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Splicing factor SRSF1 controls T cell hyperactivity and systemic autoimmunity

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Conflict of Interest Statement: The authors have declared that no conflict of interest exists.
ABSTRACT

Systemic lupus erythematosus (SLE) is a devastating autoimmune disease, in which hyperactive T cells play a critical role. Understanding molecular mechanisms underlying the T cell hyperactivity will lead to identification of specific therapeutic targets. Serine/arginine-rich splicing factor (SRSF)1 is an essential RNA-binding protein which controls posttranscriptional gene expression. We have demonstrated that SRSF1 levels are aberrantly decreased in T cells from SLE patients and correlate with severe disease, yet the role of SRSF1 in T cell physiology and autoimmune disease is largely unknown. Here we show that T cell-restricted Srsf1-deficient mice develop systemic autoimmunity and lupus-nephritis. Mice exhibit increased frequencies of activated/effector T cells producing proinflammatory cytokines, and an elevated T cell activation gene signature. Mechanistically, we noted increased activity of the mechanistic target of rapamycin (mTOR) pathway and reduced expression of its repressor PTEN. The mTOR complex (mTORC)1 inhibitor rapamycin suppressed proinflammatory cytokine production by T cells and alleviated autoimmunity in Srsf1-deficient mice. Of direct clinical relevance, PTEN levels correlated with SRSF1 in T cells from SLE patients, and SRSF1 overexpression rescued PTEN, suppressed mTORC1 activation and proinflammatory cytokine production. Our studies reveal the role of a previously unrecognized molecule SRSF1 in restraining T cell activation and averting the development of autoimmune disease and a potential therapeutic target for lupus.
INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic debilitating multi-system autoimmune disease, which disproportionately afflicts women, causing significant morbidity and mortality (1, 2). Failure of the immune system to recognize self-antigens leads to inappropriate immune responses, systemic autoimmunity and organ damage. The identification of novel molecules and mechanisms underlying the pathogenic immune response in SLE will guide the development of appropriate therapeutic targets.

While autoreactive B cells, autoantibodies, and immune complexes are important in disease pathogenesis (3), and elevated type I interferons (IFN) have been implicated in disease (4, 5), therapeutic strategies targeting B cells and Type I IFN signaling in SLE have shown limited success. In this regard, the role of T cells in disease pathogenesis is beginning to be understood and therapeutic strategies targeting them are emerging (6). Aberrantly activated effector T (Teff) cells and cytokines which orchestrate not only germinal center B cell responses for autoantibody production (7–9), but also promote inflammatory infiltration in target organs (10, 11), are critical players in disease pathogenesis and are found both in lupus-prone mice and in patients with SLE (12–15) and therefore are potential promising therapeutic targets. Yet the molecules and mechanisms which control the activated T cell phenotype in SLE are poorly understood.

A key pathway involved in the activation of T cells is the phosphoinositide-3 kinase (PI3K)/AKT/mechanistic target of rapamycin (mTOR) pathway. The mTOR pathway determines cell fate decisions of T cell activation, differentiation, proliferation and cell death (16). Resting quiescent T cells have low levels of mTOR activity, while mTOR complex (C)1 activation leads to increased proportions of activated/effector cells and a decreased pool of naïve cells (17). Recently mTOR activation has emerged as a central regulator of T cell dysfunction in systemic autoimmune diseases and as a promising therapeutic target for SLE (15, 18). Increased activity of the mTORC1 pathway in T cells is known to promote the aberrant production of cytokines IFN-γ, IL-17, IL-4 (19, 20), and
contribute to systemic autoimmunity (9). Furthermore, activation of mTORC1 is associated with altered aberrantly activated T cell responses in patients with SLE (21, 22). The phosphatase and tensin homolog (PTEN), a lipid phosphatase, negatively regulates the PI3K/Akt signaling pathway (23) and inhibits the activity of the mTOR pathway (24). PTEN-deficient T cells are hyper-responsive to TCR stimulation (25), and Pten deletion in T cells in mice leads to autoimmune lymphoproliferative disease and aberrant production of cytokines (26, 27). PTEN expression is decreased in B cells from SLE patients and contributes to B cell hyperactivity (28). However, it is still unknown whether PTEN is altered in T cells from SLE patients and whether it contributes to their hyperactivity.

Serine arginine-rich splicing factor 1 (SRSF1), previously known as splicing factor 2/alternative splicing factor (SF2/ASF), is the prototype member of the highly conserved serine arginine (SR) family of RNA-binding proteins. SRSF1 controls post-transcriptional gene expression via pre-mRNA alternative splicing, mRNA stability and translation (29). While SRSF1 is an essential ubiquitous molecule which controls genes involved in cell survival and proliferation (30), little is known of its role in the immune system. T cells from SLE patients display hyperactive T cell receptor (TCR)/CD3 signaling and proinflammatory cytokine production (1, 31). This overactive lupus T cell phenotype is associated with a reduced expression of the CD3 zeta (ζ) chain (32), attributed in part to defective alternative splicing of its 3′ untranslated region (UTR) (33). Using discovery approaches, we found SRSF1 to bind to the 3′UTR of the CD3ζ mRNA in human T cells (34, 35). We showed that SRSF1 promotes normal expression of CD3ζ by limiting expression of an unstable isoform of its 3′UTR (35) and that SRSF1 levels decrease upon T cell activation (36). We demonstrated that SRSF1 expression is aberrantly decreased in T cells from patients with SLE and correlates with severe disease (37)(38). In addition, decreased SRSF1 expression levels have been linked to the defective expression of multiple autoantigen transcripts in the organ-specific autoimmune inflammatory myositis (39). Thus, studies in humans suggest that the reduced expression of SRSF1 correlates with an activated T cell phenotype and the expression of autoimmune disease. However, evidence of a causal in vivo relationship between SRSF1, T cell dysfunction and autoimmune disease is still lacking.
Here we show that mice which lack Srsf1 in mature T cells develop systemic autoimmunity and lupus nephritis with increased proportions of activated/effectector T cell populations and proinflammatory cytokine-producing T cells. Mechanistically, we show that T cells from Srsf1-deficient mice exhibit increased mTOR activity and reduced expression of its negative regulator PTEN while rapamycin and overexpression of PTEN normalize the inflammatory cytokine production. Importantly PTEN levels are reduced in SLE patients with lower SRSF1 expression and SRSF1 rescues PTEN levels, suppresses mTORC1 activity and reduces proinflammatory cytokine production by T cells from SLE patients. Our studies demonstrate that SRSF1 is a previously unrecognized molecule, which restrains T cell activation and controls the development of autoimmune disease.
RESULTS

*Srsf1*-cKO mice develop autoimmunity and lupus nephritis

Because germline *Srsf1* deletion is embryonic lethal (40), we generated conditional distal.*Lck*\textsuperscript{cre}.*Srsf1*\textsuperscript{flx/flx} (*Srsf1*-cKO hereafter) mice to study the role of SRSF1 in T cells (Supplemental Figure 1, A and B). At birth, *Srsf1*-cKO mice were viable with normal external features and body weight (Supplemental Figure 1C). As expected (the distal Lck promoter is expressed late in thymic development and mainly in mature T cells), T cell development was normal in *Srsf1*-cKO mice (Supplemental Figure 1, C, D, E and F). We asked whether the *Srsf1*-cKO mice develop signs of autoimmunity. We measured autoantibodies in the sera of *Srsf1*-cKO mice using microarray analyses of serum IgG reactivity against a 128-autoantigen panel, which includes the lupus-associated autoantigens dsDNA, histones, and a number of nuclear, membrane, phospholipid and cytoplasmic protein autoantigens (Figure 1A). Serum from a lupus-prone MRL/lpr mouse, which develops spontaneously an autoimmune lupus-like disease, was included as positive control. We found that *Srsf1*-cKO mice develop a wide array of lupus-associated autoantibodies at levels comparable to those seen in MRL/lpr mice (Figure 1A). We confirmed by ELISA, the presence of anti-nuclear (ANA) antibodies in sera from *Srsf1*-cKO mice (Supplemental Figure 2A). In aged (12 - 18 months old) mice, we observed a lymphoproliferative phenotype (Figure 1B and Supplemental Figure 2B). We also found that T cells from *Srsf1*-cKO mice exhibit increased proliferative capacity as assessed by Ki67 staining (Figure 1C). The kidneys of aged *Srsf1*-cKO mice showed histopathologic evidence of lupus nephritis with glomerulonephritis, mesangial and endocapillary proliferation and interstitial inflammatory cell infiltration (Figure 1D) with significantly higher histopathology scores compared to control mice (Figure 1E). Immunohistochemistry staining showed T cell infiltration in kidneys of *Srsf1*-cKO mice (Supplemental Figure 2C). *Srsf1*-cKO mice developed significant proteinuria compared to wild type mice (Figure 1F). These results demonstrate that the loss of SRSF1 in T cells leads to systemic autoimmunity and lupus-like disease.
T cells from Srsf1-cKO mice exhibit a hyperactive phenotype and produce proinflammatory
cytokines

Examination of peripheral lymphoid T cells from spleen revealed significantly increased proportions of
activated CD69hi cells (Figure 2, A and B), and effector/memory CD44hiCD62Llo and
CD44hiCD62Lhi populations in the CD4 and CD8 compartments (Figure 2, C, and D). In parallel, we
recorded a significant reduction in the naïve T cell pool in both CD4 and CD8 compartments (Figure
2E). Further, we found increased proportions of IL-17-, IFN-γ- and IL-4-producing CD4 T cells, and
IFN-γ- producing CD8 T cells from Srsf1-cKO mice (Figure 2F). In addition, we found that the
frequencies of CD4+ CXCR5+PD1+ T follicular helper (Tfh) cells were increased in Srsf1-cKO mice
(Figure 2G). We also detected the increased presence of spontaneous germinal centers in the
spleens from Srsf1-cKO mice (Supplemental Figure 2D). We found that the frequencies of GL7+Fas+
erginal center B cells were increased in Srsf1-cKO mice (Supplemental Figure 2E). In addition, IgD-
memory/plasma B cells were also increased in Srsf1-cKO mice (Supplemental Figure 2F). There was
no difference in the frequencies and absolute numbers of B cells, CD11b+ cells, CD11c+ cells in
spleen of control wild type (WT) and Srsf1-cKO mice (Supplemental Figure 3). The frequencies of
CD4+CD25+FoxP3+ Treg cells in Srsf1-cKO mice were comparable to that of control mice
(Supplemental Figure 4, A and B) and suppressed the proliferation of conventional T (Tconv) cells
normally in in vitro Treg suppression assays for three days (Supplemental Figure 4, C and D). These
results show that loss of SRSF1 induces the development of activated, inflammatory cytokine
producing T cells.

Transcriptomics analysis of CD4 effector T cells from Srsf1-cKO mice reveals an elevated T
cell activation gene signature

Because the Srsf1-cKO mice develop autoimmune inflammatory disease, and exhibit increased
proportions of proinflammatory Teff cells, we asked whether SRSF1 controls genes and pathways
involved in T cell activation and differentiation. We performed transcriptomics analysis by RNA-
sequencing of CD4 effector T (Teff) cells derived by stimulation of naïve CD4 T cells with anti-CD3 and anti-CD28 for 72h, from 7-week-old control WT or Srsf1-cKO mice with 3 mice per group (Figure 3 and Table 1). At the fold change >1, p value <0.05, 890 genes were upregulated and 1021 genes downregulated in Srsf1-cKO mice compared to control mice whereas at the fold change >2 cutoff with p value <0.05, 312 were genes upregulated and 300 genes downregulated in Srsf1-cKO mice compared to normal mice (Figure 3A). Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses revealed the top pathways to include genes associated with T cell activation, regulation of protein secretion, cytokine-cytokine receptor interaction, cell cycle, Th17 differentiation, Th1 and Th2 differentiation (Table 1). Cluster analysis revealed predominantly genes involved in T cell activation, protein secretion, cytokine production, ribosome assembly, cell cycle and chromatin modifications (Figure 3B). The expression levels of multiple cytokines including Ifng, Il17, Il4 and Il21 were found to be increased (Figure 3C). Because IL-21 is known to play important roles in Tfh differentiation, the increased Il21 expression may contribute to the observed increase in Tfh cells in Srsf1-cKO mice. These data indicate that SRSF1 controls genes involved in T cell activation, differentiation, and cytokine production, and the loss of SRSF1 leads to unrestrained activation and pro-inflammatory phenotype of T cells.

T cells from Srsf1-cKO mice display increased activation of the mTORC1 pathway and reduced expression of PTEN

It is known that Pten-deficient mice exhibit features of T cell hyperactivity with increased proinflammatory cytokines and autoimmunity (27). Further, activation of the mTOR pathway is associated with hyperactive T cell responses with inflammatory cytokine production in SLE patients (15, 21, 22). In our transcriptomics analysis from Srsf1-cKO mice, a number of genes of the PI3K/AKT/mTOR pathway were differentially expressed, and the expression of its repressor PTEN was found to be downregulated (Figure 4A). Given these findings, we examined the activity of the mTOR pathway in the Srsf1-cKO mice. We found increased expression of phosphorylated (p)-S6 protein levels in CD4 Teff cells derived by stimulation of naïve CD4 T cells with anti-CD3 and anti-
CD28 for 72h, indicating an increased activity of the mTORC1 pathway (Figure 4B). It was previously reported that SRSF1 regulates the splicing of S6K1, an mTORC1 downstream signaling molecule, and modulates the mTOR pathway in cell lines (41). We found that deletion of Srsf1 in T cells results in the decrease of the short isoform of S6K1 (Supplemental Figure 5) consistent with previous reports. Although this short isoform of S6K1 associates with activation of the mTORC1 pathway (42), in the present study we found that serine-threonine kinase AKT, upstream of S6K1, is activated in CD4 effector T cells from Srsf1-cKO mice (Figure 4C). Therefore, we hypothesized that decreased PTEN levels result in the activation of mTOR pathway. We confirmed the decreased mRNA (Figure 4D) and protein (Figure 4E) expression levels of PTEN in T cells from Srsf1-cKO mice. Because SRSF1 controls gene expression at the posttranscriptional level, which is frequently mediated through the 3’UTR, we investigated the effect of SRSF1 on the 3’UTR activity of PTEN. Co-transfection of a PTEN 3’UTR-luciferase construct along with an Srsf1-expression vector in HEK293T cells revealed an increase in PTEN 3’UTR activity (Figure 4F). Transfection of the PTEN 3’UTR into freshly isolated total T cells from Srsf1-cKO mice led to decreased 3’UTR activity which was rescued by the overexpression of SRSF1 (Figure 4G). These results show that SRSF1 controls the expression of PTEN and loss of SRSF1 leads to reduced PTEN expression and increased activity of the mTOR pathway.

Rapamycin treatment reduces proinflammatory cytokine production by T cells and alleviates autoimmunity in Srsf1-cKO mice

Recently, hyperactivity of the mTOR pathway has been associated with aberrant T cell responses in SLE (15, 43), and targeted inhibition of this pathway with the lipophilic macrolide antibiotic rapamycin has led to improved T cell function and shown efficacy in a recent clinical trial in patients with active SLE (44). To assess whether the increased mTORC1 activation of T cells from Srsf1-cKO mice contributes to their proinflammatory phenotype and autoimmune phenotype, we examined the effect of rapamycin. Treatment of spleen cells from Srsf1-cKO mice ex vivo with rapamycin reduced the production of pro-inflammatory cytokines IL-17 (Figure 5, A and B), and IFN-γ (Figure 5, C and D)
from CD4 T cells. Furthermore, to assess the effects of Rapamycin on disease in vivo, we treated
Srsf1-cKO mice with rapamycin and evaluated the effect on features of autoimmunity. We observed
reduced levels of IL-17 cytokine production ex vivo (Figure 5E), and decreased proportions of CD4+
PD1+CXCR5+ Tfh cells in the spleens from cKO mice (Figure 5F). Importantly, we observed a
reduction in the levels of serum dsDNA and histone autoantibodies (Figure 5G). In addition, treatment
with rapamycin reduced T cell infiltration into the kidneys (Figure 5H). These data indicate that
deficiency of SRSF1 leads to increased activation of the mTOR pathway, the ensuing proinflammatory
function of T cells and systemic autoimmunity, which can be corrected by rapamycin.

**PTEN overexpression reduces proinflammatory cytokine production by T cells from Srsf1-cKO mice**

To confirm that SRSF1 modulates T cell activation via regulation of PTEN expression, PTEN was
overexpressed by transient transfections in T cells from WT and Srsf1-cKO mice followed by
stimulation with PMA and Ionomycin for 4h. We confirmed the overexpression of PTEN at the mRNA
and protein levels (Supplemental Figure 6, A and B). We found that cytokine production is decreased
by PTEN overexpression in T cells from Srsf1-cKO mice but not in WT T cells (Figure 6, A and B). Of
note, transfection by electroporation is toxic to cells and accounts for the overall reduced frequencies
of IFN-γ-producing cells (Figure 6) compared to untransfected cells (Figure 5C and Supplemental
Figure 6C). These data indicate that deficiency of SRSF1 leads to decreased PTEN expression,
increased activation of the mTOR pathway and the ensuing proinflammatory function of T cells
(Supplemental Figure 7) which contribute to systemic autoimmune disease.

**PTEN is decreased in T cells from SLE patients with low SRSF1 levels**

Although PTEN expression is decreased and contributes to the hyperactivity of B cells in SLE patients
(28), its role in T cells in SLE has not been studied. Given our findings of increased mTOR activity and
the decreased expression and regulatory role of PTEN in T cells from the Srsf1-cKO mice (Figure 4, 5
and 6), we asked whether PTEN expression levels are altered in T cells from SLE patients. Therefore
we assessed PTEN and SRSF1 protein expression levels in T cells from SLE patients and compared with age-, race-, gender- matched healthy control individuals. We found that the expression of PTEN was decreased in T cells from SLE patients who had decreased expression levels of SRSF1, compared to healthy individuals (Figure 7A). Importantly, we observed a linear correlation between PTEN and SRSF1 expression levels in T cells from SLE patients ($R^2=0.226, p=0.0163$, Figure 7B). These results suggest that the low SRSF1 levels may contribute to the reduced expression of PTEN in T cells from SLE patients.

SRSF1 overexpression increases PTEN levels, suppresses mTORC1 activity and reduces proinflammatory cytokine production in T cells from SLE patients

To assess whether SRSF1 overexpression would increase PTEN expression, and reduce mTORC1 activity and the aberrant cytokine production by T cells from SLE patients, we transiently transfected T cells from SLE patients with an Srsf1-expression vector followed by stimulation with anti-CD3 and anti-CD28. We found that SRSF1 overexpression increased PTEN protein levels (Figure 7C), reduced activation of the mTORC1 pathway as evidenced by a reduction in pS6 expression levels (Figure 7D) and led to reduced frequencies of IL-17- and IFN-γ-producing CD4 T cells (Figure 7E). These findings show that decreased SRSF1 expression contributes to mTORC1 activation and proinflammatory cytokine production in T cells from SLE patients through the control of PTEN.
DISCUSSION

In this study, we demonstrate that selective deletion of SRSF1 in T cells in mice leads to aberrantly activated proinflammatory cytokine-producing Teff cells leading to systemic autoimmunity and lupus nephritis (Figure 1 and 2). We report that SRSF1 controls the expression of genes involved in T cell activation, differentiation and cytokine signaling (Figure 3). At the mechanistic level we demonstrate that SRSF1 regulates the 3'UTR of the mRNA of the mTOR inhibitor PTEN and limits its expression causing increased mTOR activity (Figure 4). Rapamycin treatment corrects proinflammatory cytokine production in T cells and alleviates autoimmunity in Srsf1-deficient mice (Figure 5). Of clinical relevance is our finding that T cells from patients with SLE with low expression levels of SRSF1 had decreased levels of PTEN, which correlate with decreased expression levels of SRSF1 (Figure 7). Further, restitution of SRSF1 levels corrected the proinflammatory cytokine production by hyperactive SLE T cells (Figure 7). Thus, we uncover SRSF1 as a novel molecular brake in the control of T cell hyperactivity and prevention of autoimmune disease. Our findings highlight an important role for SRSF1 in the immune system and in the control of immune-mediated disease. SRSF1 deficiency unrestrains T cell activation, unleashes the production of proinflammatory cytokines IL-17 and IFN-γ by CD4 Teff cells and leads to activation of B cells, inflammatory infiltration in target organs leading to systemic autoimmunity and organ inflammation. Increased activity of the mTOR pathway contributes to the proinflammatory phenotype, which is rescued by rapamycin administration.

The mTOR pathway is a recently emerging crucial therapeutic target in autoimmune diseases including SLE (15, 43). Increased mTORC1 activity has been demonstrated in T cells from patients with SLE and correlates with expansion of proinflammatory cytokine producing T cells (21, 22). Treatment of lupus-prone MRL/lpr mice with rapamycin, an inhibitor of mTORC1 signaling, improved disease parameters (45). Importantly, a recent clinical trial of rapamycin in patients with SLE has shown efficacy in active SLE patients (44). Here, we find that SRSF1 deficiency in mice results in
increased activation of mTORC1 pathway, which parallels the observed increased mTORC1 activity
(15) and the decreased SRSF1 expression in patients with SLE (37).

Although PTEN is known to suppress the mTOR pathway and the expression of PTEN is decreased
in B cells from patients with SLE (28), the role of PTEN in T cells from SLE patients remains unknown.
We previously showed that the expression levels of SRSF1 are decreased in SLE T cells and
inversely correlate with disease activity suggesting the important role of SRSF1 in SLE T cells (37).
Our current study extends this concept with a number of crucial new findings. Here we show a linear
correlation between PTEN and SRSF1 in T cells from SLE patients and that lupus patients with low
SRSF1 expression levels exhibit lower expression levels of PTEN. Furthermore, the overexpression
of SRSF1 rescues PTEN expression in lupus T cells (Figure 7). This is the first evidence showing the
reduction of PTEN in T cells in a subset of SLE patients. Moreover, the overexpression of SRSF1 in
lupus T cells reduces mTOR activity and the production of proinflammatory cytokines, suggesting that
the SRSF1-PTEN-mTOR pathway may be a novel therapeutic target in SLE patients.

T cell quiescence is enforced by molecules able to restrain T cell activation, and the expression of
such molecules generally subside upon TCR stimulation to enable effector differentiation programs.
These genes include among others, the Kruppel-like factor (KLF) genes, Schlafen-2, Tsc2, and the
Forkhead box (Foxo) family (46). T cells from the Srsf1-cKO mice preferentially expressed genes
involved in the Foxo1 signaling pathway, cell cycle, T cell activation, cytokine-cytokine receptor
interaction, and Th differentiation programs. Therefore it appears that SRSF1 maybe involved in the
maintenance of quiescence of resting T cells. In agreement with these findings are our previous
findings of high SRSF1 protein levels in resting human T cells which decreased following TCR
stimulation due to its degradation by the ubiquitin-proteasome pathway (36). In contrast, deficiency or
decreased expression of SRSF1 as recorded in our Srsf1-cKO mice and in patients with SLE,
respectively, contributes to T cell activation, loss of tolerance, autoimmunity and organ inflammation.
Of direct clinical relevance is the observation that T cells from SLE patients exhibit a hyperactive phenotype and functional defects (13), linked mechanistically to decreased SRSF1 expression (37).

A limitation of our study is that the T cell restricted deletion of Srsf1 is under the distal Lck promoter, which is expressed late during thymic development as opposed to the proximal Lck promoter, which is expressed early in the double negative thymocytes. Therefore, the role of SRSF1 in early thymic development is bypassed which may differentially influence T cell physiology and function. Also, Srsf1 is absent since birth and does not address the possibility of altering T cell function should its levels decrease later in life. Deletion of Srsf1 in adult mice may result in differential physiological and pathophysiological outcomes. SRSF1 levels are decreased in T cells from SLE patients but not completely absent. Therefore, while the homozygous Srsf1-cKO mice are used in proof-of-concept studies here, the heterozygous (distal.Lck^{cre}.Srsf1^{flox/wt} mice) Srsf1-cKO mice, which also develop some features of T cell activation, and signs of autoimmune disease (V.R.Moulton, unpublished observations) may serve as a better model for human disease in future studies. Because SRSF1 regulates target genes via various mechanisms, it is possible that other pathways also contribute T cell activation and autoimmunity. In addition, although the frequency of Tregs and functional results from short-term in vitro suppression assays are comparable between WT and Srsf1-cKO mice, further studies including generating Treg conditional Srsf1-cKO mice are required to assess precisely the role of SRSF1 in Tregs. Furthermore, the differences observed in the distribution of activated/memory T cell populations within the CD4 and CD8 compartments imply a potentially differential role of SRSF1 in CD4 and CD8 T cells (Figure 2, C-E). Besides these limitations, our patient numbers in the human studies are small, and a larger cohort of SLE patients is needed to systematically define the clinical characteristics of patients stratified based on molecular levels of SRSF1 and PTEN. In addition, whether T cells from SLE patients with and without low SRSF1 expression exhibit differential response to rapamycin needs to be examined. Increased activation of the mTOR pathway is associated with altered metabolism in T cells in mice and in patients with SLE (47, 48) and it would be interesting to assess whether Srsf1-deficient T cells exhibit such defects.
In conclusion, we have uncovered a previously unrecognized role of SRSF1 in T cell physiology and demonstrated the impact of its absence in the expression of autoimmune disease. Loss of SRSF1 limits the expression of the mTOR repressor PTEN leading to aberrant T cell activation and the expression of systemic autoimmunity. Our results suggest that prevention of the loss of SRSF1 should be of therapeutic value to autoimmune diseases.

METHODS

Human Subjects

Patients with SLE, all fulfilling the American College of Rheumatology classification criteria (49), were recruited at the Rheumatology clinic at Beth Israel Deaconess Medical Center (BIDMC), Boston, MA (Supplemental Table 1). Age-, race-, and gender- matched healthy individuals were recruited as controls. Peripheral blood was drawn from patients by venipuncture.

Mice

C57BL/6J (stock 000664), B6.129S4-Srsf1-flox (stock 018020) and B6.dLck.Cre (stock 012837) mice were purchased from the Jackson Laboratory (Bar Harbor, Maine). In most experiments, sex-matched littermates were used as WT controls. All mice were maintained in the specific pathogen free animal facility at Beth Israel Deaconess Medical Center (BIDMC).

Antibodies and Reagents

Flow cytometry antibodies anti-mouse-CD4 (GK1.5), CD8a (53-6.7), CD11b (M1/70), CD11c (N418), CD19 (6D5), CD25 (PC61), CD44 (IM7), B220 (RA3-6B2), CD62L (MEL14), CD69 (H1.2F3), CD90.2 (53-2.1), TCR-β (H57-597), IgD (11-26c.2a), IL-4 (11B11), IL-17A (JC11-18F10.1), IFN-γ (XMG1.2), CXCR5 (L138D7), Ki67 (16A8), CD127 (A019D5), PD-1 (29F.1A12), GL7 (GL7), Brilliant Violet 421 conjugated Streptavidin, purified anti-mouse-CD3 (145-2C11), CD28 (37.51), CD16/32 (Fc block), anti-human CD3 (OKT3), CD4 (OKT4), CD8 (SK1), IFN-γ (4S.B1), Zombie Aqua Fixable Viability kit
mouse IL-17A ELISA kit and carboxyfluorescein succinimidyl ester (CFSE) were from Biolegend. Anti-mouse Foxp3 (FJK-16s) antibody, mouse regulatory T-cell staining kit and anti-human IL-17A antibody (eBIO64DEC17) were from eBioscience. Anti-mouse Fas (Jo2) antibody, Cytofix/Cytoperm, Perm/Wash buffer and Monensin were from BD Biosciences. ACK lysing buffer was from Fisher Scientific. SRSF1 rabbit polyclonal antibody for flow cytometry was from LifeSpan Biosciences and monoclonal antibody for western blot (clone 96) from ThermoFisher Scientific. Anti-S6 ribosomal protein phospho-Ser 235 and Ser 236 (D57.2 2E), anti-S6 ribosomal protein (5G10), anti-PTEN (138G6), anti-phospho-AKT (Ser473) (D9E), anti-phospho-AKT (Thr308) (244F9), anti-AKT (C67E7) antibodies were from Cell Signaling Technology. Phorbol myristic acid (PMA), Ionomycin, Mitomycin C and β-Actin (AC-74) antibody was from Sigma-Aldrich and goat anti-rabbit IgG-horseradish peroxidase (HRP) and goat anti-mouse IgG-HP antibodies were from ThermoFisher. Rapamycin and goat anti-mouse IgG were from Millipore. HEK293T cells were purchased from American Type Culture Collection (ATCC, Manassas). The pcDNA3.1-SRSF1 expression plasmid was a gift from Dr. James Manley (Columbia University, NY), and the pGL3-PTEN 3’UTR plasmid was a gift from Dr. Jeffrey Rosen (Addgene plasmid 28104). The pcDNA3-FLAG PTEN plasmid was a gift from Jaewhan Song (Addgene plasmid #78777).

Cell isolation and tissue processing

Spleens and lymph nodes were homogenized using a syringe plunger and mesh cell strainer. After perfusion, kidneys were homogenized and digested in HBSS medium with 1.5mg/ml collagenase B (Boehringer Mannheim) at 37 °C for 30 minutes. RBC lysis was performed with ACK lysing buffer. All cell cultures were in RPMI complete medium (RPMI plus 10% FBS plus penicillin and streptomycin antibiotics). Blood samples from mice were collected by tail vein incision and capillary tube collection, and serum separated by centrifugation. Urine samples were collected from mice in individual metabolic cages and urine protein measured by Multistix (Siemens). Human T cells from peripheral blood were isolated using theRosette Sep human T cell enrichment cocktail (STEMCELL Technologies). For histopathology, tissues were immediately fixed in 10% formalin overnight,
processed in an automated tissue processor, embedded into paraffin blocks and sent to the BIDMC Histopathology core for sectioning and slide staining with hematoxylin and eosin (H and E). Slides were evaluated blind for histopathology scoring utilizing semi-quantitative scoring criteria for lupus nephritis (50).

Flow cytometry

Zombie aqua viability dye was used for live/dead cell staining. Surface staining was performed in FACS staining buffer (phosphate buffered saline (PBS) plus 2% fetal bovine serum (FBS)) on ice for 20 min with Fc block. For cytokine production, cells from mice were stimulated for 4h in culture medium with PMA (100ng/mL), Ionomycin (1µM) and Monensin (1μl/mL). Human T cells were stimulated with PMA (10ng/mL), Ionomycin (400ng/mL) and Monensin (1μl/mL) for 4h. Cells were surface stained followed by fixation and permeabilization. Appropriate antibodies were used for intracellular staining for cytokines or Foxp3 and Ki67. Flow cytometry data were acquired on a BD LSRII or CytoFLEX LX and analyzed with FlowJo software. All procedures were performed according to the manufacturer's instructions.

Autoantibody detection

Sera from 12-week old WT and Srsf1-cKO mice and one 16-week old MRL/lpr mouse (as positive control) were analyzed for the presence of autoantibodies using the Autoantigen Microarray Super Panel (128-antigen panel) at the Genomics and Microarray Core facility at the University of Texas, Southwestern Medical Center (Dallas, TX). Heat-maps were prepared using R software. Anti-nuclear antibody (ANA) in serum was measured using an ELISA kit (Alpha Diagnostic International) following the manufacturer’s instructions. Briefly, the serum was diluted and assayed for antigen reactivity on pre-coated plates followed by incubation for 60 min. Bound IgG was detected with anti-mouse IgG-HRP antibody and developed with a tetramethylbenzidine (TMB) substrate. Plates were read at 450 nm on a spectrophotometer. To detect anti-double stranded (ds)DNA and anti-Histone antibodies, Immulon II plates (Dynatech) precoated with BSA were coated individually with 50µg/mL calf thymus
DNA (Sigma-Aldrich) or 50μg/mL calf thymus histone (Sigma-Aldrich). The serum was diluted (1:500) and assayed for autoantigen reactivity against the antigens described above by incubation overnight at 4°C. Bound IgG was detected with a goat polyclonal HRP–anti-mouse IgG detection antibody (SouthernBiotech) and visualized at 450nm using a tetramethylbenzidine (TMB) substrate (Sigma-Aldrich).

**mRNA Expression and RT-PCR**

Total RNA was isolated using the RNeasy mini kit (Qiagen) and reverse-transcribed into cDNA using the RNA to cDNA premix (Clontech). Real-time PCR amplification was carried out with SYBR Green I using a LightCycler 480 (Roche) and the following program: initial denaturation at 95°C for 5 min; 40 cycles of amplification (denaturation at 95°C for 15 sec, annealing at 60°C for 15 sec, extension at 72°C for 30 sec); one cycle of melting curves (95°C for 15 sec, 65°C for 2 min, and 97°C continuous), and a final cooling at 37°C. Threshold cycle values were used to calculate relative mRNA expression by the ΔCt relative quantification method. Primer sequences are listed in Supplemental Table 2.

**RNA sequencing**

Naïve CD4 T cells were isolated from spleen of 7-week-old WT and Srsf1-cKO mice (n=3 each) and stimulated with anti-CD3 (0.5μg/mL) and anti-CD28 (1μg/mL) for 72 hours. Cells were re-stimulated with PMA (100ng/mL) and Ionomycin (1μM) for 4 hours. Total RNA was extracted using the RNeasy mini kit (Qiagen) and submitted for sequencing to the Molecular Biology Core Facility (MBCF) at the Dana-Farber Cancer Institute (DFCI). Libraries were prepared using Illumina TruSeq Stranded mRNA sample preparation kits according to the manufacturer’s protocols. Samples were sequenced on an Illumina NextSeq500 run with single-end 75-bp reads. Data analyses were performed by the MBCF at DFCI and the Harvard T. H. Chan School of Public Health Biostatistics Core. The data have been deposited at the NCBI Gene Expression Omnibus (GEO) database under accession number GSE136286.
Western blot

To detect phosphorylated S6, naïve CD4 T cells were isolated from spleen of 9-10-week old WT and Srsf1-cKO mice and stimulated with anti-CD3 (0.5µg/mL) and anti-CD28 (1µg/mL) for 72 hours. Human T cells were stimulated with soluble anti-CD3 (5µg/mL), anti-CD28 (2.5µg/mL) and cross linker goat anti-mouse IgG (10µg/mL) for 5 minutes. Cells were lysed with radioimmunoprecipitation assay (RIPA) buffer (Boston Bioproducts). Lysates were electrophoresed on NuPAGE 4-12% Bis-Tris gels (Life Technologies) and transferred to PVDF membrane. Membranes were blocked with 5% (wt/vol) nonfat milk in Tris-buffered saline with 0.05% Tween 20 (TBS-T) for 1h, incubated with primary antibody (1:1000, or 1:10000 for β-actin antibody) in 5% milk in TBS-T or Hikari solution A (Nacalai Tesque) at 4°C overnight or at room temperature for 2h for β-actin antibody. Membranes were washed three times with TBS-T, incubated with HRP-conjugated secondary antibody (1:2000 for ECL detection reagents or 1:4000 for ECL prime detection reagents; GE Healthcare) for 1h, washed three times with TBS-T, developed with ECL reagents, and visualized by a Fujifilm LAS-4000 imager or Bio-Rad ChemiDoc imager. Densitometry was performed using Quantity One software (Bio-Rad).

Transfections

HEK293T cells were cultured in DMEM complete medium. One day prior to transfection, 0.3 x 10⁶ cells were seeded in a 12-well plate. Cells were co-transfected with the pGL3-PTEN 3’UTR luciferase plasmid and either pcDNA3.1 empty vector (EV) or pcDNA3.1-Srsf1 vector using Lipofectamine (Invitrogen). All transfections included a pRL-TK Renilla luciferase plasmid as internal control. Human T cells and mouse T cells were transfected using the Amaza human T cell nucleofector kit and the mouse T cell nucleofector kit (Lonza, Germany) respectively following manufacturer’s instructions. Briefly, 3-6 x 10⁶ cells were resuspended in 100µL of nucleofector solution, plasmid DNA added, cells transferred into a cuvette and electroporated using the U-014 program for human cells or the X-001 program for mouse cells in the nucleofector device. Cells were immediately rescued into prewarmed medium and cultured overnight.
Luciferase Assays

Cells were transfected as described above. After 24h culture, cells were collected, lysed in passive lysis buffer (Promega) and luciferase activity was quantified on a luminometer using the dual-luciferase assay system (Promega) according to manufacturer’s instructions.

Immunofluorescence and Immunohistochemistry staining

For immunofluorescence staining, spleens were embedded in OCT tissue media (Tissue-Tek) and frozen on dry ice. Frozen sections (7-µM thickness) were fixed to slides in ice-cold acetone for 15 minutes and air dried for 30 seconds. The sections were blocked with 10% horse serum for 30 minutes at room temperature, then stained for 30 minutes at room temperature in a humidified chamber with fluorescent-conjugated anti-IgD (405717; Biolegend), anti-CD4 (100533; Biolegend), anti-B220 (103207; Biolegend) and biotinylated PNA (B-1075; Vector laboratories). Biotinylated PNA was detected using streptavidin-Alexa fluor 350 (S-11249; Life Technologies). For immunohistochemistry staining, slides of formalin-fixed paraffin-embedded kidneys were incubated with purified anti-mouse CD3 antibody (100201; Biolegend) overnight at 4°C after antigen retrieval. The primary antibody was detected with rabbit anti-rat HRP secondary antibody (ab6734, Abcam).

In vitro suppression assays

Total T cells were isolated from spleens by magnetic assisted cell sorting (MACS), using the Pan T cell isolation kit (Miltenyi Biotech). Conventional CD4 T cells (Tconv) (CD4+CD25-CD127hi) and Treg cells (CD4+CD25+CD127lo) were sorted by flow cytometry (BD FACS Aria II). Tconv cells were labeled with CFSE and co-cultured with Treg cells at increasing ratios for 3 days, in presence of Mitomycin C treated splenocytes and anti-CD3 and anti-CD28, and proliferation of Tconv analyzed by flow cytometry.

Rapamycin treatment
Rapamycin was dissolved in DMSO and diluted in PBS. For in vitro culture treatment experiments, a final concentration of 1 or 10 nM rapamycin was added for the duration of cell cultures. For in vivo treatment experiments, rapamycin (2 mg/kg dissolved in DMSO, diluted in 150 µl PBS) or PBS (equal volume DMSO in 150 µl PBS) was administered to mice by intraperitoneal injection once every two days for four weeks. After four weeks treatment, mice were euthanized and spleen cells analyzed by flow cytometry, and sera collected to measure autoantibodies by ELISA. In some experiments, mice were treated with Rapamycin every day for one week. Kidneys were collected to analyze T cell infiltration by flow cytometry. Spleen cells were cultured with anti-CD3 (2 µg/ml) and anti-CD28 (2 µg/ml) for 24 hours and IL-17A measured in supernatants by ELISA (Biolegend).

Statistics

Student’s two-tailed t-test, Mann-Whitney U test, and one-way analysis of variance (ANOVA) were used to calculate statistical significance among groups. A p value of less than 0.05 was considered significant. In all graphs, data represent mean ± standard error of mean (SEM).

Study Approval

For human studies written informed consent was obtained from all participants and all studies were approved by the institutional review board (Committee on Clinical investigations) at BIDMC. All animal studies were approved by the Institutional Animal Care and Use Committee at BIDMC.
AUTHOR CONTRIBUTIONS

VRM conceptualized the study, TK, HL, DC, VRM designed, performed the experiments, analyzed and interpreted data and TK, HL, GCT, VRM prepared the manuscript.

ACKNOWLEDGEMENTS

We thank Andrew Gillooly, Michael Mosho, Melissa Carr-Reynolds for excellent technical assistance and Ms. Suzanne Krishfield for recruiting SLE patients and healthy individuals, Dr. Donna Farber and Dr. Martin Flajnik for critical reading of the manuscript and Dr. Amir Sharabi and Dr. Abel Suarez-Fueyo for helpful discussions. **Funding:** This work was funded by NIH grants K01AR060781 and R01AR068974 to VRM, R01AI42269 to GCT and an SICPA Foundation grant to DC. **Competing interests:** Authors have no competing financial interests.
REFERENCES


21. Kato H, Perl A. Mechanistic target of rapamycin complex 1 expands Th17 and IL-4+ CD4-CD8-
   2014;192(9):4134–4144.

22. Lai Z-W et al. Mechanistic target of rapamycin activation triggers IL-4 production and necrotic

23. Stambolic V et al. Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor


25. Buckler JL, Walsh PT, Porrett PM, Choi Y, Turka LA. Cutting edge: T cell requirement for CD28
costimulation is due to negative regulation of TCR signals by PTEN. J. Immunol. 2006;177(7):4262–
   4266.

26. Di Cristofano A et al. Impaired Fas response and autoimmunity in Pten+/- mice. Science

27. Suzuki A et al. T cell-specific loss of Pten leads to defects in central and peripheral tolerance.

28. Wu X et al. Defective PTEN regulation contributes to B cell hyperresponsiveness in systemic

29. Howard JM, Sanford JR. The RNAissance family: SR proteins as multifaceted regulators of gene

30. Li X, Manley JL. New talents for an old acquaintance: the SR protein splicing factor ASF/SF2


38. Kono M et al. Decreased expression of Serine/arginine-rich splicing factor 1 in T cells from patients with active systemic lupus erythematosus accounts for reduced expression of RasGRP1 and DNA methyltransferase 1. *Arthritis & Rheumatology (Hoboken, N.J.)* [published online ahead of print: June 14, 2018]; doi:10.1002/art.40585


FIGURE LEGENDS

Figure 1. Srsf1-cKO mice develop systemic autoimmunity and lupus nephritis

(A) Heat maps show autoantibody microarray data from sera of 12-week old WT and Srsf1-cKO mice (n=3 each) and one MRL/lpr mouse, analyzed for IgG reactivity against a 128-autoantigen panel. (B) Spleen images from aged (12-18 month old) WT and Srsf1-cKO mice. Data are representative from at least four independent experiments. (C) Flow cytometry plots and graphs show Ki67 staining on live-gated CD4 and CD8 T cells from spleen of WT and Srsf1-cKO mice (2-18 month old, n= 11WT, 8KO). (D) Representative light microscopy images of hematoxylin and eosin (H and E) stained paraffin sections of kidneys from aged (9-18 month old) mice. (E) Graph shows semiquantitative scores for kidney histopathology (n= 12WT, 9KO). (F) Graph shows proteinuria from WT and Srsf1-cKO mice (n=7 each). (E: Mann-Whitney U test, C and F: unpaired t-test, *p<0.05, ***p<0.005).

Figure 2. T cells from Srsf1-cKO mice have a hyperactive phenotype and produce proinflammatory cytokines

Spleen cells were isolated from WT and Srsf1-cKO mice and analyzed by flow cytometry. (A) Plots show CD69 expression gated on live CD4 T cells. (B) Graph shows percent of CD69+CD4 T cells (n=13WT, 14KO, <20wks old mice). (C) Plots show CD62L and CD44 staining gated on live CD4 and CD8 T cells in spleen (D) Graphs show percent of activated effector/memory subsets (CD44hiCD62Lhi and CD44hi CD62Llo) of CD4 and CD8 T cells (n=CD4 11WT, 13KO, CD8 18WT, 16KO, <20wks old mice). (E) Graphs show percent of naïve (CD44lo CD62Lhi) CD4 and CD8 T cells (n=CD4 11WT, 13KO, CD8 18WT, 16KO, <20wks old mice). (F) Spleen cells were stimulated for 4h with PMA, Ionomycin with Monensin followed by surface and intracellular cytokine staining for flow cytometry. Plots show IL-17, IL-4 and IFN-γ staining gated on live CD4 and CD8 T cells. Graphs below show percent cytokine-producing CD4 and CD8 T cells from spleen (n=IL-17:8 each, IL-4:6-7, CD4 IFN-γ:13 each, CD8 IFN-γ:3-4; CD4: <20wks old mice, CD8: 9-40wks old mice). (G) Splenocytes from WT or Srsf1-cKO mice were stained for PD1 and CXCR5 by flow cytometry. Plots show cells
gated on live CD4 T cells. Graph below shows percent Tfh cells (n=20WT, 21KO, 10-28 wks old mice) (unpaired t-test, *p<0.05, **p<0.005).

Figure 3. Transcriptomics analysis of CD4 effector T cells from Srsf1-cKO mice reveals an elevated T cell activation gene signature

(A) CD4 effector T (Teff) cells were generated as follows. Naïve CD4 T cells were sorted from spleens of WT and Srsf1-cKO mice (n=3 each), and stimulated with anti-CD3 (0.5 µg/mL) and anti-CD28 (1.0 µg/mL) for 72h. RNA-sequencing data analysis shows differentially expressed (DE) genes with fold change (FC) differences at p<0.05. (B) GO terms enrichment map of DE genes. The size of the red circles indicates the number of genes within a given pathway, and the color represents the p-values relative to the other displayed terms. Outlines (added manually) indicate groups of similar GO terms. (C) Heat map showing average expression of DE cytokine genes in WT and Srsf1-cKO mice.

Figure 4. T cells from Srsf1-cKO mice exhibit increased activation of the mTORC1 pathway and reduced expression of PTEN. (A) Fold expression of the mTOR pathway genes from RNA-sequencing data. (B-D) Naïve CD4 T cells were isolated from spleens of mice, and stimulated with anti-CD3 (0.5 µg/mL) and anti-CD28 (1.0 µg/mL) for 72h. (B) Total protein was immunoblotted for p-S6 and total-S6. Data are from one representative of three independent experiments. Graph shows relative densitometry quantitation of pS6 and S6 (n=3 each). (C) After 72h, additional stimulation with anti-CD3 (10µg/mL) and anti-CD28 (10µg/mL) for 5min was performed. Total protein was immunoblotted for p-AKT and total-AKT. Data are from one representative of four independent experiments. Graph shows relative densitometry quantitation of p-AKT and AKT (n=4 each). (D) Total RNA was isolated and reverse transcribed. Pten expression was measured by real time qPCR and normalized to housekeeping gene cyclophilin A (n=5 each). (E) Total protein was isolated from total T cells and immunoblotted for PTEN and β-actin. Data are from one representative of four independent experiments. Graph shows densitometric quantitation of PTEN normalized to β-actin (n=4 each). (F) HEK293T cells were transfected with a pGL3-PTEN 3`UTR luciferase plasmid and co-transfected with
either a pcDNA3.1 empty vector (EV) or an Srsf1 (pSrsf1) plasmid, and included an internal control pRL-TK luciferase plasmid. Cells were lysed and dual luciferase assays performed. Graph shows relative luciferase units normalized to the internal luciferase control from n=4 in four independent experiments. (G) T cells from spleens of mice were transfected with a PTEN-3'UTR-Luc plasmid and either EV or pSrsf1 plasmid. 24h later, cells were lysed and luciferase activity measured as in (F) (n=7). (B-E: unpaired t-test, F and G: One-way ANOVA with Bonferroni correction, *p<0.05, **p<0.005, ***p<0.0005 mean± SEM).

**Figure 5. Rapamycin treatment reduces proinflammatory cytokine production by T cells and alleviates autoimmunity in Srsf1-cKO mice. (A - D) Spleen cells from WT or Srsf1-cKO mice were cultured for 4h with PMA plus Ionomycin in the presence of Monensin. Rapamycin (1 or 10nM) was added for the duration of cultures. Cells were collected, surface stained, fixed and permeabilized for intracellular cytokine staining. (A) and (C) Plots show IL-17 and IFN-γ intracellular staining gated on live CD4 T cells. (B) Graphs show data from n=8 mice in seven independent experiments. (D) Graph shows data from n=15 mice each in ten independent experiments. (E - H) Rapamycin (2 mg/kg) or PBS was administered to WT and Srsf1-cKO mice by intraperitoneal injection once every two days for four weeks (F and G) or every day for one week (E and H). (E) Spleen cells were cultured with anti-CD3 (2 µg/ml) and anti-CD28 (2 µg/ml) for 24h and IL-17A measured in the supernatants by ELISA. Data are shown as fold-values normalized to controls (n=5 mice in two independent experiments). (F) Spleen cells were analyzed by flow cytometry, and graph shows the frequency of Tfh cells in spleen (n=5-6 mice in two independent experiments). (G) Serum was collected and autoantibodies measured by ELISA. (n=5-6 mice in two independent experiments). (H) Cells from kidneys were analyzed by flow cytometry, and graph shows the frequency of T cells in kidneys (n=5 mice in two independent experiments). (B, D, E and F: One-way ANOVA with Bonferroni correction, G and H: two-tailed unpaired t-test, *p<0.05, **p<0.005, n.s.: no significant difference, mean ± SEM).
Figure 6. PTEN overexpression reduces proinflammatory cytokine production by T cells from Srsf1-cKO mice (A) T cells were isolated from spleens of WT or Srsf1-cKO mice and transfected by electroporation with empty vector (EV) or PTEN overexpression plasmid (pPTEN). 16h later, cells were stimulated with PMA plus ionomycin in the presence of Monensin for 4h and cells were collected, surface stained, fixed and permeabilized for intracellular cytokine staining. (B) Graphs show average data from n=9 mice in five independent experiments. (two-tailed paired t-test, *p<0.05, n.s.: no significant difference, mean ± SEM).

Figure 7. PTEN is decreased in T cells from SLE patients and correlates with SRSF1 levels. SRSF1 overexpression increases PTEN, suppresses mTORC1 activity and reduces proinflammatory cytokine production. (A and B) Peripheral blood T cells were isolated from patients with SLE and normal (N) healthy control individuals. Total protein was immunoblotted for PTEN, SRSF1 and β-actin. (A) Data are from one representative of ten independent experiments. Graph shows relative quantitation by densitometry (n=14 SRSF1 low and 12 SRSF1 normal patients). (B) Graph shows a linear correlation between relative PTEN and SRSF1 expression. (C) Peripheral blood T cells were isolated from patients with SLE, and transfected with empty vector (EV) or SRSF1 overexpression plasmid (pSrsf1). At 16-18h after transfections, SLE T cells were stimulated with anti-CD3, anti-CD28 and crosslinker for 5min. Total protein was immunoblotted for PTEN. Data are from one representative of five independent experiments (n=10). Graph shows relative quantitation by densitometry. (D) Total protein was immunoblotted for phosphorylated S6 (p-S6) and total S6 in stimulated SLE T cells after transfection as described in (C). Data are from one representative of four independent experiments (n=4). Graph shows relative quantitation by densitometry. (E) At 16-18h after transfection, cells were stimulated with PMA and Ionomycin in the presence of Monensin. After 4h, cytokine production was analyzed by flow cytometry. Graphs show quantitation of cytokine data (n=8). (Paired t-test, *p<0.05, n.s.: no significant difference, mean± SEM).

SUPPLEMENTAL FIGURE LEGENDS AND TABLES
Supplemental Figure 1. Generation of Srsf1-cKO mice. (A) Tail genomic DNA was used for PCR amplification of Srsf1-flox and dLck-Cre genes. WT (Srsf1 +/+ Cre+, or Srsf1 flox/flox Cre-) and Srsf1-cKO (Srsf1 flox/flox Cre+) mice are indicated. (B) Spleen cells were isolated from WT and Srsf1-cKO mice, surface stained for Thy1.2, TCRβ, CD4, CD8 and intracellular stained for SRSF1. Plots show SRSF1 staining gated on live T cells, CD4 and CD8 T cells. (C) Body weight and thymus weight of 4-14 week old mice (Body Weight: n= 11WT, 10KO, Thymus weight: n= 14WT, 13KO). (D) Cells were isolated from thymus and analyzed by flow cytometry. Graph shows thymus cell counts (n= 10WT, 9KO). (E) Flow cytometry plots show thymocyte populations gated on live Thy1.2+ cells. (F) Graph shows absolute numbers of DN, DP, and SP CD4 and CD8 T cells in thymus (n=10WT, 9KO).

Supplemental Figure 2. Srsf1-cKO mice develop systemic autoimmune disease (A) Sera from WT and Srsf1-cKO mice (24 week-old) were analyzed for anti-nuclear antibodies (ANA) by ELISA (n=6-7 each). (B) Graphs show spleen weights (n= 11WT, 8KO) and absolute numbers of T cells (n=5WT, 4KO) from aged (12 - 18 month old) mice. Data are representative from at least four independent experiments. (C) Representative images of immunohistochemistry staining of kidneys from WT and Srsf1-cKO (12 month old) mice. Original magnification, x4 (upper); x20 (lower). (D) Confocal imaging analysis of frozen spleen sections from wild type or Srsf1-cKO mice stained with fluorescent-conjugated anti-IgD, B220, CD4 and PNA-biotin followed by an Alexa 350–conjugated secondary streptavidin as described in the Methods. Original magnification, ×20. Graph shows the frequency of follicles with GCs (n=4 each). (E) Plots show GL-7 and Fas staining for germinal center (GC) B cells gated on live CD19+ B cells. Graph shows percent GL-7+ Fas+ B cells among live B cells (4-12wks, n= 6-7 each). (F) Splenocytes were stained for B220 and IgD. Plots show B220 and IgD staining gated on live B cells. Graph shows percent IgD- B cells among live B cells (n=3 each). (unpaired t-test, *p<0.05, **p<0.005).

Supplemental Figure 3. Non-T cell populations in WT and Srsf1-cKO mice. Spleen cells were isolated from WT and Srsf1-cKO mice and analyzed by flow cytometry. (A) Plots show CD19+ B cells,
CD11b+ cells and CD11c+ cells. (B) Graph shows percent (upper panels) and absolute numbers (lower panels) of these cells (n=18-20).

Supplemental Figure 4. Examination of Treg cells from Srsf1-cKO mice. (A) Spleen cells from wild-type or Srsf1-cKO young (6-20 weeks) and aged (12-18 months) mice were surface stained for CD4 and CD25, and intracellular stained for FoxP3 and analyzed by flow cytometry (B) Graphs show Treg cell frequencies (n=young 8WT, 9KO, aged 16WT, 12KO). (C) Conventional CD4 T (Tconv) cells were labeled with CFSE and co-cultured with Treg cells at increasing ratios for 3 days and proliferation of Tconv analyzed by flow cytometry. Representative plots are shown. (D) Graph shows proliferation of Tconv cells from four independent experiments (n= 4WT, 5KO).

Supplemental Figure 5. Short isoform of S6K1 is decreased in T cells from Srsf1-cKO mice. Naïve CD4 T cells were isolated from spleen of WT and Srsf1-cKO mice, and stimulated with anti-CD3 (0.5 µg/ml) and CD28 (1.0 µg/ml) for 3 days. Total RNA was isolated and reverse transcribed. S6K1 (Rps6kb1) short isoform expression was measured by real time qPCR and normalized to housekeeping gene cyclophilin A (n=4 each). (unpaired t-test, *p<0.05)

Supplemental Figure 6. PTEN overexpression by transfection in mouse T cells. (A-C) T cells were isolated from spleens of WT or Srsf1-cKO mice and transfected by electroporation with empty vector (EV) or PTEN overexpression plasmid (pPTEN) or left untransfected. Sixteen hours later, cells were collected, and Pten mRNA expression levels were assessed by qPCR (A) and protein levels by Western blotting (B) (n=4 each). (C) After 16h culture, cells were stimulated with PMA plus ionomycin in the presence of Monensin for 4h. Cells were collected, surface stained, fixed and permeabilized for intracellular cytokine staining. Graph shows IFN-γ-producing CD4+ cells in WT T cells without transfection and with EV transfection (n=3 each) (two-tailed paired t-test, *p<0.05).

Supplemental Figure 7. Schematic summary of the role of SRSF1
in T cells from Srsf1-cKO mice and SLE patients. SRSF1 contributes to the control of activation of
the mTOR pathway via the regulation of PTEN expression. In T cells from KO mice and SLE patients,
reduced SRSF1 levels contribute to reduced PTEN levels and increased activity of the mTOR
pathway. Rapamycin blocks the mTORC1 pathway and reduces proinflammatory cytokines.
Table 1. Pathway analysis of RNA-sequencing of CD4 effector T cells from Srsf1-cKO mice

Table shows pathways and gene counts within, identified by the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and gene ontology (GO) enrichment analyses of differentially expressed (DE) genes.

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Supplemental Table 1. Demographics and clinical characteristics of SLE patients.

Table shows demographic and clinical features of SLE patients. Values shown are mean ± SD (minimum - maximum).

SLEDAI: systemic lupus erythematosus disease activity index, WBC: white blood cells

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Supplemental Table 2. Table shows list of primers and gene sequences

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Figure 1. 

(A) Heat maps show autoantibody microarray data from sera of 12-week old WT and Srsf1-cKO mice (n=3 each) and one MRL/lpr mouse, analyzed for IgG reactivity against a 128-autoantigen panel. 

(B) Spleen images from aged (12-18 month old) WT and Srsf1-cKO mice. Data are representative from at least four independent experiments. 

(C) Flow cytometry plots and graphs show Ki67 staining on live-gated CD4 and CD8 T cells from spleen of WT and Srsf1-cKO mice (2-18 month old, n=11 WT, 8KO). 

(D) Representative light microscopy images of hematoxylin and eosin (H and E) stained paraffin sections of kidneys from aged (9-18 month old) mice. 

(E) Graph shows semiquantitative scores for kidney histopathology (n=12 WT, 9 KO). 

(F) Graph shows proteinuria from WT and Srsf1-cKO mice (n=7 each). 

(E: Mann-Whitney U test, C and F: unpaired t-test, *p<0.05, ***p<0.005).
Figure 2.

A

CD69 - CD4+ (%)

WT KO

B

CD69+ CD4+ (%)

WT KO

C

CD44 - CD62L (%)

WT KO

D

Activated T cells

CD44hi CD62Llo (%)

KO WT

CD44hi CD62Lhi (%)

KO WT

E

Naive T cells

CD44lo CD62Lhi (%)

KO WT

CD44lo CD62Llo (%)

KO WT

F

CD4+ CD8+ (%)

IL-17+ (%)

IFN-γ+ (%)

WT KO

G

CD4+ CD8+ (%)

PD1+ CXCR5+ CD4 (%)

KO WT

IL-17+ CD4+ (%)

IFN-γ+ CD4+ (%)

CD4+ CD8+ (%)

**
Figure 2. T cells from Srsf1-cKO mice have a hyperactive phenotype and produce proinflammatory cytokines

Spleen cells were isolated from WT and Srsf1-cKO mice and analyzed by flow cytometry. **(A)** Plots show CD69 expression gated on live CD4 T cells **(B)** Graph shows percent of CD69+CD4 T cells (n=13WT, 14KO, <20wks old mice) **(C)**. Plots show CD62L and CD44 staining gated on live CD4 and CD8 T cells in spleen **(D)** Graphs show percent of activated effector/memory subsets (CD44hiCD62Lhi and CD44hi CD62Llo) of CD4 and CD8 T cells (n=CD4 11WT, 13KO, CD8 18WT, 16KO, <20wks old mice). **(E)** Graphs show percent of naïve (CD44lo CD62Lhi) CD4 and CD8 T cells (n=CD4 11WT, 13KO, CD8 18WT, 16KO, <20wks old mice). **(F)** Spleen cells were stimulated for 4h with PMA, Ionomycin with Monensin followed by surface and intracellular cytokine staining for flow cytometry. Plots show IL-17, IL-4 and IFN-γ staining gated on live CD4 and CD8 T cells. Graphs below show percent cytokine-producing CD4 and CD8 T cells from spleen (n=IL-17:8 each, IL-4:6-7, CD4 IFN-γ:13 each, CD8 IFN-γ:3-4; CD4: <20wks old mice, CD8: 9-40wks old mice). **(G)** Splenocytes from WT or Srsf1-cKO mice were stained for PD1 and CXCR5 by flow cytometry. Plots show cells gated on live CD4 T cells. Graph below shows percent Tfh cells (n=20WT, 21KO, 10-28 wks old mice) (unpaired t-test, *p<0.05, **p<0.005).
Figure 3. Transcriptomics analysis of CD4 effector T cells from Srsf1-cKO mice reveals an elevated T cell activation gene signature

(A) CD4 effector T (Teff) cells were generated as follows. Naïve CD4 T cells were sorted from spleens of WT and Srsf1-cKO mice (n=3 each), and stimulated with anti-CD3 (0.5 µg/ml) and anti-CD28 (1.0 µg/ml) for 72h. RNA-sequencing data analysis shows differentially expressed (DE) genes with fold change (FC) differences at p<0.05. (B) GO terms enrichment map of DE genes. The size of the red circles indicates the number of genes within a given pathway, and the color represents the p-values relative to the other displayed terms. Outlines (added manually) indicate groups of similar GO terms. (C) Heat map showing average expression of DE cytokine genes in WT and Srsf1-cKO mice.
Figure 4.

A. Log2FC (KO / WT) for mTOR signal related genes.

B. Western blot analysis showing pS6/S6 ratio in WT and KO.

C. Western blot analysis showing pAKT (Ser473) and pAKT (Thr308) normalized to AKT in WT and KO.

D. Relative Pten mRNA expression in WT and KO.

E. Western blot analysis showing PTEN and β-Actin in WT and KO.

F. Luciferase activity for PTEN 3'UTR-Luc (RLU) with different pSrsf1 concentrations.

G. Luciferase activity for PTEN 3'UTR-Luc (RLU) with different conditions.
Figure 4. T cells from Srsf1-cKO mice exhibit increased activation of the mTORC1 pathway and reduced expression of PTEN. (A) Fold expression of the mTOR pathway genes from RNA-sequencing data. (B-D) Naïve CD4 T cells were isolated from spleens of mice, and stimulated with anti-CD3 (0.5 µg/mL) and anti-CD28 (1.0 µg/mL) for 72h. (B) Total protein was immunoblotted for p-S6 and total-S6. Data are from one representative of three independent experiments. Graph shows relative densitometry quantitation of pS6 and S6 (n=3 each). (C) After 72h, additional stimulation with anti-CD3 (10µg/mL) and anti-CD28 (10µg/mL) for 5min was performed. Total protein was immunoblotted for p-AKT and total-AKT. Data are from one representative of four independent experiments. Graph shows relative densitometry quantitation of p-AKT and AKT (n=4 each). (D) Total RNA was isolated and reverse transcribed. Pten expression was measured by real time qPCR and normalized to housekeeping gene cyclophilin A (n=5 each). (E) Total protein was isolated from total T cells and immunoblotted for PTEN and β-actin. Data are from one representative of four independent experiments. Graph shows densitometric quantitation of PTEN normalized to β-actin (n=4 each). (F) HEK293T cells were transfected with a pGL3-PTEN 3’UTR luciferase plasmid and co-transfected with either a pcDNA3.1 empty vector (EV) or an Srsf1 (pSrsf1) plasmid, and included an internal control pRL-TK luciferase plasmid. Cells were lysed and dual luciferase assays performed. Graph shows relative luciferase units normalized to the internal luciferase control from n=4 in four independent experiments. (G) T cells from spleens of mice were transfected with a PTEN-3’UTR-Luc plasmid and either EV or pSrsf1 plasmid. 24h later, cells were lysed and luciferase activity measured as in (F) (n=7). (B-E: unpaired t-test, F and G: One-way ANOVA with Bonferroni correction, *p<0.05, **p<0.005, ***p<0.0005 mean± SEM).
Figure 5.

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Figure 5. Rapamycin treatment reduces proinflammatory cytokine production by T cells and alleviates autoimmunity in Srsf1-cKO mice. (A - D) Spleen cells from WT or Srsf1-cKO mice were cultured for 4h with PMA plus Ionomycin in the presence of Monensin. Rapamycin (1 or 10nM) was added for the duration of cultures. Cells were collected, surface stained, fixed and permeabilized for intracellular cytokine staining. (A) and (C) Plots show IL-17 and IFN-γ intracellular staining gated on live CD4 T cells. (B) Graphs show data from n=8 mice in seven independent experiments. (D) Graph shows data from n=15 mice each in ten independent experiments. (E - H) Rapamycin (2 mg/kg) or PBS was administered to WT and Srsf1-cKO mice by intraperitoneal injection once every two days for four weeks (F and G) or every day for one week (E and H). (E) Spleen cells were cultured with anti-CD3 (2 µg/ml) and anti-CD28 (2 µg/ml) for 24h and IL-17A measured in the supernatants by ELISA. Data are shown as fold-values normalized to controls (n=5 mice in two independent experiments). (F) Spleen cells were analyzed by flow cytometry, and graph shows the frequency of Tfh cells in spleen (n=5-6 mice in two independent experiments). (G) Serum was collected and autoantibodies measured by ELISA (n=5-6 mice in two independent experiments). (H) Cells from kidneys were analyzed by flow cytometry, and graph shows the frequency of T cells in kidneys (n=5 mice in two independent experiments). (B, D, E and F: One-way ANOVA with Bonferroni correction, G and H: two-tailed unpaired t-test, *p<0.05, **p<0.005, n.s.: no significant difference, mean ± SEM).
Figure 6. PTEN overexpression reduces proinflammatory cytokine production by T cells from Srsf1-cKO mice (A) T cells were isolated from spleens of WT or Srsf1-cKO mice and transfected by electroporation with empty vector (EV) or PTEN overexpression plasmid (pPTEN). 16h later, cells were stimulated with PMA plus ionomycin in the presence of Monensin for 4h and cells were collected, surface stained, fixed and permeabilized for intracellular cytokine staining. (B) Graphs show average data from n=9 mice in five independent experiments. (two-tailed paired t-test, *p<0.05, n.s.: no significant difference, mean ± SEM).
Figure 7.

A. Western blots showing PTEN, SRSF1, and β-Actin levels in N and SLE samples. Low SRSF1 levels are indicated in N samples. Normal SRSF1 levels are indicated in SLE samples.

B. Scatter plot showing the correlation between PTEN expression and SRSF1 expression in N and SLE samples. The regression line is shown with an R² value of 0.226 and a p-value of 0.0163.

C. Western blots showing PTEN and β-Actin levels in EV and pSrsf1 samples.

D. Western blots showing pS6 and S6 levels in EV and pSrsf1 samples.

E. Bar charts showing the percentage of IL-17+ and IFN-γ+ CD4+ cells in EV and pSrsf1 samples. The results are shown as mean ± SEM, with * indicating statistical significance (n.s. for non-significant).
Figure 7. PTEN is decreased in T cells from SLE patients and correlates with SRSF1 levels. SRSF1 overexpression increases PTEN, suppresses mTORC1 activity and reduces proinflammatory cytokine production. (A and B) Peripheral blood T cells were isolated from patients with SLE and normal (N) healthy control individuals. Total protein was immunoblotted for PTEN, SRSF1 and β-actin. (A) Data are from one representative of ten independent experiments. Graph shows relative quantitation by densitometry (n=14 SRSF1 low and 12 SRSF1 normal patients). (B) Graph shows a linear correlation between relative PTEN and SRSF1 expression. (C) Peripheral blood T cells were isolated from patients with SLE, and transfected with empty vector (EV) or Srsf1 overexpression plasmid (pSrsf1). At 16-18h after transfections, SLE T cells were stimulated with anti-CD3, anti-CD28 and crosslinker for 5 min. Total protein was immunoblotted for PTEN. Data are from one representative of five independent experiments (n=10). Graph shows relative quantitation by densitometry. (D) Total protein was immunoblotted for phosphorylated S6 (p-S6) and total S6 in stimulated SLE T cells after transfection as described in (C). Data are from one representative of four independent experiments (n=4). Graph shows relative quantitation by densitometry. (E) At 16-18h after transfection, cells were stimulated with PMA and Ionomycin in presence of Monensin. After 4h, cytokine production was analyzed by flow cytometry. Graphs show quantitation of cytokine data (n=8). (Paired t-test, *p<0.05, n.s.: no significant difference, mean± SEM).