MDSC) and

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$CD11B^{+}Ly6G^{-}Ly6C^{lo}$ monocytic MDSCs (M-MDSC) in the bone marrow and spleen, using flow cytometric markers as previously described [44]. We observed that $Srsf1^{fl/fl}dLck^{cre}$ mice had higher frequencies of M-MDSC in the bone marrow and higher frequencies of PMN-MDSC in the spleen at naive state (Fig. 4C,D). However, 24 h after acute LCMV infection, unlike the WT mice, the splenic PMN-MDSC frequency of the $Srsf1^{fl/fl}dLck^{cre}$ mice failed to expand (Fig. 4E). Both WT and $Srsf1^{fl/fl}dLck^{cre}$ mice exhibited decreased PMN-MDSC frequency in the bone marrow 24 h after acute LCMV infection (Fig. 4F). Collectively our data suggest that ablation of SRSF1 in mature T cells affects innate immune responses.

3.5. SRSF1 is associated with human psoriasis and IL-17A

In order to understand the clinical implications of SRSF1 in the context of psoriasis, SRSF1 gene expression was evaluated in psoriasis lesional and healthy control skin. Two independently acquired datasets were used for this analysis and results are presented as dot-plots of log2 transformed read counts [23–25]. In each case, SRSF1 was markedly elevated in psoriasis skin compared to healthy controls (Fig. 5A). Moreover, SRSF1 gene expression was significantly and positively correlated with *IL17A* expression (Fig. 5B-D).

4. Discussion

SRSF1 is regulating adaptive and innate immune functions pertinent to the pathogenesis of autoimmune and autoinflammatory diseases. Since SRSF1 is a splicing factor it would be expected to contribute to autoimmunity by gene expression regulation through alternatively spicing of mRNAs [12,45–49]. However, in our case, although we observed elevated gene expression levels of *Il-17a* and *Il-17 f* in our transcriptomic analysis, we did not detect any difference in the splicing patterns between WT and the $Srsf1^{fl/fl}dLck^{cre}$ mice in IL-17A or IL-17F, or any of the transcription factors that regulate their expression. A recent study that looked at alternatively splicing events in the $Srsf1^{fl/fl}dLck^{cre}$ mice detected changes in IRF7 and IL-27RA but no indication on IL-17A or IL-17F [50]. These data suggest that the elevated *Il-17a* and *Il-17 f* mRNA levels might be regulated by other mechanisms independent of alternative splicing.

An increase of *Il-17a* and *Il-17 f* mRNA levels could also be achieved indirectly by inhibition of immune tolerance. Immune tolerance is achieved by multiple cell types including regulatory T cells (Tregs) and myeloid suppressor cells. Tregs play an important role in restraining skin inflammation [51], and psoriatic arthritis patients have low circulating Treg numbers [52]. Moreover, Treg-deficient mice exhibit autoimmune phenotypes that resemble symptoms of systemic autoimmunity with hyperkeratotic skin lesions [50,53]. Indeed, deficiency of SRSF1 in Tregs is associated with autoimmunity [8]. An effect on Tregs is highly plausible as the distal Lck promoter-driven Cre, also affects other cell types including innate-like T cells, including invariant NK T cells, mucosal-associated invariant T (MAIT) cells, and γδT cells [54]. In this study, however, we observed no difference in Treg frequency or $IL\text{-}17A^{+}$Tregs in $Srsf1^{fl/fl}dLck^{cre}$ mice. The discrepancy of IL-17A and IL-17F expression on the protein and mRNA level observed in this study supports previous findings that SRSF1 associates with translating ribosomes and

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stimulates translation [55,56]. Without SRSF1, the elevation in the expression of *Il-17a* and *Il-17 f* mRNA levels is diminished on the protein level. Since the changes in transcripts and proteins are not synchronized in the absence of SRSF1, proteomic studies are needed to comprehensively investigate the effect of SRSF1.

An additional mechanism that SRSF1 may break tolerance is via the inhibition of myeloid-derived suppressor cells (MDSCs), which are major negative regulators of immune responses. MDSCs are categorized into two groups of cells, termed polymorphonuclear (PMN-MDSCs) and monocytic (M-MDSCs), which are phenotypically and morphologically similar to neutrophils and monocytes, respectively [57]. MDSCs suppress the immune responses of $CD8^+$ T cells [20]. Our gene ontology pathway analysis showed many differentially expressed genes in the $Srsf1^{fl/fl}dLck^{cre}$ mice that are involved in innate immunity pathways including cytokine receptors, neutrophil, and monocyte chemotaxis pathways that are pertinent to the function of MDSCs [58]. Moreover, our experiments with 24-h acute LCMV infection highlighted the impaired immune response to viral challenge and clearly demonstrated that the SRSF1 splicing factor regulates autoimmune processes through indirect effects on other cell types to prolong CD8 responses leading to autoimmunity. These data suggest an additional mechanism by which SRSF1 contributes to chronic inflammation leading to autoimmunity.

In psoriatic patient skin lesions, SRSF1 gene expression is consistently elevated across different bulk RNA-seq datasets. This observation is positively correlated to the elevated expression of IL-17A in psoriatic lesions [59]. However, psoriatic lesional skins encompass a variety of resident cells, including keratinocytes, mesenchymal cells, myeloid, in addition to resident T cells. In psoriatic skin, inflammatory T cells are found in the dermis and CD8+ T cells and neutrophils are found in the epidermis [59]. The skin samples collected from healthy donors and psoriatic patients for the analyses of this study were extracted by a punch biopsy method, which collected epidermis, dermis and superficial fat. Due to this heterogeneity it is unclear the high expression of SRSF1 in which cell type(s) has a dominant effect in the pathogenesis of psoriasis. It is also unclear, whether the elevation of SRSF1 expression is the cause or the consequence of the disease. Nor is it clear whether SRSF1 plays a pathogenic or a protective role in the pathophysiology of the disease. Other paradoxical observations have also been reported in psoriasis studies. For example, the mRNA level of CXCL17 is significantly higher in psoriatic skin than in normal skin, but it has been found that CXCL17 can attenuate Imiquimod-induced psoriasis-like skin inflammation by recruiting MDSCs and Tregs [60]. Therefore, our study provided new evidence to the potential SRSF regulation of autoimmunity by immunosuppressive cells. Although the exact function of SRSF1 in these cells remains elusive, the pleiotropic functions of SRSF1 in motility, cell division, and activation of mTOR pathway have been implicated with psoriasis [61–63]. A more global effect on inflammatory signaling may be perturbated by the aberrant signaling of SRSF1.

In addition to being a splicing factor, SRSF1 also functions as a shuttling protein that exports mRNA from the nucleus to the cytoplasm [65]. Eukaryotic cells produce mRNA in the nucleus through a series of events including 5′ capping, 3′-end processing and splicing, which are coupled with transcription and exported from the nucleus to the cytoplasm where

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it can be translated to generate proteins. SRSF1 is found to reduce the nuclear export of inflammatory mRNAs [65]. It is therefore possible that the elevated *Il-17a* and *Il-17 f* expression we observed in the RNA-seq data was the result of increased nuclear export in the absence of Srsf1 rather than altered AS events [66].

Collectively, understanding the role of SRSF1 in IL-17A signaling will pave the way to a detailed understanding of the pathophysiology of psoriasis that can be explored to develop novel therapeutic approaches for our patients.

5. Conclusion

In this study, we observed elevated Il-17a and Il-17 f mRNA expression level and reduced innate immune response in T cell specific Srsf1 knockout mice. We did not detect significant AS events in these mice, suggesting SRSF1 might regulate inflammation via its role as a shuttling protein over splicing factor. Although there is a clear correlation between SRSF1 gene expression and psoriasis, the mechanisms by which SRSF1 contributes to the pathogenesis of psoriasis requires further investigation.

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References

[1]. Nestle FO, Kaplan DH, Barker J, Mechanisms of disease: psoriasis, N. Engl. J. Med 361 (2009) 496–509, 10.1056/NEJMra0804595. [PubMed: 19641206]

[2]. Miossec P, Korn T, Kuchroo VK, Interleukin-17 and type 17 helper T cells, N. Engl. J. Med 361 (2009) 888–898, 10.1056/nejmra0707449. [PubMed: 19710487]

[3]. Ghoreschi K, Balato A, Enerbäck C, Sabat R, Therapeutics targeting the IL-23 and IL-17 pathway in psoriasis, Lancet. 397 (2021) 754–766, 10.1016/S0140-6736(21)00184-7. [PubMed: 33515492]

[4]. Khan D, Ahmed SA, Regulation of IL-17 in autoimmune diseases by transcriptional factors and microRNAs, Front. Genet 6 (2015) 236, 10.3389/fgene.2015.00236. [PubMed: 26236331]

[5]. Bonnal SC, López-Oreja I, Valcárcel J, Roles and mechanisms of alternative splicing in cancer — implications for care, Nat. Rev. Clin. Oncol 178 (17) (2020) 457–474, 10.1038/s41571-020-0350-x.

[6]. Mailer RKW, Joly A-L, Liu S, Elias S, Tegner J, Andersson J, IL-1β promotes Th17 differentiation by inducing alternative splicing of FOXP3, Sci. Rep 51 (5) (2015) 1–9, 10.1038/srepl4674.

[7]. Katsuyama T, Li H, Comte D, Tsokos GC, Moulton VR, Splicing factor SRSF1 controls T cell hyperactivity and systemic autoimmunity, J. Clin. Invest 129 (2019) 5411–5423, 10.1172/JCI127949. [PubMed: 31487268]

[8]. Katsuyama T, Moulton VR, Splicing factor SRSF1 is indispensable for regulatory T cell homeostasis and function, Cell Rep. 36 (2021), 109339, 10.1016/J.CELREP.2021.109339. [PubMed: 34233194]

[9]. Wang Y, Liu J, Huang B, Xu Y, Li J, Huang L, Lin J, Zhang J, Min Q, Yang W, Wang X, Mechanism of alternative splicing and its regulation, Biomed. Reports 3 (2015) 152–158, 10.3892/br.2014.407.

[10]. Scotti MM, Swanson MS, RNA mis-splicing in disease, Nat. Rev. Genet 17 (2016) 19–32, 10.1038/nrg.2015.3. [PubMed: 26593421]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

[11]. Graveley BR, Maniatis T, Arginine/serine-rich domains of SR proteins can function as activators of pre-mRNA splicing, Mol. Cell 1 (1998) 765–771, 10.1016/SI097-2765(00)80076-3. [PubMed: 9660960]

[12]. Sun Q, Mayeda A, Hampson RK, Krainer AR, Rottman FM, General splicing factor SF2/ASF promotes alternative splicing by binding to an exonic splicing enhancer, Genes Dev. 7 (1993) 2598–2608, 10.1101/GAD.7.12B.2598. [PubMed: 8276242]

[13]. Das S, Krainer AR, Emerging functions of SRSF1, splicing factor and oncoprotein, in RNA metabolism and cancer, Mol. Cancer Res 12 (2014) 1195–1204, 10.1158/1541-7786.MCR-14-0131. [PubMed: 24807918]

[14]. Fregoso OI, Das S, Akerman M, Krainer AR, Splicing-factor Oncoprotein SRSF1 stabilizes p53 via RPL5 and induces cellular senescence, Mol. Cell 50 (2013) 56–66, 10.1016/j.molcel.2013.02.001. [PubMed: 23478443]

[15]. Lemaire R, Prasad J, Kashima T, Gustafson J, Manley JL, Lafyatis R, Stability of a PKCI-1-related mRNA is controlled by the splicing factor ASF/SF2: a novel function for SR proteins, Genes Dev. 16 (2002) 594–607, 10.1101/gad.939502. [PubMed: 11877379]

[16]. Katsuyama T, Martin-Delgado IJ, Krishheld SM, Kyttaris VC, Moulton VR, Splicing factor SRSF1 controls T cell homeostasis and its decreased levels are linked to lymphopenia in systemic lupus erythematosus, Rheumatology (Oxford) 59 (2020) 2146–2155, 10.1093/rheumatology/keaa094. [PubMed: 32206811]

[17]. Sun D, Novotny M, Bulek K, Liu C, Li X, Hamilton T, Treatment with IL-17 prolongs the half-life of chemokine CXCL1 mRNA via the adaptor TRAF5 and the splicing-regulatory factor SF2 (ASF), Nat. Immunol 12 (2011) 853–860, 10.1038/ni.2081. [PubMed: 21822258]

[18]. Swaidani S, Liu C, Zhao J, Bulek K, Li X, TRAF regulation of IL-17 cytokine signaling, Front. Immunol 10 (2019) 1293, 10.3389/fimmu.2019.01293. [PubMed: 31316496]

[19]. Gabrilovich DI, Nagaraj S, Myeloid-derived suppressor cells as regulators of the immune system, Nat. Rev. Immunol 9 (2009) 162–174, 10.1038/nri2506. [PubMed: 19197294]

[20]. Norris BA, Uebelhoer LS, Nakaya HI, Price AA, Grakoui A, Pulendran B, Chronic but not acute virus infection induces sustained expansion of myeloid suppressor cell numbers that inhibit viral-specific T cell immunity, Immunity. 38 (2013) 309–321, 10.1016/j.immuni.2012.10.022. [PubMed: 23438822]

[21]. Zeng Y, Li Y, Xu Z, Gan W, Lu L, Huang X, Lin C, Myeloid-derived suppressor cells expansion is closely associated with disease severity and progression in HBV-related acute-on-chronic liver failure, J. Med. Virol 91 (2019) 1510–1518, 10.1002/JMV.25466. [PubMed: 30905084]

[22]. Moulton VR, Gillooly AR, Perl MA, Markopoulou A, Serine arginine-rich splicing factor 1 (SRSF1) contributes to the transcriptional activation of CD3ζ in human T cells, PLoS One 10 (2015), e0131073, 10.1371/journal.pone.0131073. [PubMed: 26134847]

[23]. Li B, Tsoi LC, Swindell WR, Gudjonsson JE, Tejasvi T, Johnston A, Ding J, Stuart PE, Xing X, Kochkodan JJ, Voorhees JJ, Kang HM, Nair RP, Abecasis GR, Elder JT, Transcriptome analysis of psoriasis in a large case-control sample: RNA-seq provides insights into disease mechanisms, J. Invest. Dermatol 134 (2014) 1828–1838, 10.1038/JID.2014.28. [PubMed: 24441097]

[24]. Tsoi LC, Iyer MK, Stuart PE, Swindell WR, Gudjonsson JE, Tejasvi T, Sarkar MK, Li B, Ding J, Voorhees JJ, Kang HM, Nair RP, Chinnaiyan AM, Abecasis GR, Elder JT, Analysis of long non-coding RNAs highlights tissue-specific expression patterns and epigenetic profiles in normal and psoriatic skin, Genome Biol. 16 (2015) 1–15, 10.1186/S13059-014-0570-4/TABLES/3. [PubMed: 25583448]

[25]. Tsoi LC, Rodriguez E, Degenhardt F, Baurecht H, Wehkamp U, Volks N, Szymczak S, Swindell WR, Sarkar MK, Raja K, Shao S, Patrick M, Gao Y, Uppala R, Perez White BE, Getsios S, Harms PW, Maverakis E, Elder JT, Franke A, Gudjonsson JE, Weidinger S, Atopic dermatitis is an IL-13-dominant disease with greater molecular heterogeneity compared to psoriasis, J. Invest. Dermatol 139 (2019) 1480–1489, 10.1016/JJID.2018.12.018. [PubMed: 30641038]

[26]. Andrews S, Krueger F, Seconds-Pichon A, Biggins F, Wingett S, FastQC. A quality control tool for high throughput sequence data. Babraham Bioinformatics, Babraham Inst. 1 (2015) 1. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/ (accessed January 24, 2022).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

[27]. Martin M, Cutadapt removes adapter sequences from high-throughput sequencing reads, EMBnet.Journal 17 (2011) 10, 10.14806/ej.17.l.200.

[28]. Conesa A, Madrigal P, Tarazona S, Gomez-Cabrero D, Cervera A, McPherson A, Szcześniak MW, Gaffney DJ, Elo LL, Zhang X, Mortazavi A, A survey of best practices for RNA-seq data analysis, Genome Biol. 171 (17) (2016) 1–19, 10.1186/S13059-016-0881-8.

[29]. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR, STAR: ultrafast universal RNA-seq aligner, Bioinformatics. 29 (2013) 15–21, 10.1093/BIOINFORMATICS/BTS635. [PubMed: 23104886]

[30]. Liao Y, Smyth GK, Shi W, featureCounts: an efficient general purpose program for assigning sequence reads to genomic features, Bioinformatics. 30 (2014) 923–930, 10.1093/BIOINFORMATICS/BTT656. [PubMed: 24227677]

[31]. Love MI, Huber W, Anders S, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2, Genome Biol. 15 (2014) 1–21, 10.1186/S13059-014-0550-8/FIGURES/9.

[32]. Yu G, Wang LG, Han Y, He QY, clusterProfiler: an R package for comparing biological themes among gene clusters, OMICS. 16 (2012) 284–287, 10.1089/OMI.2011.0118. [PubMed: 22455463]

[33]. Storey JD, Tibshirani R, Statistical significance for genomewide studies, Proc. Natl. Acad. Sci 100 (2003) 9440–9445, 10.1073/PNAS.1530509100. [PubMed: 12883005]

[34]. Anders S, Reyes A, Huber W, Detecting differential usage of exons from RNA-seq data, Genome Res. 22 (2012) 2008–2017, 10.1101/GR.133744.111. [PubMed: 22722343]

[35]. Reyes A, Anders S, Weatheritt RJ, Gibson TJ, Steinmetz LM, Huber W, Drift and conservation of differential exon usage across tissues in primate species, Proc. Natl. Acad. Sci. U. S. A 110 (2013) 15377–15382, 10.1073/PNAS.1307202110/-/DCSUPPLEMENTAL. [PubMed: 24003148]

[36]. Wickham H, ggplot2: create elegant data visualisations using the grammar of graphics, Wiley Interdiscip, Rev. Comput. Stat 3 (2011) 180–185. https://ggplot2.tidyverse.org/ (accessed January 24, 2022).

[37]. Gu Z, Eils R, Schlesner M, Complex heatmaps reveal patterns and correlations in multidimensional genomic data, Bioinformatics. 32 (2016) 2847–2849, 10.1093/BIOINFORMATICS/BTW313. [PubMed: 27207943]

[38]. Garrido-Martín D, Palumbo E, Guigó R, Breschi A, Ggsashimi: sashimi plot revised for browser- and annotation-independent splicing visualization, PLoS Comput. Biol 14 (2018), e1006360, 10.1371/journal.pcbi.1006360. [PubMed: 30118475]

[39]. Xu X, Yang D, Ding JH, Wang W, Chu PH, Dalton ND, Wang HY, Bermingham JR, Ye Z, Liu F, Rosenfeld MG, Manley JL, Ross J, Chen J, Xiao RP, Cheng H, Fu XD, ASF/SF2-regulated CaMKIIδ alternative splicing temporally reprograms excitation-contraction coupling in cardiac muscle, Cell. 120 (2005) 59–72, 10.1016/j.cell.2004.ll.036. [PubMed: 15652482]

[40]. Zhang DJ, Wang Q, Wei J, Baimukanova G, Buchholz F, Stewart AF, Mao X, Killeen N, Selective expression of the Cre recombinase in late-stage Thymocytes using the distal promoter of the Lck gene, J. Immunol 174 (2005) 6725–6731, 10.4049/JIMMUNOL.174.ll.6725. [PubMed: 15905512]

[41]. Laurence A, Tato CM, Davidson TS, Kanno Y, Chen Z, Yao Z, Blank RBB, Meylan F, Siegel R, Hennighausen L, Shevach EM, O'Shea JJJ, Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation, Immunity 26 (2007) 371–381, 10.1016/J.IMMUNI.2007.02.009. [PubMed: 17363300]

[42]. Lazarevic V, Chen X, Shim JH, Hwang ES, Jang E, Bolm AN, Oukka M, Kuchroo VK, Glimcher LH, T-bet represses TH 17 differentiation by preventing Runx1-mediated activation of the gene encoding RORγt, Nat. Immunol 12 (2011) 96–104, 10.1038/ni.1969. [PubMed: 21151104]

[43]. van Hamburg JP, de Bruijn MJW, Ribeiro de Almeida C, van Zwam M, van Meurs M, de Haas E, Boon L, Samsom JN, Hendriks RW, Enforced expression of GATA3 allows differentiation of IL-17-producing cells, but constrains Th17-mediated pathology, Eur. J. Immunol 38 (2008) 2573–2586, 10.1002/EJI.200737840. [PubMed: 18792410]

[44]. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, Mandruzzato S, Murray PJ, Ochoa A, Ostrand-Rosenberg S, Rodriguez PC, Sica A, Umansky V, Vonderheide RH,

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Gabrilovich DI, Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards, Nat. Commun 71 (7) (2016) 1–10, 10.1038/ncommsl2150.

[45]. Caceres JF, Krainer AR, Functional analysis of pre-mRNA splicing factor SF2/ASF structural domains, EMBO J. 12 (1993) 4715–4726, 10.1002/j.1460-2075.1993.tb06160.x. [PubMed: 8223480]

[46]. Das S, Anczuków O, Akerman M, Krainer AR, Oncogenic splicing factor SRSF1 is a critical transcriptional target of MYC, Cell Rep. 1 (2012) 110–117, 10.1016/j.celrep.2011.12.001. [PubMed: 22545246]

[47]. Anczuków O, Akerman M, Cléry A, Wu J, Shen C, Shirole NH, Raimer A, Sun S, Jensen MA, Hua Y, Allain FHT, Krainer AR, SRSF1-regulated alternative splicing in breast Cancer, Mol. Cell 60 (2015) 105–117, 10.1016/j.molcel.2015.09.005. [PubMed: 26431027]

[48]. Zhou X, Wang R, Li X, Yu L, Hua D, Sun C, Shi C, Luo W, Rao C, Jiang Z, Feng Y, Wang Q, Yu S, Splicing factor SRSF1 promotes gliomagenesis via oncogenic splice-switching of MYO1B, J. Clin. Invest 129 (2019) 676–693, 10.1172/JCU20279. [PubMed: 30481162]

[49]. Qi Z, Wang F, Yu G, Wang D, Yao Y, You M, Liu J, Liu J, Sun Z, Ji C, Xue Y, Yu S, SRSF1 serves as a critical posttranscriptional regulator at the late stage of thymocyte development, Sci. Adv 7 (2021) eabf0753, 10.1126/SCIADV.ABF0753. [PubMed: 33863728]

[50]. Sharma R, Sung SSJ, Fu SM, Te Ju S, Regulation of multi-organ inflammation in the regulatory T cell-deficient scurfy mice, J. Biomed. Sci. 16 (2009) 20, 10.1186/1423-0127-16-20. [PubMed: 19272184]

[51]. Hartwig T, Zwicky P, Schreiner B, Yawalkar N, Cheng P, Navarini A, Dummer R, Flatz L, Conrad C, Schlapbach C, Becher B, Regulatory T cells restrain pathogenic T helper cells during skin inflammation, Cell Rep. 25 (2018) 3564–3572.e4, 10.1016/J.CELREP.2018.12.012. [PubMed: 30590032]

[52]. Wang J, Zhang SX, Hao YF, Qiu MT, Luo J, Li YY, Gao C, Li XF, The numbers of peripheral regulatory T cells are reduced in patients with psoriatic arthritis and are restored by low-dose interleukin-2, Ther. Adv. Chronic Dis 11 (2020), 10.1177/2040622320916014, 2040622320916014.

[53]. Hadaschik EN, Wei X, Leiss H, Heckmann B, Niederreiter B, Steiner G, Ulrich W, Enk AH, Smolen JS, Stummvoll GH, Regulatory T cell-deficient scurfy mice develop systemic autoimmune features resembling lupus-like disease, Arthritis Res. Ther 17 (2015) 35, 10.1186/sl3075-015-0538-0. [PubMed: 25890083]

[54]. Figueroa MG, Parker LM, Krol K, Zhao M, Distal Lck promoter-driven Cre shows cell type-specific function in innate-like T cells, ImmunoHorizons. 5 (2021) 772–781, 10.4049/IMMUNOHORIZONS.2100079. [PubMed: 34583938]

[55]. Sanford JR, Gray NK, Beckmann K, Cáceres JF, A novel role for shuttling SR proteins in mRNA translation, Genes Dev. 18 (2004) 755–768, 10.1101/GAD.286404. [PubMed: 15082528]

[56]. Michlewski G, Sanford JR, Cáceres JF, The splicing factor SF2/ASF regulates translation initiation by enhancing phosphorylation of 4E-BP1, Mol. Cell 30 (2008) 179–189, 10.1016/J.MOLCEL.2008.03.013. [PubMed: 18439897]

[57]. Gabrilovich DI, Myeloid-derived suppressor cells, Cancer, Immunol. Res 5 (2017) 3–8, 10.1158/2326-6066.CIR-16-0297. [PubMed: 28052991]

[58]. Veglia F, Sanseviero E, Gabrilovich DI, Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity, Nat. Rev. Immunol 218 (21) (2021) 485–498, 10.1038/s41577-020-00490-y.

[59]. Schön MP, Boehncke W-H, Psoriasis, NEJM 352 (2009) 1899–1912, 10.1056/NEJMRA041320.

[60]. Oka T, Sugaya M, Takahashi N, Takahashi T, Shibata S, Miyagaki T, Asano Y, Sato S, CXCL17 attenuates Imiquimod-induced psoriasis-like skin inflammation by recruiting myeloid-derived suppressor cells and regulatory T cells, J. Immunol 198 (2017) 3897–3908, 10.4049/JIMMUNOL.1601607. [PubMed: 28389593]

[61]. Ghigna C, Giordano S, Shen H, Benvenuto F, Castiglioni F, Comoglio PM, Green MR, Riva S, Biamonti G, Cell motility is controlled by SF2/ASF through alternative splicing of the Ron protooncogene, Mol. Cell 20 (2005) 881–890, 10.1016/J.MOLCEL.2005.10.026. [PubMed: 16364913]

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[62]. Maslon MM, Heras SR, Bellora N, Eyras E, Cáceres JF, The translational landscape of the splicing factor SRSF1 and its role in mitosis, Elife. 3 (2014), e02028, 10.7554/ELIFE.02028. [PubMed: 24842991]

[63]. Cibrian D, de la Fuente H, Sánchez-Madrid F, Metabolic pathways that control skin homeostasis and inflammation, Trends Mol. Med 26 (2020) 975–986, 10.1016/J.MOLMED.2020.04.004. [PubMed: 32371170]

[65]. Zhou H, Bulek K, Li X, Herjan T, Yu M, Qian W, Wang H, Zhou G, Chen X, Yang H, Hong L, Zhao J, Qin L, Fukuda K, Flotho A, Gao J, Dongre A, Carman JA, Kang Z, Su B, Kern TS, Smith JD, Hamilton TA, Melchior F, Fox PL, Li X, IRAK2 directs stimulus-dependent nuclear export of inflammatory mRNAs, Elife. 6 (2017), e29630, 10.7554/eLife.29630. [PubMed: 28990926]

[66]. Huang Y, Gattoni R, Stévenin J, Steitz JA, SR splicing factors serve as adapter proteins for TAP-dependent mRNA export, Mol. Cell 11 (2003) 837–843, 10.1016/SI097-2765(03)00089-3. [PubMed: 12667464]

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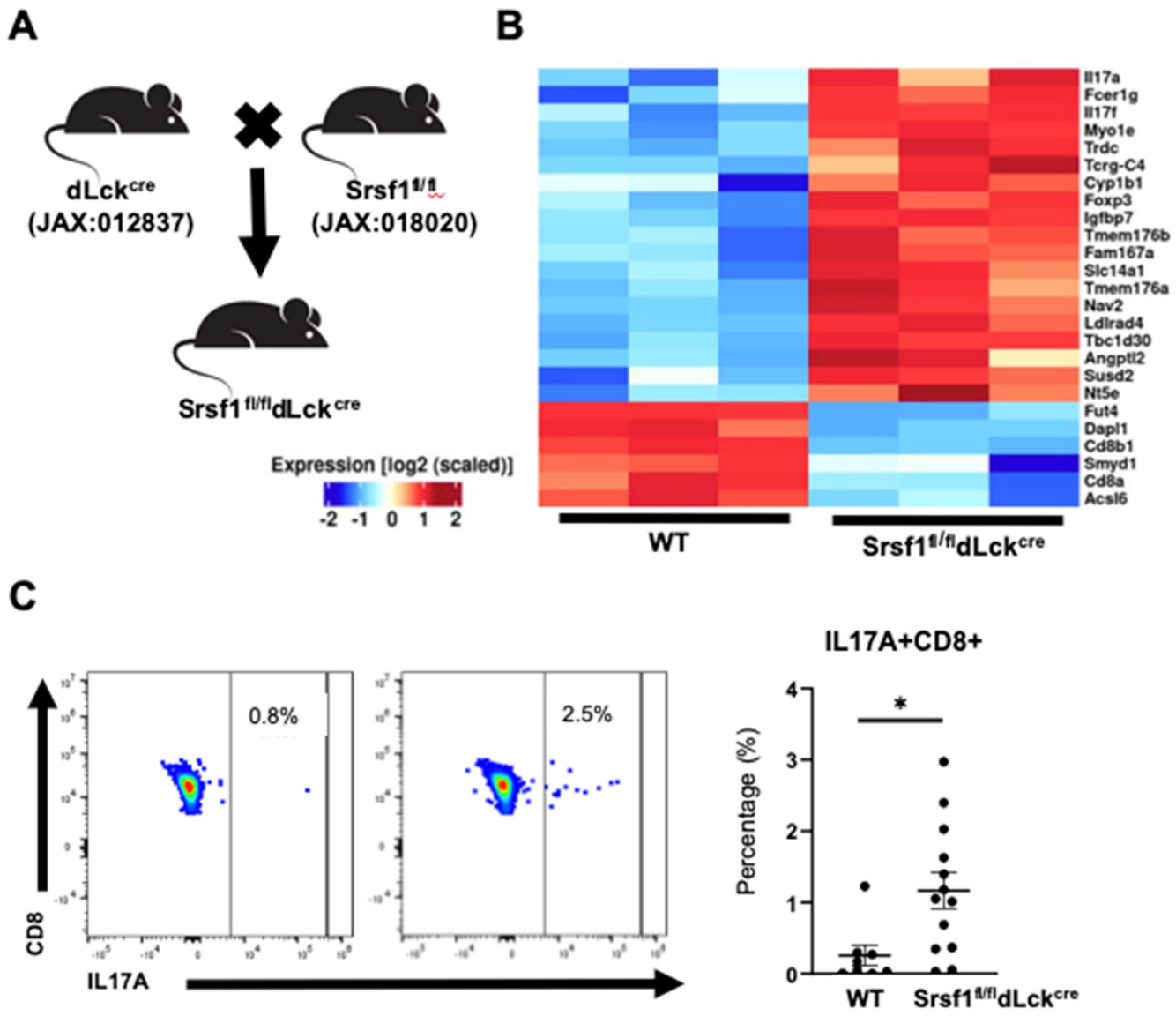

Fig. 1.

Ablation of Srsf1 in T cells increases the mRNA expression and protein levels of IL-17A. (A) Schematic representation showing the strategy for generating the mouse model (Srsf1$^{fl/fl}$dLck^{cre}). (B) Heatmap of differential expressed genes showing top 25 significantly differentially expressed genes in CD4^{+}CD25^{hlgh}CD127^{low} nTregs from the splenocytes of WT and Srsf1$^{fl/fl}$dLck^{cre} mice (n = 3) by RNA-seq data (GSE 173268). (C) Representative pseudocolor plots showing IL-17A^{+} CD8^{+} T cells from the spleens. (WT: n = 8, Srsf1$^{fl/fl}$dLck^{cre}: n = 13). The percentage of respective population of each group is shown on the right. Student t-test, unpaired, nonparametric. * $p < 0.05$).

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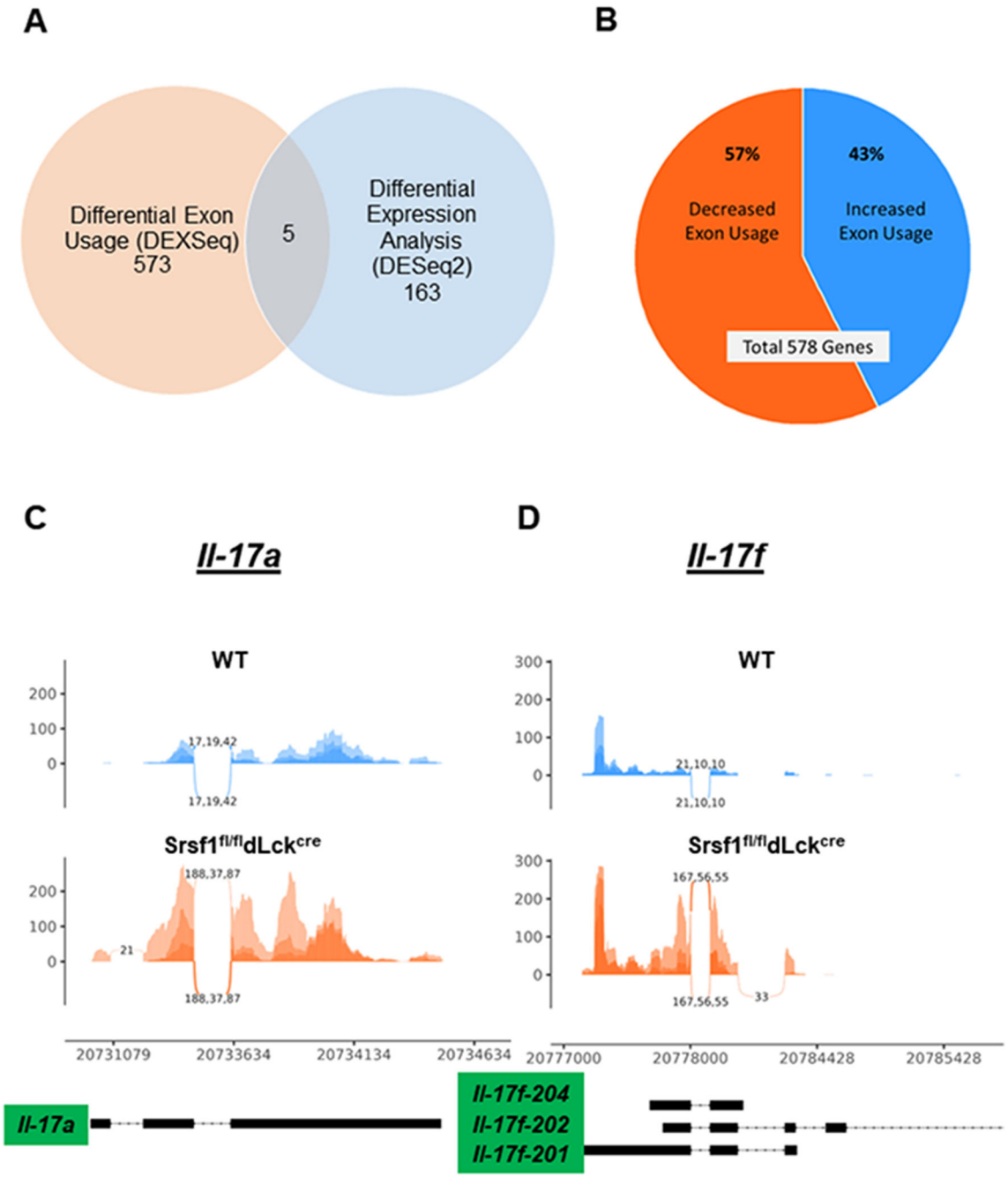

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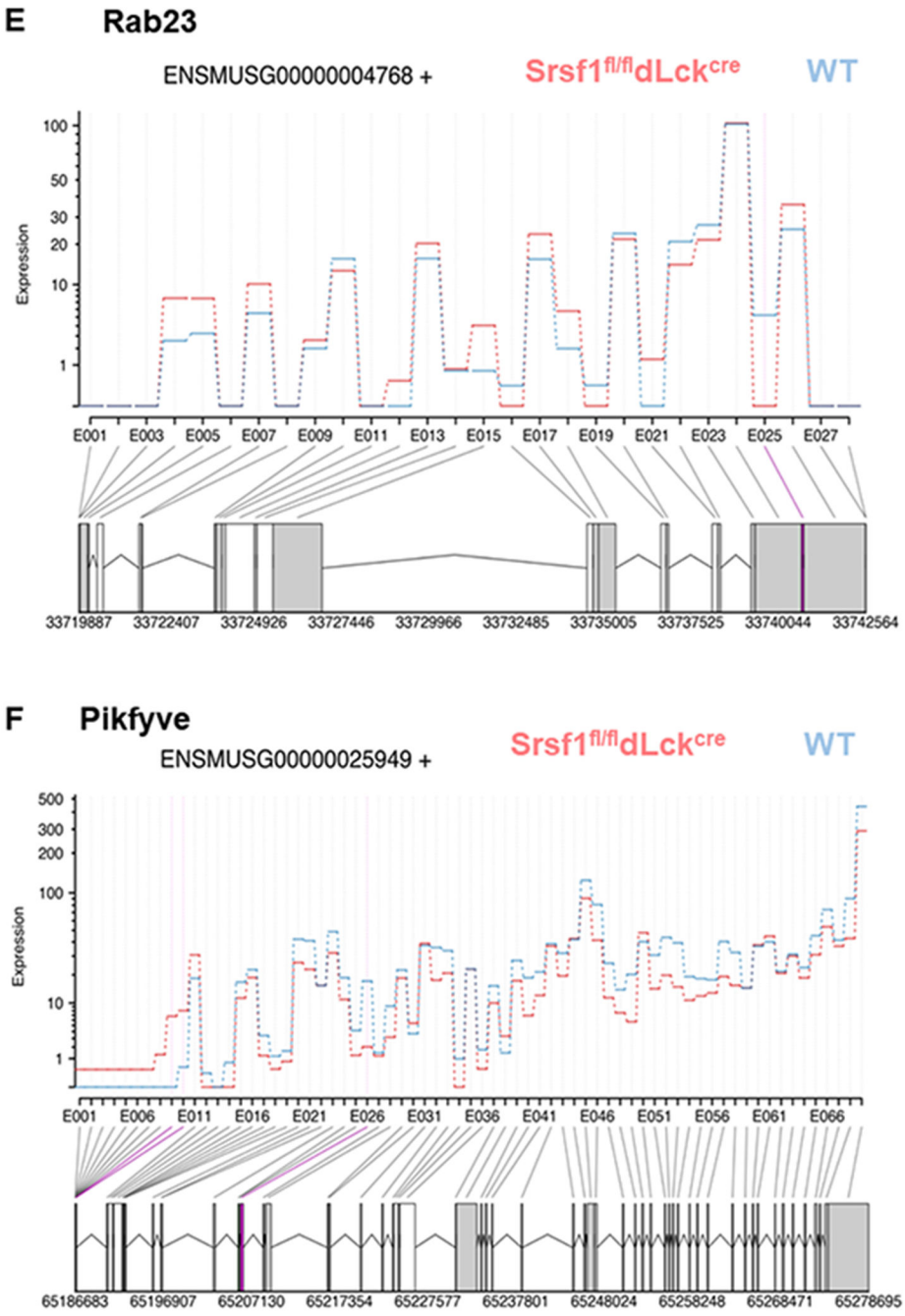

Fig. 2.

Alternative splicing does not regulate the expression of IL-17a/IL-17 f in Srsf1$^{fl/fl}$dLck^{cre} mice. (A) Venn diagram shows the overlap between differentially expressed genes and genes with differential exon usage. (B) Pie chart shows the proportion of genes with increased or decreased exon usage out of a total of 578 genes with differential usage of exons in Srsf1$^{fl/fl}$dLck^{cre} (FDR < 0.1). (C—D) Sashimi plots show read coverage and exon-exon junctions (numbers on arches indicate junction reads) of Il-17a (C) and Il-17 f (D). (E-F)

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Plots showing differential exon usage in *Rab23* (E) and *Pikfyve* (F) between WT and $Srsf1^{fl/fl}dLck^{cre}$.

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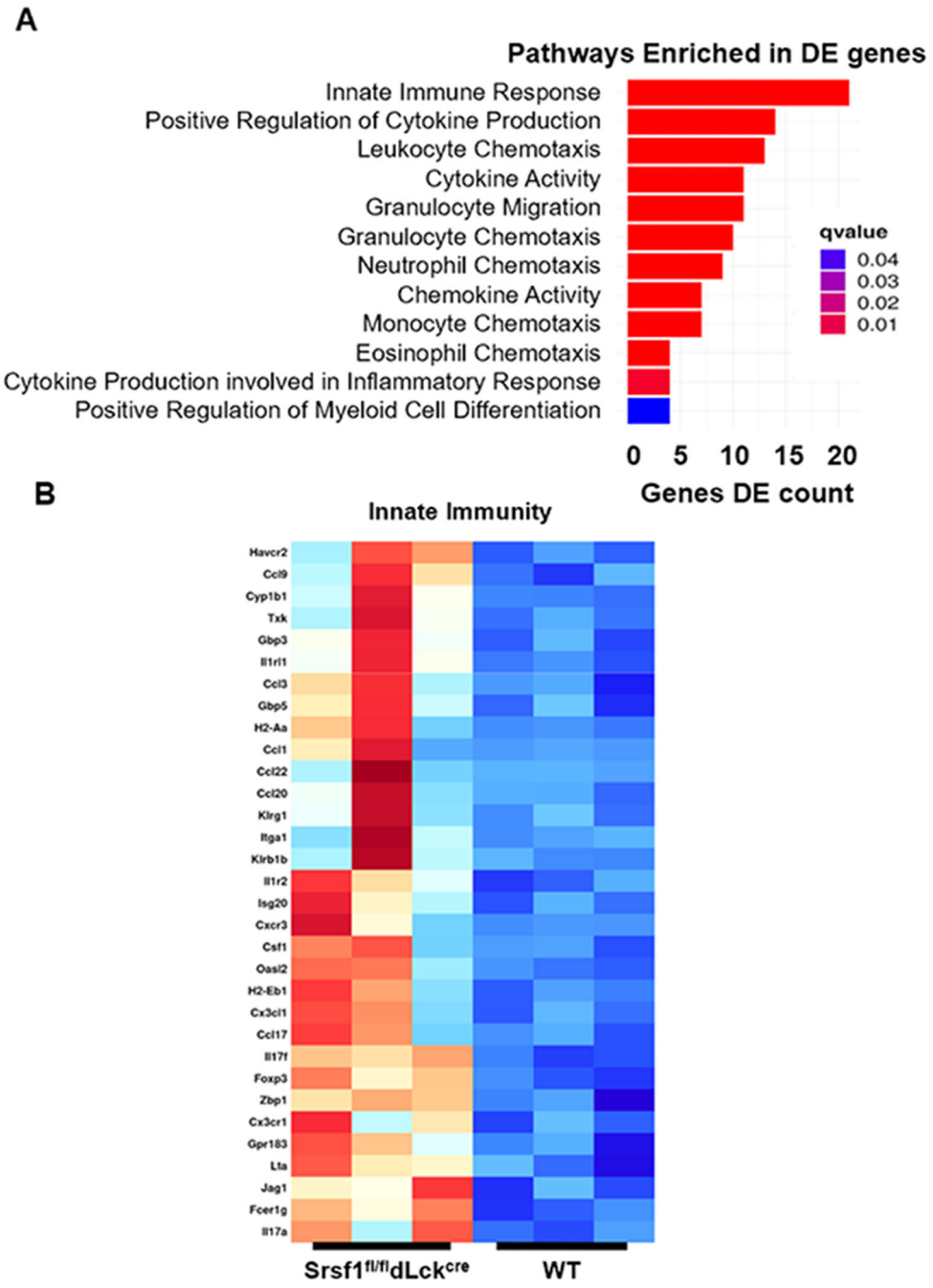

Fig. 3.

Srsf1$^{fl/fl}$.dLck^{cre} Treg cells possess distinct gene signatures associated with myeloid cells. (A) Top enriched innate immune pathways of differentially expressed genes (DEGs) between WT and Srsf1$^{fl/fl}$dLck^{cre}. (B) Heatmaps showing DEGs related to innate immunity.

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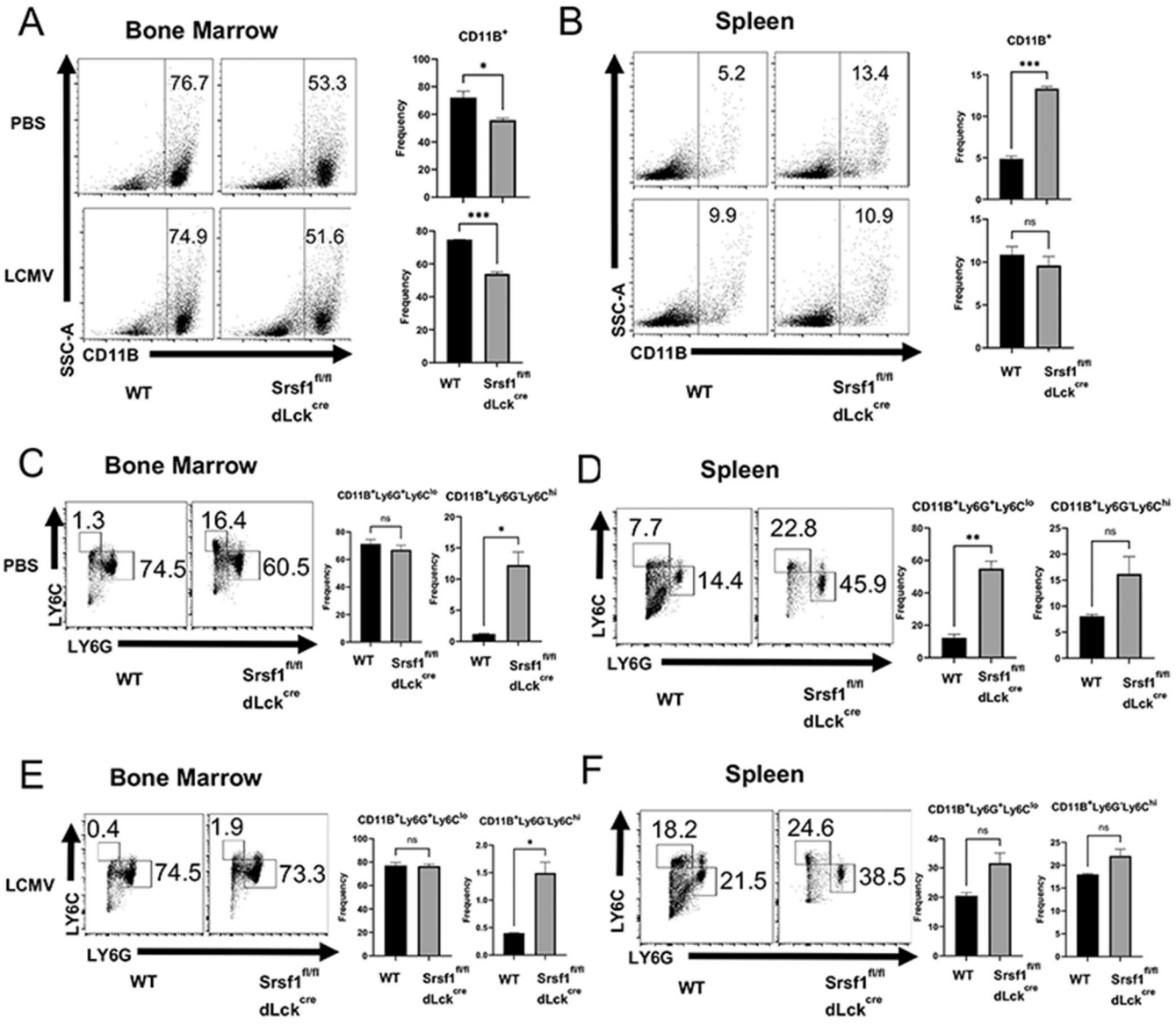

Fig. 4.

Srsf1 deficiency in T cells affects innate immune cell distribution and migration. Dot plots and graphs showing the populations of $CD11B^+$ myeloid cells in bone marrow and spleen of WT and $Srsf1^{fl/fl}dLck^{cre}$ mice in naive state (A, B) and 24 h after acute LCMV infection (C, D). Dot plots and graphs showing the populations of PMN-MDSC ($CD11b^+Ly6G^+Ly6C^{lo}$) and M-MDSC ($CD11b^+Ly6G^-Ly6C^{lo}$) in the bone marrow and spleen of WT and $Srsf1^{fl/fl}dLck^{cre}$ mice in naive state (E, F) and 24 h after acute LCMV infection (G, H). Experiments were performed twice with at least 3 mice per group. Student t-test, unpaired, *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$).

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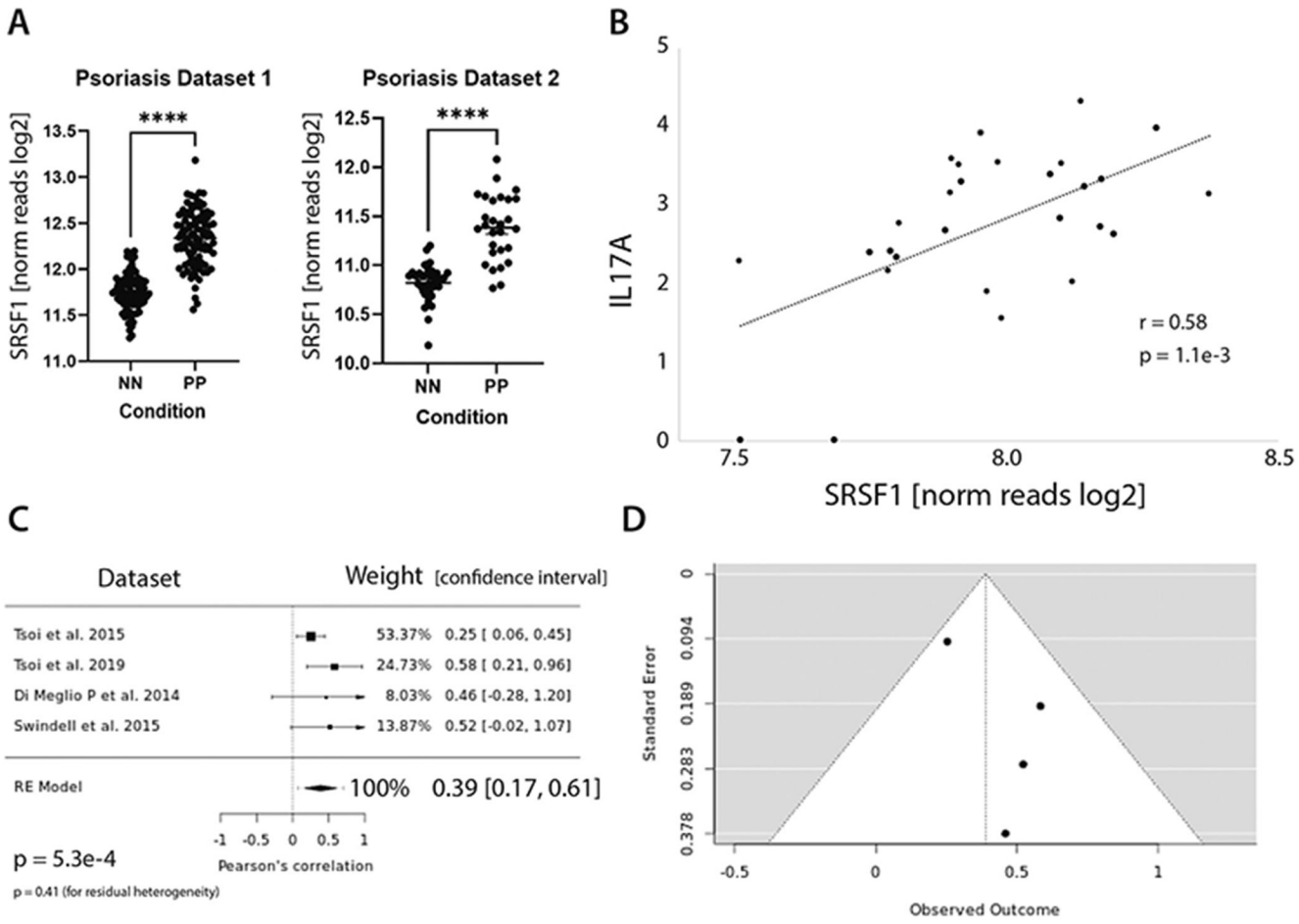

Fig. 5.

SRSF1 gene expression is associated with human psoriasis and IL-17A. A) Graphs comparing SRSF1 gene expression (normalized log2 reads) in the skin biopsies of Psoriatic Patients (PP) and Non-lesional Normal skin biopsies of healthy donors (NN) from two independent studies. (Student t-test, unpaired, ****, $P < 0.0001$.) B) representative scatter plot of SRSF1 association with *IL17A* C) Forest and D) Funnel plots of meta-analysis across all four RNAseq datasets.