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Splicing factor SRSF1 controls distinct molecular programs in regulatory and effector T cells implicated in systemic autoimmune disease

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Abstract

Systemic autoimmune diseases are characterized by hyperactive effector T cells (Teffs), aberrant cytokines and chemokines, and dysfunctional regulatory T cells (Tregs). We previously uncovered new roles for serine/arginine-rich splicing factor 1 (SRSF1) in the control of genes involved in T cell signaling and cytokine production in human T cells. SRSF1 levels are decreased in T cells from patients with systemic lupus erythematosus (SLE), and low levels correlate with severe disease. Moreover, T cell-conditional *Srsf1*-deficient mice recapitulate the autoimmune phenotype, exhibiting CD4 T cell hyperactivity, dysfunctional Tregs, systemic autoimmunity, and tissue inflammation. However, the role of SRSF1 in controlling molecular programs in Teffs and Tregs and how these pathways are implicated in autoimmunity is not known. Here, by comparative bioinformatics analysis, we demonstrate that SRSF1 controls largely distinct gene programs in Tregs and Teffs in vivo. SRSF1 regulates 189 differentially expressed genes (DEGs) unique to Tregs, 582 DEGs unique to Teffs, and 29 DEGs shared between both. Shared genes included IL-17A, IL-17F, CSF1, CXCL10, and CXCR4, and were highly enriched for inflammatory response and cytokine-cytokine receptor interaction pathways. SRSF1 controls distinct pathways in Tregs, which include chemokine signaling and immune cell differentiation, compared with pathways in Teffs, which include cytokine production, T cell homeostasis, and activation. We identified putative mRNA binding targets of SRSF1 which include CSF1, CXCL10, and IL-17F. Finally, comparisons with transcriptomics profiles from lupus-prone MRL/lpr mice reveal that SRSF1 controls genes and pathways implicated in autoimmune disease. The target genes of SRSF1 and putative binding targets we discovered, have known roles in systemic autoimmunity. Our findings suggest that SRSF1 controls distinct molecular pathways in Tregs and Teffs and

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aberrant SRSF1 levels may contribute to their dysfunction and immunopathogenesis of systemic autoimmune disease.

Keywords

Autoimmune disease; transcriptomics; bioinformatics; gene expression; SRSF1; regulatory T cells; effector T cells

1. Introduction

Autoimmune diseases result from a loss of self-tolerance, leading to the attack of healthy cells and tissues by the immune system. One classic example is systemic lupus erythematosus (SLE), characterized by systemic autoimmunity and multi-organ damage that can manifest as arthritis, skin disease, blood cell abnormalities, and renal involvement (1). SLE patients exhibit aberrant T cell function and signaling and overproduction of inflammatory cytokines including IL-17 (2). Furthermore, IL-2 is necessary for the maintenance of properly functioning regulatory T cells (Tregs); the reduced production thereof in SLE leads to Treg dysfunction and an inability to attenuate the immune response (2). Compounding this issue is the finding that CD4 effector T cells (Teffs) from SLE patients are intrinsically less sensitive to suppression by Tregs either from the same diseased individual or healthy donors (3) and exhibit hyperactivity. This imbalance of Teff and Treg cell subsets extends to T helper (Th) 17 cells. SLE patients are known to exhibit increased Th17/Treg cell ratios in both quiescent disease and during flares (4,5), and this ratio correlates with disease severity (6). Yet the molecules responsible for Treg dysfunction, aberrant cytokine production, and T cell subset imbalances in systemic autoimmune diseases are not fully known. Better understanding of the molecular controls in Teffs and Tregs in autoimmune diseases is necessary for the development of biomarkers and improved therapeutic targets.

Treg dysfunction and their inability to suppress inflammation is observed in a number of autoimmune diseases and is often attributed to the plasticity of Tregs and their altered migration to tissues. Treg plasticity can be defined as the re-differentiation of Tregs into other cell subsets, notably with the acquisition of pro-inflammatory properties (7). Th1-like Tregs, identified by expression of T-box transcription factor TBX21 (T-bet), highly express interferon gamma (IFN-γ), C-X-C motif chemokine receptor 3 (CXCR3), and C-C motif chemokine receptor 5 (CCR5). Binding of CXCR3 and its ligands induces integrin activation and chemotaxis, which permits leukocyte extravasation to inflamed tissues. Likewise, CCR5, found on T cells and macrophages, is the receptor for several chemokines. Th1 -like Tregs are involved in multiple sclerosis (MS), type 1 diabetes, and SLE (7). Alternatively, Th2-like Tregs express IL-4, IL-5, and IL-13, which are involved in B cell differentiation and immunoglobulin class switching to IgE, class switching to IgA, and allergic responses respectively. The Th2-like Treg cell subset demonstrates activity in collagen-induced arthritis (CIA) and systemic sclerosis (SSc) (7). Finally, Th17-like Tregs, primarily induced by IL-6, express IL-17 and are involved in the pathogenesis of rheumatoid arthritis (RA), MS, psoriasis, and SSc (7). Elucidation of gene expression in

Treg lineages and other T cell subsets in such diseases is pivotal for understanding their immunopathogenesis and potential therapeutic targets.

Chemokine signaling is crucial for proper migration of immune cells to target tissues, including Tregs to ameliorate tissue inflammation and maintain organ-specific homeostasis (8,9). Aberrant chemokine/receptor expression and dysfunctional chemokine signaling can lead to impaired migration of Tregs and coupled with altered/inflammatory phenotype of Tregs contribute to tissue inflammation. There is immune cell imbalance in chronic inflammatory autoimmune diseases and SLE, including cutaneous SLE in which Tregs are the major T cell subset in the skin (10). Indeed, SLE patients exhibit impaired Treg migration via altered signaling of CCR4 and its ligands CCL17 and CCL22 (11), leading to the inability to suppress tissue inflammation. However, molecular control over chemokine signaling molecules in SLE and other autoimmune diseases remains unresolved.

Serine/arginine-rich splicing factor 1 (SRSF1) is the prototype member of the SR family of splicing factors known for its role in posttranscriptional gene regulation, including control of alternative splicing and regulation of mRNA decay and translation (12). We have uncovered new roles for SRSF1 in the control of genes involved in T cell signaling and function, and its relevance in SLE (13–17). We have shown that SRSF1 levels are decreased in T cells from SLE patients (15,16), and low levels associate with severe disease and comorbidities (15,18,19). In addition, overexpression of SRSF1 can rescue deficient IL-2 production in T cells from SLE patients (15). We have shown that deletion of SRSF1 exclusively in T cells in mice yields a lupus-like phenotype; mice develop T cell hyperactivity with increased frequencies of inflammatory cytokine producing Teffs (20,21). Furthermore, these mice develop systemic autoimmunity and lupus nephritis (20). Besides its role in Teffs, SRSF1 is essential for the homeostasis and function of Tregs, and mice with Treg-conditional deficiency of *Srsf1* exhibit an early lethal systemic autoimmune disease phenotype with multi-organ inflammation (22). These studies reveal that low levels of SRSF1 lead to T cell dysfunction-mediated pathogenesis of systemic autoimmune disease. However, the differential role of SRSF1 in the molecular control of Teffs and Tregs is not known.

In this study, we investigated the gene programs and molecular pathways controlled by SRSF1 in Tregs and Teffs. Using RNA-sequencing (RNA-seq) transcriptomics datasets (20,22) and subsequent comparative bioinformatics differential gene expression analysis, we found unique gene programs controlled by SRSF1 in each T cell subset. To further our understanding of the molecular implications of these genes, we conducted pathway analysis using Metascape (23) and created protein-protein interaction (PPI) networks with the bioinformatics tools Cytoscape and MCODE (24,25). We then probed the RNA-binding protein (RBP) database oRNAment to discover new putative RNA binding targets of SRSF1 (26), in order to determine specific genes controlled by SRSF1. Finally, our comparison to transcriptomics data from MRL/lpr mice (27) allowed us to evaluate the relevance of SRSF1 in controlling gene programs that mediate autoimmunity. Our data demonstrate that SRSF1 controls distinct key molecular pathways underlying the effector and regulatory functions of T cells implicated in systemic autoimmune disease. Therefore, SRSF1 may be a potential molecular target and correcting its expression may offer a therapeutic strategy for autoimmune diseases.

2. Materials and Methods

2.1. Transcriptomics profiling datasets from Srsf1-ko mice

We used previously generated RNA-sequencing transcriptomics profiling data from Tregs and Teffs from T cell-conditional *Srsf1-ko* mice (20,22). Briefly, Teffs were generated by stimulating naïve CD4 T cells from spleens of T cell-conditional *Srsf1-ko* or control WT (n=3) mice for 72h with CD3/CD28 antibodies (20). $CD4^{+}CD25^{+}CD127^{lo}$ natural Tregs (nTregs) were sorted by flow cytometry and stimulated for 72h with CD3/CD28 antibodies and recombinant IL-2 (22). Total RNA from Tregs and Teffs was submitted for RNA-seq to the Molecular Biology Core Facility (MBCF) at the Dana-Farber Cancer Institute (DFCI). Libraries were prepared using Roche Kapa mRNA HyperPrep sample preparation kits from 100ng of purified total RNA according to the manufacturer's protocol. The finished dsDNA libraries were quantified by Qubit fluorometer, Agilent TapeStation 2200, and RT-qPCR using the Kapa Biosystems library quantification kit according to manufacturer's protocols. Uniquely indexed libraries were pooled in equimolar ratios and sequenced on an Illumina NextSeq500 with single-end 75bp reads. Sequenced reads were aligned to the UCSC mm9 reference genome assembly and gene counts were quantified using STAR v2.7.3a (28).

2.2. Transcriptomics profiling dataset from MRL/lpr mice

We used the previously generated RNA-seq dataset of splenic CD4 T cells from the MRL/lpr mouse (27) under the identifier GSE139283 from the NCBI GEO database. Our RNA-seq dataset of Teffs from WT B6–129 mice (20) was used as a control for differential expression analysis conducted in R (29).

2.3. Comparative bioinformatics analysis of transcriptomics data

Differential expression analysis of raw counts was conducted using the DESeq2 v1.22.1 (30) and SummarizedExperiment v1.22.0 (31) packages in R. RNA-seq analysis was performed using the VIPER snakemake pipeline (32). To compare the differentially expressed genes (DEGs) derived from RNA-seq data from Tregs and Teffs, we used the MIT comparison tool BaRC (http://barc.wi.mit.edu/tools/compare). Next, pathway enrichment analysis for mutually enriched genes and genes unique to each T cell subset was performed using Metascape (23). We included gene ontology (GO) biological processes and Kyoto encyclopedia of genes and genomes (KEGG) pathways in the criteria and required a statistical significance of $p<0.05$. Protein-protein interaction (PPI) networks were constructed for both shared and unique DEGs in Cytoscape (24) with the MCODE application to determine clusters of highly interconnected regions (25). Default parameters were used for MCODE, with the exception of setting the node score cutoff to 0.3 to include more nodes in the cluster. We queried RNA targets of SRSF1 using oRNAment, a database of putative RNA-binding protein (RBP) binding sites. oRNAment uses a computational algorithm to survey putative RBP target motifs across coding mRNAs and non-coding RNAs (26). We cross-referenced our list of DEGs to their database of RBP binding sites to obtain a list of putative binding sites of SRSF1.

2.4. Heatmaps

All heatmap figures were created using the ComplexHeatmap v2.8.0 package in R (29,33).

2.5. Statistical Analysis

Student's two-tailed t-test was used to calculate statistical significance for DEGs among groups. For RNA-seq data, p-values were adjusted for false discovery rate (FDR) using the Benjamini-Hochberg procedure. Significance testing for pathway analysis was done using Metascape (23). A p-value of less than 0.05 was considered significant.

3. Results

3.1. SRSF1 controls largely distinct transcriptomics profiles in Tregs and Teffs

To determine the role of SRSF1 in effector and regulatory T cell subsets, we used comparative bioinformatics strategies to evaluate transcriptomics profiles of Tregs and Teffs from T cell-conditional *Srsf1-knockout (ko)* mice (20,22). Our criteria for differentially expressed genes (DEGs) required a statistical significance of $p_{adj}<0.05$ and gene expression fold change (FC) $|\log_2 FC>1|$ compared to control genes from wild-type mice. There were a total of 218 DEGs identified in Tregs (22) and 612 DEGs in Teffs (20) (Figure 1A). Our comparative analysis revealed 189 DEGs unique to Tregs, 582 DEGs unique to Teffs, and 29 mutually enriched genes across both cell types. To better understand the expression and magnitude of regulation, we created a heatmap of all DEGs. The majority of DEGs were up-regulated in Tregs (Figure 1B), suggesting the normal function of SRSF1 as an inhibitory regulator of mRNA expression in that cell subset. However, there was a nearly equal number of up- and down-regulated genes in Teffs (Figure 1B), suggesting that the function of SRSF1 is not as one-sided and includes both activator and repressor roles. Across both cell types, genes were regulated with similar magnitudes, shown by their $\log_2 FC$. Our data also show that the majority of mutually enriched genes (19/29 genes) are regulated in the same direction in both Tregs and Teffs (Figure 1B). Ranking these 29 shared genes revealed RPL22L1, H2-AA, SERPINE1, SLC2A6, and TNIP3 as the highest ranked according to average |t-statistic| (Table 1).

To discriminate any possible confounding effects from cell stimulation alone, we performed the same differential gene expression analysis across four comparisons: (1) unstimulated WT Tregs vs stimulated WT Tregs, (2) unstimulated *Srsf1-ko* Tregs vs stimulated *Srsf1-ko* Tregs, (3) unstimulated WT naïve CD4 T cells vs stimulated WT Teffs, and (4) unstimulated *Srsf1-ko* naïve CD4 T cells vs stimulated *Srsf1-ko* Teffs. By comparing DEGs between comparison groups [(1) and (2)] and [(3) and (4)], we can determine the relative effect of stimulation alone on differential gene expression. Our data revealed that the majority of DEGs (2946 genes) between unstimulated and stimulated Tregs are shared across WT and *Srsf1-ko* strains (Supplementary Figure 1A). These 2946 DEGs also seem to be regulated in a very similar direction across strains (Supplementary Figure 1B). Likewise, the majority of DEGs (3809 genes) between unstimulated and stimulated Teffs are shared across WT and *Srsf1-ko* strains (Supplementary Figure 2A) and are regulated in a similar direction (Supplementary Figure 2B). These results indicate that differences in gene expression between the WT and *Srsf1-ko* are more likely mediated by SRSF1, rather than cell

stimulation alone. In other words, the effects seen from cell stimulation are consistent across WTs and KOs for each cell subset.

Further evidence for this clarification can been seen by looking at pathway enrichment. DEGs unique to WT Tregs and WT Teffs mostly corresponded to pathways involved in immune cell activation, migration, homeostasis, and signaling (Supplementary Figure 1C, 2C). DEGs unique to KO Tregs and KO Teffs mostly comprised pathways related to DNA/RNA modification and expression (Supplementary Figure 1C, 2C). This is consistent with the function of SRSF1 as an RNA-binding protein involved in mRNA splicing and translation.

3.2. Mutually enriched genes in Srsf1-ko Tregs and Teffs represent inflammatory pathways

Our next goal was to analyze the pathways that are constituted by the 29 mutually enriched genes shared between *Srsf1-ko* Tregs and Teffs. We identified pathways that were significantly represented, along with genes contributing to each pathway. We included GO biological processes and KEGG pathways in the pathway enrichment analysis and required a statistical significance of $p<0.05$. “Cytokine-cytokine receptor interaction” and “inflammatory response” were the top two highly enriched pathways according to p-value (Figure 2A). The remaining pathways pertained to other aspects of the immune response, such as leukocyte differentiation, regulation of defense response, and response to external stimuli. Narrowing our scope, we analyzed pathway enrichment for the 15 mutually up-regulated DEGs between Tregs and Teffs. “Regulation of leukocyte differentiation” and “inflammatory response” were the top two highly enriched pathways (Figure 2B). Analysis of the four mutually down-regulated DEGs yielded no significant pathway enrichment. To gain insight into the cooperativity between these 29 mutually enriched genes, we created a protein-protein interaction (PPI) network with clustering using Cytoscape and MCODE (24,25). One cluster consisted of the cytokines IL-17A, IL-17F, colony stimulating factor 1 (CSF1), C-X-C motif chemokine ligand 10 (CXCL10), and C-X-C chemokine receptor type 6 (CXCR6) (Figure 2C). Boxplots of the transcripts per kilobase million (TPM) for the shared genes shown in the PPI have been included as an example (Figure 2D).

3.3. SRSF1 controls cytokines and immune cell differentiation molecules in Tregs distinct from Teffs

A major aim of this investigation was to explore the transcriptomic differences between Tregs and Teffs to identify molecular functions of SRSF1 within these distinct subsets. To accomplish this, we used Metascape (23) to discover pathways enriched by DEGs unique to Tregs (189 genes) and Teffs (582 genes). Implementing the same criteria as before, we found only two overlapping pathways between Tregs and Teffs and 18 pathways unique to each cell subset (Figure 3A). Pathways related to cytokines and the immune response were most heavily represented once again. This indicates that SRSF1 regulates different pathways in cellular immunity within Tregs and Teffs. Our results demonstrate that Tregs more prominently mediate immune cell proliferation and differentiation, while Teffs have a slight predisposition for metabolic and homeostatic pathways. We then selected the top 30 DEGs by p-value for each cell type. Overall, the top 30 genes demonstrated a similar proportion of

up- to down-regulated genes as the entire DEG set (Figure 3B). Across both cell types, these top 30 unique genes encapsulated different cytokines, receptors, transcription factors for cell cycle/differentiation, and cell adhesion molecules.

Creating PPI networks with clustering allowed us to evaluate any interactions between the highly expressed DEGs. Tregs showed one cluster containing the chemokines/receptors C-C motif chemokine 22 (CCL22) and C-X-C motif chemokine receptor 3 (CXCR3), along with integrin subunit alpha E (ITGAE/CD103) (Figure 3B, 3C). The *Srsf1-ko* in Tregs also down-regulated interleukin 12 receptor, beta 2 subunit (IL-12RB2). Within the top 30 unique DEGs and PPI cluster for Teffs was the up-regulation of the key Th2 cytokines IL-4, IL-5, and IL-13 (Figure 3B, 3C). The same Teff cluster contained C-C motif chemokine receptor 5 (CCR5) and C-C motif chemokine receptor 7 (CCR7).

3.4. The majority of identified DEGs are potential binding sites of SRSF1

One goal of this investigation was to discover new potential target sites of the RNA-binding protein (RBP) SRSF1, which would expand our knowledge of its molecular function and role in pathology. We queried oRNAment, a database of putative RBP binding sites, for the DEGs from our studies. The database calculates a matrix similarity score (MSS), ranging from 0 to 1, which serves as a proxy for the confidence of RBP-RNA interaction (26). An MSS of 1 indicates that the RNA transcript contains the canonical binding motif of the given RBP. We retrieved the MSS's for our respective DEGs from the oRNAment database of potential binding sites of SRSF1 in *M. musculus*. Without any restriction to the MSS, we found that 174/189 genes and 500/582 genes unique to Tregs and Teff respectively were potential binding sites of SRSF1 (Figure 4A, 4B). Even using stringent guidelines by restricting the MSS to 1 (that is, only mapping RNAs that contain the canonical binding motif), we classified 141/189 genes and 410/582 genes unique to Tregs and Teff respectively as putative targets of SRSF1 (Figure 4A, 4B).

Our next objective was to query oRNAment for mutually enriched genes. We reasoned that if genes are differentially expressed in both cell types, this would strongly suggest that SRSF1 indeed binds to these mRNAs. Implementing the same criteria as above, we established that 19/29 mutually enriched genes are putative binding sites (MSS=1) of SRSF1 (Figure 4C). More specifically, nearly half of these 19 putative targets are up-regulated in both *Srsf1-ko* Tregs and Teffs (Figure 4D), indicating that SRSF1 normally inhibits these mRNAs. These up-regulated genes include cytokines that were described previously such as CSF1, CXCL10, and IL-17F. Another putative target was sulfatase 2 (SULF2), a heparan endosulfatase which modulates heparan sulfate proteoglycans (HSPGs) that act as co-receptors for cytokine signaling. Although unknown, it is likely that SULF2 may have the ability to amplify downstream effects of cytokines. We also note the presence of interleukin 1 receptor like 1 (IL1RL1), the receptor for IL-33 that mediates activation of nuclear factor kappa B (NF-κB), mitogen-activated protein (MAP) kinases, and Th2 cytokine production (34). Moreover, granzyme C (GZMC) was an up-regulated putative binding site, most likely involved in inducing apoptosis of target cells. Finally, to understand how these genes may be inter-regulated, we created a PPI network of our 19 putative targets of SRSF1. We found that 6/19 putative targets of SRSF1 interact with each other at the protein level,

including CSF1, CXCL10, and IL-17F (Figure 4E). We have encountered these three genes repeatedly throughout our study, suggesting that they are likely directly regulated by SRSF1 and mediate the T cell dysfunction and contribute to the pathogenesis of disease observed in the *Srsf1-ko* mice.

3.5. SRSF1 controls molecular programs implicated in autoimmune disease

To validate the relevance of our findings to autoimmune disease, we evaluated our SRSF1-controlled DEGs by drawing comparisons to gene profiles from CD4 T cells from the well-studied lupus-prone MRL/lpr mice. Publicly available RNA-seq data from MRL/lpr mice (27) were compared to CD4 Teff cells from control WT mice to determine DEGs enriched in the MRL/lpr mice. We then compared these MRL/lpr DEGs to our *Srsf1-ko* CD4 Teffs DEGs to evaluate the transcriptomic profiles controlled by SRSF1 that are relevant in autoimmune disease pathophysiology. We found that just over half (307) of the DEGs from *Srsf1-ko* Teffs are shared with splenic CD4 T cells from the MRL/lpr strain (Figure 5A). These 307 shared DEGs were generally regulated in the same direction, however MRL/lpr CD4 T cells exhibited a greater magnitude of fold change (Figure 5B). Some of these genes included the up-regulation of CSF1, CXCR6, and CXCL10 which were consistently differentially expressed across both *Srsf1-ko* Teff and Tregs. CCR5 was also mutually up-regulated between MRL/lpr CD4 T cells and *Srsf1-ko* Teffs.

We verified our pathway enrichment by comparing *Srsf1-ko* Teffs to MRL/lpr CD4 T cells once again. The 307 shared DEGs yielded “inflammatory response” and “cytokine-cytokine receptor interaction” as the top two enriched pathways (Figure 5C). These were the same top two pathways among the 29 shared DEGs between *Srsf1-ko* Tregs and Teffs. Other pathways pertained to leukocyte differentiation, regulation of the immune response, regulation of cell killing, and regulation of biosynthetic processes. Overall, shared genes between the *Srsf1-ko* and MRL/lpr mice revealed pathways that overlapped with both *Srsf1-ko* Teffs and Tregs. These data suggest that SRSF1 may be a key regulator of gene programs in CD4 T cells implicated in autoimmunity.

4. Discussion

In this study, we examined the role of splicing SRSF1, whose low levels in T cells are implicated in the pathogenesis of SLE (15,18–20). Specifically, we evaluated Tregs and Teffs from T cell conditional *Srsf1-ko* mice, using bioinformatics strategies to compare transcriptomic profiles in these two subsets. We found that although SRSF1 regulates few genes which are common to both Tregs and Teffs (Figure 1), SRSF1 mainly regulates distinct gene programs and pathways in each cell subset (Figure 1, 3B). *Srsf1-ko* Tregs are enriched for genes corresponding to pathways of chemokine-signaling cytokines, immune cell differentiation, and immune responses (Figure 3A). *Srsf1-ko* Teffs are enriched for pathways in cytokine signaling and activation, but also contain some homeostatic and metabolic pathways (Figure 3A).

Our results shed light on several genes regulated by SRSF1 and thus may be potential binding sites of this RBP. In the context of the 29 mutually enriched DEGs across Tregs and Teffs, our PPI network revealed the cluster of IL-17A, IL-17F, CSF1, CXCL10, and

CXCR6 (Figure 2C). IL-17A-producing Tregs are pathogenic in several inflammatory diseases such as psoriasis, inflammatory bowel disease (IBD), and SLE (35). IL-17F is often co-produced with IL-17A and the two have some redundancy in their downstream effects (36). Importantly, IL-17 is known to be elevated in the serum and kidneys of SLE patients (37,38), illustrating the congruency between *Srsf1-ko* mice and human SLE patients. CXCL10 and CXCR6 are both chemotactic for lymphocytes and have been shown to be significantly elevated in the serum of SLE patients (39) and urinary CD4 T cells in lupus nephritis (40).

In Tregs, we described the PPI cluster of CCL22, CXCR3, and ITGAE (Figure 3C). CXCR3 is the receptor for CXCL10, which we found to be up-regulated in both *Srsf1-ko* Tregs and Teffs and was deemed a putative binding site of SRSF1 (Figure 4D). CXCR3 has been shown to be expressed on the majority of skin-infiltrating CD4 and CD8 T cells in SLE (41). Likewise, CXCR3-inducing chemokines are produced locally, leading to the recruitment of T cells to those inflammatory lesions (41). Next, ITGAE (CD103) is expressed on Tregs that migrate to and are retained in inflamed tissues (42). We noted the down-regulation of IL12RB2 as well, which normally plays a role in Th1 differentiation. This change may indicate the ability of Tregs to induce a rampant Th2 phenotype in *Srsf1-ko* mice, which is similar to the pathogenesis of SLE (43). Perhaps the most interesting candidate for pharmacotherapy in SLE could be CCL22. Although its role in SLE remains largely unresolved, serum CCL22 is increased in patients with rheumatoid arthritis (44). Importantly, recombinant CCL22 down-regulates FoxP3 and decreases the number of Tregs, while anti-CCL22 antibody yields the opposite effects (44). These findings, along with our data, suggest SRSF1 as a mediator of dysregulation of CCL22 in autoimmune disease and CCL22 as a potential therapeutic target.

Among the DEGs unique to *Srsf1-ko* Teffs, we noted the up-regulation and PPI cluster containing the Th2 cytokines IL-4, IL-5, and IL-13 (Figure 3C). These seem to be major stimulators of the Th2 immune response in SLE. Specifically, IL-4 is known to provide positive regulation of Th2 differentiation, B cell differentiation, and class-switching to IgE, while IL-13 is involved in allergic processes (45). Within the same cluster was CCR5, a chemokine receptor present on T cells and macrophages, and CCR7, a chemokine receptor involved in homing of T cells to lymphoid tissue (46). CCR5 was up-regulated in the *Srsf1-ko* and CCR7 was down-regulated, likely permitting Teffs to migrate out of lymphoid organs and into inflamed tissues. Although CCR7 does not necessarily correlate with disease activity, CCR7 expression in $CD4^{+}CD95^{+}$ T cells does correlate with antibody immune responses and is associated with ANA positivity (47). However, we noticed the down-regulation of CCR7 in our Teffs and, alternatively, $CD4^{+}CD95^{+}CCR7^{-}$ T cells are associated with cell-mediated inflammatory responses and inflammatory cytokines (47). Insight into this interesting dichotomy may help illuminate the pathogenesis of SLE. With respect to CCR5, the fraction of $CD4^{+}CCR5^{+}$ T cells correlates with carotid atherosclerosis in SLE patients independently of disease severity (48). Therefore, this molecule may be a potentially useful biomarker to stratify patients in terms of cardiovascular risk or even prophylactically intervene.

Finally, we ascertained several mRNAs that are likely to be binding sites of SRSF1. The most promising may be CSF1, a crucial regulator in macrophage differentiation, especially in steady-state microglia and renal macrophages (49). Renal failure still remains one of the top causes of death in SLE patients, with increased CSF1 expression being found in those with lupus nephritis (50). To combat this, the testing of interventional options has been initiated. A CSF1R kinase inhibitor has been shown to alleviate renal and neuropsychiatric disease (depression-like behavior) in MRL/lpr mice (51). Thus, our data contribute evidence that this molecule holds hope as a useful biomarker and pharmacologic target. Another gene of interest as a binding site of SRSF1 that we found is CXCL10, which is actually implicated similarly to CSF1. Serum levels are increased and correlate with SLE disease activity and possibly with renal involvement, while intrathecal levels are elevated in neuropsychiatric SLE (41). Despite this knowledge, the mechanism of control of CXCL10 remains unclarified. Our study suggests SRSF1 may be a direct regulator of CXCL10.

While evaluating our results and their relevance to autoimmune disease using RNA-seq data from well-characterized lupus-prone mice, we discovered that approximately half of the DEGs from *Srsf1-ko* Teffs are shared with MRL/lpr splenic CD4 T cells (Figure 5A). This suggests that SRSF1 controls gene programs implicated in autoimmune disease and the *Srsf1-ko* mice share similarities with mice strains that develop autoimmune disease, but that the *Srsf1-ko* strain still demonstrates unique mechanisms of autoimmunity.

While our data point to the regulatory role of SRSF1 in both CD4 Teffs and Tregs and in controlling key molecular pathways to autoimmunity, we require validation of our RNA-seq data through RT-qPCR assays. It would be advantageous in the future to conduct these assays for highly enriched genes in *Srsf1-ko* Tregs and Teffs, genes shared with the lupus-prone mice, as well as the genes with potential binding sites of SRSF1. To further solidify these mRNAs as binding sites, and even discover additional sites, performing RNA immunoprecipitation (RIP)-Seq, cross-linking immunoprecipitation (CLIP)-Seq, or photoactivatable ribonucleoside-enhanced (PAR)-CLIP experiments would pinpoint these interactions.

Overall, our data indicate the distinct role of SRSF1 in Tregs and Teffs and implications in the molecular mechanisms underlying the pathogenesis of systemic autoimmune disease. The deficiency of SRSF1 in both cell subsets largely induces mediators of inflammation and cytokine-receptor interaction, suggesting its role as a brake for T cell hyperactivity. Yet while the gene programs unique to Teffs mainly represent cytokine production, those in the *Srsf1-ko* Tregs promote chemokines and aspects of immune cell differentiation implying an altered plasticity of Tregs. Probing of mutually enriched DEGs in the oRNAment database allowed us to discover highly promising binding targets of SRSF1. When validating our results with the lupus-prone mice, we found a large overlap in DEGs between *Srsf1-ko* Teffs and MRL/lpr CD4 T cells, including the consistent up-regulation of CSF1, CXCR6, and CXCL10. Shared DEGs between these two strains contribute to pathways involved in inflammatory processes, cytokine-receptor interactions, leukocyte differentiation, and biosynthetic processes, depicting the relevance of SRSF1 in molecular mechanisms leading to autoimmunity. Many of these consistently enriched genes and prospective binding targets are already known to have roles in SLE pathogenesis or potential treatment and therefore

indicate that low SRSF1 levels observed in SLE patients may dysregulate a number of genes thus contributing to pathogenesis of SLE. In conclusion, SRSF1 controls multiple molecular and cellular pathways to autoimmunity and may be a useful therapeutic target.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Bioinformatics reveals that SRSF1 controls discrete gene programs in effector and regulatory T cell subsets
- SRSF1 regulates chemokine signaling and immune cell differentiation pathways in regulatory T cells
- SRSF1 regulates cytokine production, T cell homeostasis, and activation in effector T cells
- SRSF1 controls gene programs that are implicated in systemic autoimmunity
- SRSF1 controls distinct cellular and molecular pathways to autoimmunity and may be a potential molecular target for therapy

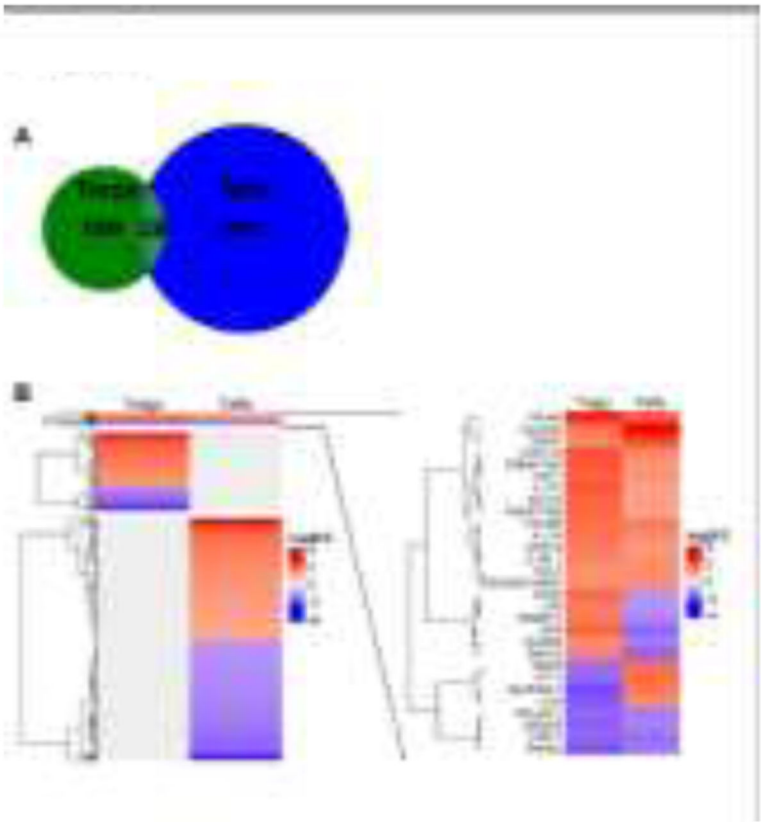

Figure 1. SRSF1 controls largely distinct transcriptomics profiles in Tregs and Teffs

(**A**) Unique and mutually enriched DEGs from RNA-seq data from WT (n=3) and KO (n=3) mice for Teffs and WT (n=3) and KO (n=3) mice for Tregs, with the criteria $p_{adj}<0.05$ and $|\log_2 FC>1|$. Data show genes differentially expressed in the KO mice compared to WT as controls.

(**B**) Heatmap of $\log_2 FC$ of DEGs from RNA-seq data. Data is split by mutually enriched genes, genes unique to Tregs, and genes unique to Teffs. Common genes are expanded (right) with hierarchical clustering.

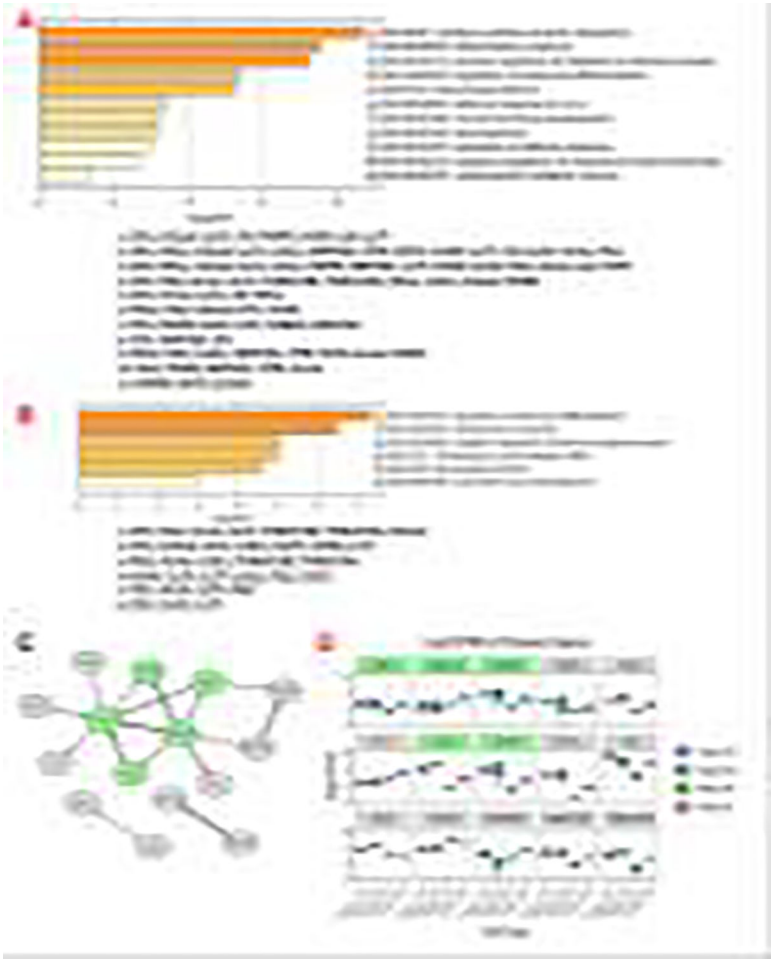

Figure 2. Mutually enriched genes in Srsf1-ko Tregs and Teffs represent inflammatory pathways

(**A**) Biological pathway analysis using GO and KEGG terms for 29 mutually enriched genes between Tregs and Teffs performed using Metascape with $p<0.05$. Genes contributing to each pathway are listed below the figure.

(**B**) Biological pathway analysis using GO and KEGG terms for 15 mutually up-regulated genes between Tregs and Teffs performed using Metascape with $p<0.05$. Genes contributing to each pathway are listed below the figure.

(**C**) PPI of 29 common genes using Cytoscape. Singleton genes are not shown. Cluster demonstrating a highly interconnected network is shown in green using MCODE. Directionality of interaction represented by arrow tail (source gene) and arrow head (target gene). Strength of interaction represented by arrow edge thickness.

(**D**) Boxplots of $\log_2$(TPM) for the 15 genes contained in the PPI in Figure 2C. Genes comprising the MCODE cluster are shown with a green background.

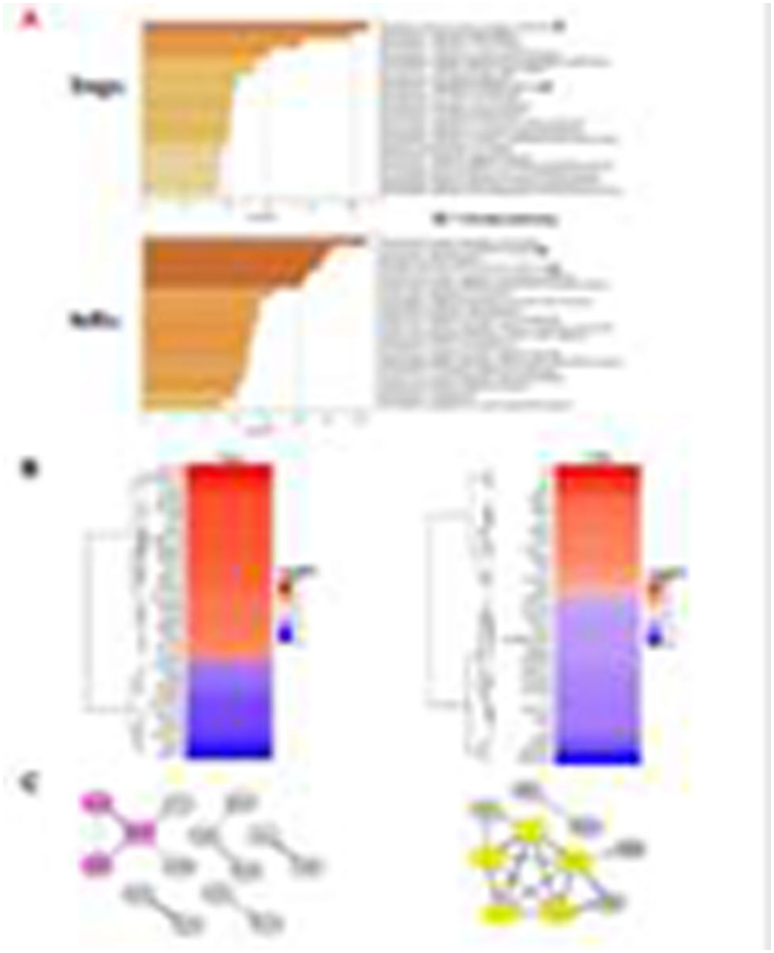

Figure 3. SRSF1 controls cytokines and immune cell differentiation molecules in Tregs distinct from Teffs

(**A**) Heatmap of pathway analysis derived from DEGs unique to Tregs and Teffs, sorted by $\log_{10}$(p-value). Shared pathways are indicated with a star.

(**B**) Heatmaps of top 30 DEGs (determined by p_{adj}) unique to Tregs and Teffs.

(**C**) PPI of top 30 DEGs unique to Tregs and Teffs. Singleton genes are not shown. Clusters demonstrating highly interconnected networks are shown in magenta and yellow. Directionality of interaction represented by arrow tail (source gene) and arrow head (target gene). Strength of interaction represented by arrow edge thickness.

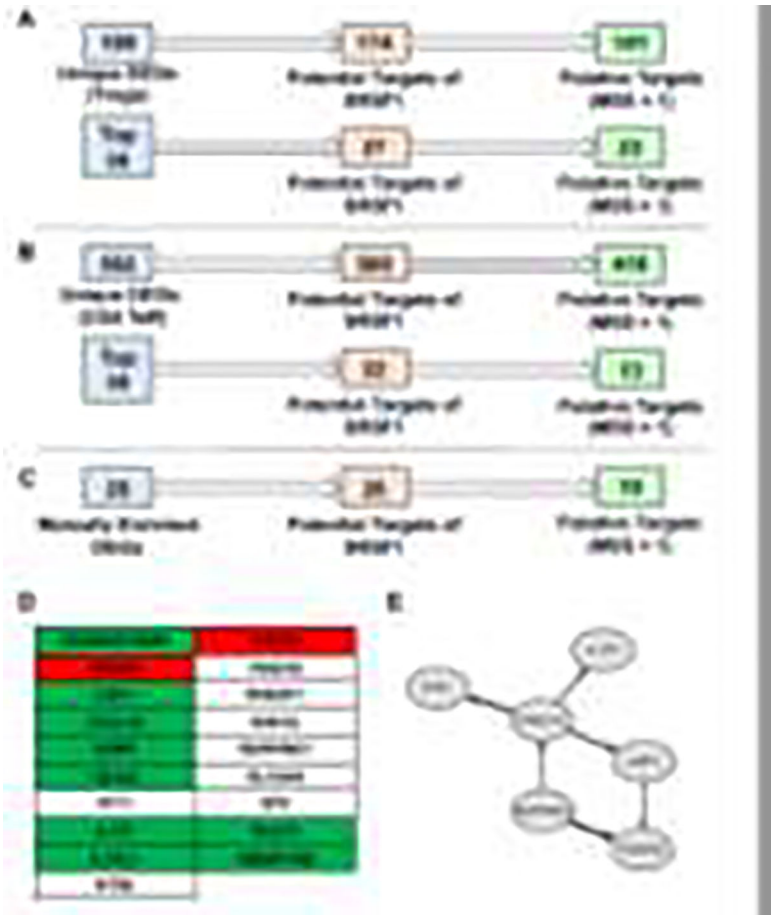

Figure 4. The majority of Srsf1-ko DEGs are potential binding sites of SRSF1

(**A**) Flowchart of RBP-RNA interaction analysis for 189 DEGs unique to Tregs using oRNAment. Potential binding sites restricted using MSS=1.

(**B**) Flowchart of RBP-RNA interaction analysis for 582 DEGs unique to Teffs. Potential binding sites restricted using MSS=1.

(**C**) Flowchart of RBP-RNA interaction analysis for 29 mutually enriched genes. Potential binding sites restricted using MSS=1.

(**D**) Table of 19 putative targets of SRSF1 in Tregs and Teffs. Green boxes indicate the corresponding gene was up-regulated in both knockout cell subsets. Red boxes indicate the corresponding gene was down-regulated in both cell subsets. White boxes indicate the gene was regulated in opposite directions in Tregs and Teffs.

(**E**) PPI of 19 putative targets of SRSF1. Singleton genes are not shown. Directionality of interaction represented by arrow tail (source gene) and arrow head (target gene). Strength of interaction represented by arrow edge thickness.

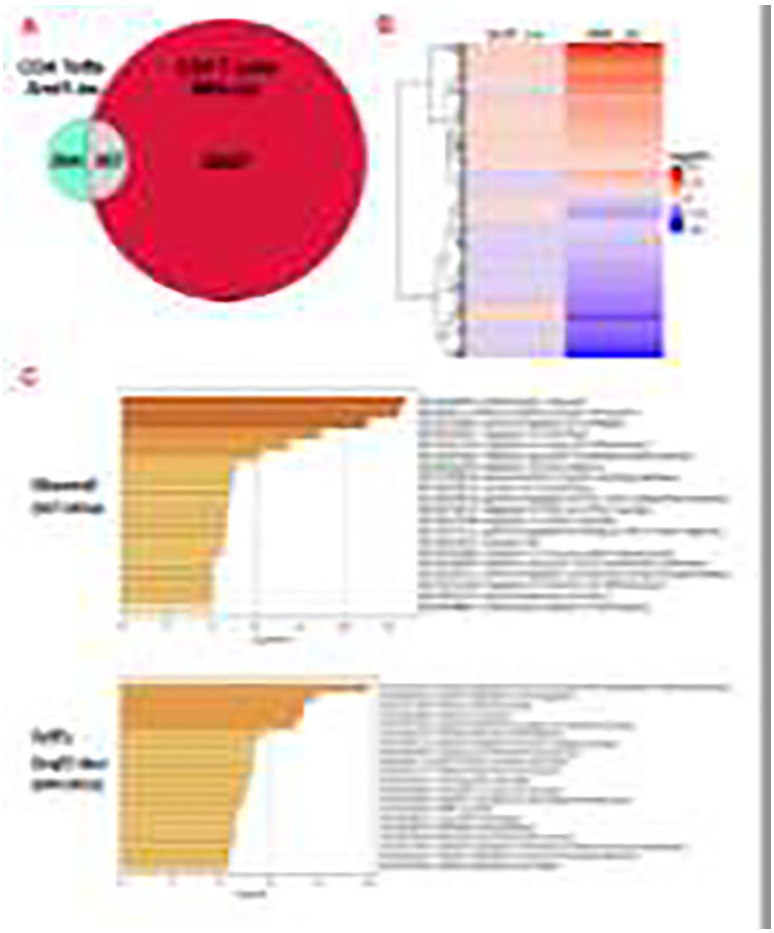

Figure 5. SRSF1 controls molecular programs implicated in autoimmune disease

(**A**) Unique and mutually enriched DEGs between *Srsf1-ko* Teffs (n=3) and MRL/lpr CD4 T cells (n=2). DEGs were held to the same criteria of $p_{adj}<0.05$ and $|\log_2 FC>1|$.

(**B**) Heatmap of $\log_2 FC$ of mutually enriched DEGs between *Srsf1-ko* Teffs and MRL/lpr CD4 T cells.

(**C**) Biological pathway analysis using GO and KEGG terms for mutually enriched DEGs and DEGs unique to *Srsf1-ko* Teffs, performed using Metascape with $p<0.05$.

Table 1.

Ranking of 29 mutually enriched genes between Tregs and Teffs

			Tregs		Teffs	
Rank	**Gene**	**Avg |t-statistic|**	**log2FC**	**padj**	**log2FC**	**padj**
1	RPL22L1	6.27	−1.51	1.17E-03	−1.25	1.01E-12
2	H2-AA	5.52	2.30	2.89E-05	1.85	2.45E-06
3	SERPINE1	5.17	−1.83	1.17E-03	1.56	7.35E-07
4	SLC2A6	5.01	1.54	2.46E-02	2.38	2.57E-08
5	TNIP3	4.96	−1.44	5.03E-03	1.68	7.12E-07
6	F2RL1	4.84	−1.51	9.83E-03	−1.41	8.10E-07
7	LTB	4.76	1.51	1.06E-02	−1.00	1.45E-06
8	GZMC	4.74	1.36	1.34E-02	2.05	1.27E-06
9	6530402F18RIK	4.63	1.25	1.38E-02	1.29	3.33E-06
10	PDGFB	4.61	1.31	1.15E-02	−1.26	5.10E-06
11	NT5E	4.45	1.72	2.50E-04	−1.02	1.35E-03
12	IL24	4.43	−1.67	2.50E-04	1.36	1.69E-03
13	CXCR6	4.36	1.63	1.17E-03	1.50	6.38E-04
14	SP6	4.36	1.79	1.92E-03	−1.5	3.93E-04
15	IFIT1	4.35	−1.45	3.33E-02	1.64	9.76E-06
16	CSF1	4.22	1.80	2.50E-04	1.25	5.75E-03
17	IL17F	4.20	1.94	1.52E-05	1.07	3.62E-02
18	BEND5	4.18	−1.58	1.11E-03	−1.22	2.39E-03
19	TMEM176A	4.12	1.92	1.58E-04	1.22	1.52E-02
20	P4HA2	4.11	−1.75	1.04E-02	−1.32	3.68E-04
21	CXCL10	4.05	1.94	3.06E-03	1.37	2.00E-03
22	RNF43	3.95	1.44	1.41E-02	−1.55	7.65E-04
23	IL17A	3.89	1.60	2.78E-03	1.36	5.74E-03
24	CHST2	3.79	1.53	7.49E-03	1.15	4.52E-03
25	TMEM176B	3.76	1.77	4.27E-04	1.04	4.07E-02
26	IL1RL1	3.69	1.42	3.59E-03	1.25	1.35E-02
27	SULF2	3.54	1.78	3.15E-03	1.07	3.09E-02
28	FGL2	3.47	1.27	4.81E-02	1.31	4.00E-03
29	RHBDF1	3.23	1.52	4.87E-02	−1.17	1.43E-02

Genes were ranked according to average |t-statistic|. Log_2FC and adjusted p-values are shown for Tregs and Teffs separately.