SRPK3 regulates alternative pre-mRNA splicing required for B lymphocyte development and humoral responsiveness

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One Sentence Summary: SRPK3 regulates alternative splicing of pre-mRNA that is crucial for B cell development, activation and antibody responses.

Abstract:

Alternative splicing (AS) of pre-mRNA is a critical component of transcriptional regulation that diversifies the cellular proteome. The Serine-Arginine Protein Kinases (SRPK) initiate early events in AS. Using conditional knockout mice (*cKO*), we demonstrated the importance of the X-linked gene *Srpk3* in B lymphocyte development and in response to immunization in vivo. Significantly decreased numbers of immature and mature B cells were observed in *Srpk3-cKO* mouse bone marrow relative to wild-type (*WT*). Immunization of *Srpk3-cKO* mice with the T lymphocyte-independent type-2 antigen 4-hydroxy-3-nitrophenylacetyl-Ficoll (NP-Ficoll) elicited greatly reduced amounts of NP-specific IgG3. *Srpk3* deletion resulted in hundreds of differentially spliced mRNAs in B cells, including mRNAs encoding proteins associated with signaling pathways and mitochondrial function. We conclude that SRPK3 is an immunomodulatory kinase that controls humoral immunity via its regulation of pre-mRNA splicing, antibody production, and metabolism in B cells.

Introduction:

Co-transcriptional mechanisms of gene regulation are emerging as important contributors to immune cell differentiation and function. One such mechanism is alternative pre-mRNA splicing (AS), which increases informational diversity, functional capacity, and the efficiency of protein expression (*1*). Recent studies have confirmed that AS influences immunity and tolerance through its roles in lymphocyte homeostasis (*2*). Additionally, B cell differentiation, activation, and maturation to antigen secreting cells (ASCs) are dependent on unique splicing patterns that modulate B cell responsiveness and function (*3, 4*). However, the linkage of AS with upstream signaling pathways including the B cell receptor (BCR) and its co-receptors has not been dissected in B cells. Therefore, understanding how pre-mRNA splicing is regulated in these cells is of critical importance.

The functional spliceosome is a dynamic machine comprised of approximately 20 protein and RNA components that regulate splice site selection in pre-mRNAs concurrently with transcription (*5*). The choice of consensus or non-consensus 5' and 3' sites for splicing of pre-mRNAs is a regulated process with multiple possible outcomes including inclusion or exclusion of exons or introns (reviewed in *6*). Upstream regulators of these pathways include the Serine-Arginine protein kinase (SRPK) family, a group (SRPK1, 2, and 3 in mammals) of highly conserved serine-threonine kinases (CMGC kinases), which phosphorylate Serine-Arginine repeat (SR)-rich proteins in response to extracellular signals (*7-10*). SR proteins include twelve SR Splicing Factors (SRSF) that promote splice site selection by bridging between initial splice site selection in the pre-spliceosome and assembly of the mature spliceosome.

In mammals, SRPK1 and SRPK2 have been extensively studied as regulators of AS and mRNA maturation (*11-13*), transduction of growth signaling (*14*), chromatin reorganization (*15*),

cell cycle (*7*) and metabolic signaling (*16*). However, the function of SRPK3 has not been elucidated. SRPK3 possesses bi-lobed kinase domains that are nearly identical with those of SRPK1 and -2, but possesses divergent N-terminal, linker, and C-terminal domains. SRPK1 is widely expressed, while SRPK2 and SRPK3 are expressed in different subsets of cells. SRPK3 was identified first as a transcriptional target of Myocyte Enhancer Factor 2C (MEF2C) in skeletal muscle and the heart (*17*). Later studies identified the X-linked *Srpk3* gene as a target of MEF2C and the B lineage master regulator PAX5 in B cells (*18-20*).

Expression patterns of *Srpk3* in BCR^+ B cells of the bone marrow and periphery suggest that the kinase is important for B cell signaling and antibody responses. Here, we use B cell-specific conditionally deleted *Srpk3* (*Srpk3-cKO*) mice to demonstrate that SRPK3 is essential for efficient development of B cells in the bone marrow, where immature and mature B cells are decreased in its absence. Decreased generation of these cells correlates with extensive changes in AS events. *Srpk3-cKO* mice exhibit depressed antigen-specific IgG3 production in response to 4-hydroxy-3-nitrophenylacetyl-Ficoll (NP-Ficoll), suggesting that marginal zone B cells, which also exhibit changes in AS, require SRPK3 for robust antibody production. Notably, *Srpk3-cKO* male and homozygous mutant female mice are partially immune deficient, while hemizygous female mice generate intermediate antibody responses. Overall, loss of SRPK3 profoundly affects B cell development and function, and cellular metabolism including mitochondrial biogenesis and function necessary for normal immune responses of B cells.

Results

Deletion of Srpk3 *in vivo results in decreased immature and mature bone marrow B cells*

To assess the physiological roles of SRPK3 in B cell development *in vivo*, we bred *Srpk3*-floxed female (*Srpk3*$^{fl/fl}$) or male (*Srpk3*$^{fl/y}$) mice with transgenic mice that express Cre recombinase in early B cell progenitors (*CD79aCre*$^{Tg/+}$) (*21, 22*). Deletion of *Srpk3* floxed alleles results in loss of exons 6-8, which encode functional kinase residues (Fig. S1A). In resulting *Srpk3* conditional knockout (*Srpk3*$^{cKO/cKO}$ females or *Srpk3*$^{cKO/y}$ males; collectively *Srpk3-cKO*) mice, Cre expression deletion initiates in early pre-pro B cells in the bone marrow prior to expression of *Srpk3* (Fig. S1 B-D).

To characterize phenotypic differences in bone marrow B cell progenitors lacking SRPK3, we used flow cytometry to determine relative frequencies of cells representing phenotypic fractions in bone marrow of wild-type and *Srpk3-cKO* male and female mice (*23*). In *Srpk3-cKO* bone marrow, early stages of B cell development are unaffected, while later stages including the number and frequency of immature and mature B cells are decreased (Fig. 1, A-E). These data suggest that *Srpk3* expression is necessary for robust development of BCR expressing cells in the bone marrow microenvironment. In contrast, we observed no differences between in splenic B cell populations of wild type (*WT*) and *Srpk3-cKO* mice (Fig. S2, A-E).

The decrease in immature bone marrow B cell numbers in *Srpk3-cKO* mice could be due to their inability to undergo appropriate Ig light chain gene rearrangements. Using flow cytometry, we observed that *Srpk3-cKO* immature and mature B cells had increased ratios of κ/λ light chains relative to *WT* cells (Fig. 2, A-D). *S*plenic B cells also trend towards skewed frequencies of κ/λ^{+} B cells (Fig. S3, A and B). No significant differences were detected between frequencies of intracellular κ^{+} cells or κ mean fluorescence intensity (MFI) in *Srpk3-cKO* versus *WT* pre-B cells (Fig. S4, A and B). The detected changes in light chain frequencies suggest that SRPK3 is important for the generation of normal BCR repertoires in mice.

SRPK3 regulates patterns of AS in immature and mature bone marrow B cells

Maximal *Srpk3* expression coincides with the upregulation of BCR expression on immature B cells during B lymphopoiesis (*24*). Therefore, we hypothesized that SRPK3 regulates the splicing of pre-mRNAs that are necessary to generate immature B cells, and/or for their positive/negative selection in the bone marrow. For comparison, we also analyzed mRNAs isolated from mature bone marrow B cells, which can be activated to secrete antibodies by blood-borne antigens (*25*). We performed high-throughput RNA-sequencing (RNA-seq) to determine genome-wide changes in transcript frequencies and patterns of AS. Purity of sorted populations was assessed (Fig. S5A) and principal component analysis of RNA-seq was performed to determine relationships between biological replicates between populations (Fig. S5B). Differential gene expression analysis identified only 5 genes that are differentially expressed between *WT* and *Srpk3-cKO* immature B cells: including *Cd79a* (due to the *Cre* knock-in allele) (≥ 1.5 fold; $P_{adj} < 0.05$). Similarly, only 18 genes were differentially expressed between *WT* and *Srpk3-cKO* mature B cells: which included *Col5a3*, a gene that encodes α chain for fibrillar collagens that regulates metabolism in mice (*26*), and *Fms-tyrosine kinase 3 (Flt3),* a gene that is essential for germinal center B cell function and class-switching (*27*) (≥ 1.5 fold, $P_{adj} < 0.05$) (Data file S1). However, analysis of AS events revealed robust changes in splicing patterns between mRNAs of *WT* and *Srpk3-cKO* immature and mature bone marrow B cells. Analysis of AS detected 290 significant AS events in immature B cells and 620 significant AS events in mature B cells ($\Delta\Psi \geq 0.1$, FDR < 0.05) (Fig. 3, A and B; Data file S2). In both immature and mature B cells, skipped exons (SE) comprised the majority of AS events, with differences in the inclusion of mutually exclusive exons (MXE) as second most frequent type of AS. Other types of events, including alternative 3' splice sites

(A3SS), alternative 5' splice sites (A5SS) and retained introns (RI) were detected at lower frequencies. When displayed in a heat map that separates *WT* from *Srpk3-cKO* AS events (Fig. 3, A and B), it is apparent that different types of AS are detected both in the absence or presence of SRPK3. Next, we used Ingenuity Pathway Analysis to identify pathways affected by AS in immature and mature B cells. AS of genes relating to several cellular pathways are affected in *Srpk3-cKO* immature B cells including Ataxia-Telanglectasia Mutated (ATM) signaling, DNA double-strand break (DSB) repair, DNA damage response (DDR) pathways and cell cycle damage checkpoints, Hypoxia signaling and AMPK (Fig. 3C). Additionally, IPA indicated that critical cellular pathways including ERK5, AMPK, mTOR and TGF-β signaling are affected by the loss of SRPK3 in mature B cells (Fig. 3D). Dysregulation of these pathways may explain the decreased frequencies of immature and mature B cells observed between *WT* and *Srpk3-cKO* mice, because these pathways are each crucial for the development and survival of these cells.

In support of RNA-seq data we validated AS of genes that are strongly affected by the loss of SRPK3, in addition to being crucial components of pathways enriched in IPA between *WT* and *Srpk3-cKO* B cells. AS of transcripts of *Tyrosine kinase 2* (*Tyk2)*, a component of type I and type III interferon signaling pathways that regulates mitochondrial function *(28, 29),* and *NIMA related kinase 4 (Nek4),* which regulates DDR *(30),* genes were confirmed (Fig.3, E and F). Together these data confirm that SRPK3 is a critical regulator of AS that contributed to immature and mature B cell development in the bone marrow.

SRPK3 is essential for T lymphocyte-independent type-2 (TI-2) immune responses in vivo

Genetic database searches [MyGene2, *31*); MARRVEL, *32*)] yielded valuable information regarding known SRPK3 mutations and phenotypes, including immunodeficiency, associated

with genetic variants in human patients. To confirm that SRPK3 deficiency attenuates immune function, we first investigated basal immunoglobulin levels in naïve mice. IgG1 serum levels were significantly elevated in naïve *Srpk3-cKO* mice, whereas IgG3 serum levels trended lower (Fig. S6A). To determine the impact of SRPK3-deficiency on immune responses *in vivo*, we immunized mice with NP-Lipopolysaccharide (LPS), a model T lymphocyte-independent type-1 (TI-1) antigen, or NP-Ficoll, a model TI-2 antigen, and measured NP-specific IgM and IgG3 antibody responses. Similar levels of NP-specific IgM and IgG3 were detected between *Srpk3-cKO* and *WT* mice in response to NP-LPS (over 28 days post-immunization; Fig. S7A). Anti-NP IgM levels were also unaffected between *Srpk3-cKO* and *WT* mice after immunization with NP-Ficoll. However, over two weeks post-immunization, *Srpk3-cKO* mice produced significantly depressed levels of NP-specific IgG3 (Fig. 4, A and B) with levels consistently reduced over a total of 21 days. Immunization with the model T lymphocyte-dependent antigen NP-chicken-gammaglobulin (NP-CGG), and NP-specific IgM and IgG1 titers were measured. Anti-NP IgM titers were decreased in *Srpk3-cKO* mice after initial challenge (Fig. S7B). Additionally, *Srpk3-cKO* mice generated increased levels of NP-specific IgG1 antibodies after primary immunization but failed to increase production after secondary immunization (Fig. S7B). Together these data confirm that SRPK3 is necessary for robust IgG3 production in response to TI-2 antigens.

We hypothesized that the dysregulation of TI-2 antibody responses is due to defective generation or proliferation of NP-specific B cells in *Srpk3-cKO* mice. Indeed, we observed decreased frequencies and overall cell numbers of NP-specific IgM^+ and $IgG3^+$ B cells in *Srpk3-cKO* mice 4 days after immunization with NP-Ficoll (Fig. 4, C-E). These data suggest that either NP-specific SRPK3-deficient B cells do not proliferate to the same extent as WT B cells in response to immunization or have fewer NP-specific B cells prior to immunization. We also

considered whether reductions in NP-specific IgG3$^+$ B cells are due to defective class switch recombination (CSR). We measured the ability of total spleen B cells to undergo CSR in response to NP-Ficoll immunization (Fig. S8A). Relative expression of transcripts associated with CSR indicated no significant differences between *WT* and *Srpk3-cKO* B cells.

SRPK3 is required for Marginal Zone B cell responses to TI-2 antigens

Given that MZ B cells have been characterized to dominate the TI-2 antibody response (*33*), we hypothesized that the inability of *Srpk3-cKO* mice to generate robust immune responses to NP-Ficoll is due, in part, to an intrinsic defect in MZ B cells. Therefore, we purified naïve MZ B cells from *Srpk3-cKO* and *WT* (all male) mice and stimulated them in vitro with dextran-conjugated anti-IgD antibody (α-δ-dex), an immunogen that elicits TI-2 type BCR signaling and activation, together with interleukin-5 (IL-5) and interferon-gamma (IFN-γ) to mimic the response to soluble TI-2 antigens in vivo (*34, 35*). Initially, we measured IgM and IgG3 antibody secretion after 6 days in culture. *Srpk3-cKO* MZ B cells produced significantly lower titers of both IgM and IgG3 compared to *WT* cells (Fig. 5, A and B). These data suggest that SRPK3 is crucial for MZ B cell response to antigen *in vitro*. We next quantitated surface expression of antigen and cytokine receptors using flow cytometry. *Srpk3-cKO* naïve mice had significantly reduced IL-5Rα$^+$ MZ B cells (Fig. S9, A and B). However, we found no relative differences between IgD or IFNγ-receptor (IFNGR1) expression on MZ B cells from *Srpk3-cKO* versus *WT* unimmunized mice (Fig. S9, C-F). We next determined whether defective antibody production was due to failure of MZ B cells to proliferate, differentiate to ASCs, or undergo CSR. *WT* and *Srpk3-cKO* MZ B cell numbers increased similarly in response to activation (α-δ-dex) and unstimulated cells were detected in comparable numbers relative to WT 3 days after in vitro

culture (Fig. S10A). Thus, *Srpk3-cKO* MZ B cells appeared to proliferate normally in response to BCR stimulation and displayed similar in vitro half-life as WT. Next, cells were harvested and analyzed for differentiation to ASCs using CD138 and TACI as plasma cell markers (*36, 37*). Both *Srpk3-cKO* and *WT* MZ B cells differentiated into ASCs similarly after 3 days in culture (Fig. S11, A and B). To assess CSR, we analyzed germline and post-switch transcripts in naïve and stimulated cells. We detected no defects in the abilities of *Srpk3-cKO* MZ B cells to undergo CSR in response to activation (Fig. S12A). These data indicate that *Srpk3-cKO* MZ B cells proliferate and differentiate normally but have additional underlying abnormalities that influence the ability of these cells to respond to antigen.

SRPK3 modulates alternative splicing patterns in naïve and stimulated MZ B cells

To further delineate mechanisms requiring SRPK3 in MZ B cells, we used RNA-seq to interrogate mRNA sequences in naïve *WT* or *Srpk3-cKO* MZ B cells or following their stimulation *in vitro* with α-δ-dex, IL-5 and IFN-γ for 8-hours (initial activation) or 72-hours (class-switched). Purity of sorted populations was assessed (Fig. S13A) and principal component analysis of RNA-seq was performed to determine relationships between biological replicates (Fig. S13B). DGE analysis yielded no significant differences in overall gene expression between the two genotypes of naïve and stimulated MZ B cells (≥ 1.5 fold, $P_{adj} < 0.05$) (Data file S3). However, analysis of AS revealed changes in splicing patterns between *WT* and *Srpk3-cKO* MZ B cells, under both naïve and stimulated conditions (Fig. 6, A and B; Fig. S14, A and B). We detected 213 significant alternative splicing events in naïve samples, 38 significant alternative splicing events in 8-hour stimulated samples, and 51 significant alternative splicing events in 72-hour stimulated samples ($\Delta\Psi \geq 0.1$, FDR < 0.05) (Data file S4).

We focused our attention on naïve and 8-hour stimulated MZ B cells, because these conditions captured splicing changes initiated downstream of BCR and co-receptor signaling in these cells. Moreover, RNA-seq confirmed that *Srpk3* gene transcription shuts off between 8- and 72-hours of stimulation (Data file S3). Ingenuity pathway analysis of differentially spliced genes identified several key cellular pathways that were enriched in *WT* and *Srpk3-cKO* MZ B cells (Fig. 6, C and D). In naïve cells, these pathways included purine metabolism, B cell development, estrogen biosynthesis and primary immunodeficiency signaling (Fig. 6C). In 8-hour stimulated cells, enriched pathways included cholesterol biosynthesis, triacylglycerol degradation and heparan sulfate biosynthesis (Fig. 6D). Because a majority of these pathways were related to cell metabolism, we addressed the hypothesis that differential splicing of genes in *Srpk3-cKO* MZ B cells impairs metabolic function. In naïve cells, validation was obtained for AS of *Solute carrier family 1 member 5 (Slc1a5),* a neutral amino acid transporter of glutamine (*38*), and *Mitochondrial elongation factor 1* (*Mief*1; independently identified as MiD51), which regulates mitochondrial fusion (*39*) (Fig. 6, E and F). In 8-hour stimulated samples, *Nicotinamide nucleotide transhydrogenase* (*Nnt*), an inner mitochondrial membrane protein that contributes to mitochondrial NADPH/$NADP^+$ ratios (*40*) and *Mitochondrial elongation factor 2* (*Mief2*; independently identified as MiD49), a mitochondrial outer membrane protein that regulates fusion with MiD51 (Fig. 6, G and H), undergo AS. Splicing differences between *WT* and *Srpk3-cKO* MZ B cells, regardless of stimulation, suggest that SRPK3 fine tunes AS events in MZ B cells.

Mitochondrial dynamics and metabolism are disrupted in splenic B cells that lack SRPK3

AS is important for the control of normal and defective cellular metabolism (*41, 42*). Notably, AS of genes important for mitochondrial maintenance and activity have detrimental effects that impact cellular metabolism (*43*). Flow cytometric analysis revealed that naïve and stimulated *Srpk3-cKO* MZ B cells each have decreased mitochondrial mass compared to *WT* MZ B cells (this analysis does not consider relative ultrastructural morphology of mitochondria, which may influence the observed decrease in mass) (Fig. 7, A and B). To determine whether the loss of mass is due to a decline in mitochondrial abundance, we stained cells with Mitotracker Red and enumerated relative numbers of mitochondria per cell in naïve and stimulated samples using high resolution microscopy. *Srpk3-cKO* naïve MZ B cells exhibited considerably decreased numbers of mitochondria per cell compared to *WT* cells (Fig. 7C). However, after stimulation mitochondrial frequencies were relatively similar between *WT* and *Srpk3-cKO* cells.

We next determined whether AS of genes involved in mitochondrial function affects mitochondrial metabolism. We performed mitochondrial stress tests and measured oxygen consumption rates (OCR) between *WT* and *Srpk3-cKO* MZ B cells. *Srpk3*-deficient naïve and stimulated cells each had increased mitochondrial respiratory capacity when compared to *WT* cells (Fig. 7, D and E). Additionally, the mutant cells had increased non-mitochondrial respiration, which is indicative of decreased mitochondrial function (*44*). Together, these data suggest that mitochondrial function is perturbed in the absence of SRPK3.

Discussion

Srpk3 was identified as a transcriptional target of MEF2C in B cells by Wilker and colleagues (*18*), but its functions in B cells remained elusive. Here, we observed striking effects of the deletion of *Srpk3* genes in *cKO* mice. Numbers of B cell progenitors in *Srpk3-cKO* mice are

reduced significantly. While numbers of peripheral B cells appeared to be unaffected by the loss of SRPK3, responses to TI-2 (and to a lesser degree, TD) antigens were greatly reduced. The strength of B cell responses to the model TI-2 antigen NP-Ficoll was proportional to the dosage of *WT Srpk3* alleles: *WT*>female hemizygous>null mice, suggesting that *Srpk3* impacts humoral responses in a dosage/sex-dependent manner.

Deletion of *Srpk3* genes in B cells resulted in hundreds of different AS events. These results were expected in light of previous studies that linked SRPK family kinases with the control of AS in multiple cell types (reviewed in *9*). Immature, mature and MZ B cells had various AS events in mRNAs linked with functions including cell signaling and metabolism. The majority of these AS events involved SE and MXE, which can result in alternative protein isoforms or lead to instability of mRNA and its nonsense-mediated decay. These event types suggest that SRPK3 regulates SR proteins that direct exon inclusion and skipping (*45, 46*). The wide range of defects in the absence of SRPK3 is likely due to lack of activation of multiple splicing factors, but further biochemical studies are necessary to identify the interactome of the kinase in B cells.

Immature B cells represent a key checkpoint in bone marrow B cell development. Reduced numbers of these cells and skewed κ/λ ratios suggest that SRPK3 is involved in generation of the B cell repertoire (*47-49*). Despite clues to the overall functions of SRPK3, the mechanism that reduces bone marrow progenitors in the absence of SRPK3 is unknown. The percentage of intracellular Igκ$^+$ and Igκ MFI is similar in pre-B cells between *WT* and *cKO* cells, however mutant immature B cells have increased surface Igκ$^+$ cells. This suggests that either the Ig light chain λ loci do not rearrange normally or BCR signaling is impaired in cells lacking SRPK3. Therefore, autoreactive immature B cells may not signal appropriately, and do not receptor edit normally. Additionally, changes in signaling pathways important for immature B cell

development were noted, including the ATM kinase, which orchestrates cellular DSB and DDR responses (*50, 51*) and is critical in regulating V(D)J recombination (*52*). Other pathways included modulators of metabolism and mitochondria. In mature B cells, ERK5 signaling, which is indispensable for BAFF-induced mature B cell survival and self-reactivity, was affected (*53, 54*), as well as the mechanistic target of rapamycin (mTOR) and transforming growth factor (TGF)-β signaling pathways (*55, 56*). Together, the large number of affected pathways suggests compound effects of mutant proteomes.

A major conclusion of our studies is that SRPK3 is required for robust humoral responses of B cells *in vivo* and *in vitro*. Although the lack of SRPK3 in peripheral B cell subsets did not affect their numbers in unimmunized mice, antibody production (i.e. IgG3) in response to challenge with the TI-2 antigen NP-Ficoll was depressed in male and female null *Srpk3-cKO* mice. Production of IgG3 in response to TI-2 antigens is a primary role of MZ cells, which are pre-activated to respond to antigens on circulating bacteria in the spleen (*57, 58*). MZ B cells undergo CSR preferentially to the IgG3 isotype, which facilitates discrimination of multivalent antigenic targets (*59, 60*). The multivalent antigen NP-Ficoll primarily elicits a λ^+-anti-NP antibody response by MZ (and also by B1) B cells. Decreased λ^+ cells in *Srpk3-cKO* mice may, in part, explain the deficit in TI-2 responses of these mice. While we have not examined antibody responses to pathogens, we hypothesize that loss of SRPK3 in mice and humans affects the ability to respond to and clear infection.

The molecular basis of B cell unresponsiveness in the absence of SRPK3 is likely complex, but we identified multiple mRNA transcripts that require SRPK3 for their differential splicing. Many of these transcripts encode proteins with functions in cellular metabolism or in the maintenance or biogenesis of mitochondria. In this regard, recent studies confirmed the

importance of metabolic reprogramming as a 'gatekeeper' of B lymphocyte development, activation and responses (*61-65*). Loss of SRPK3 in MZ B cells results in differential splicing of candidate gatekeepers *Nnt* and *Slc1a5*. *Nnt* is essential for non-mitochondrial respiration, with important roles in pyruvate metabolism and the Tricarboxylic Acid cycle (TCA) (*66*). In turn, the TCA cycle requires glutamine, which is transported by the *Slc1a5* solute carrier. Additionally, we observed reduced mitochondrial mass in the absence of SRPK3, which could be due to the differential splicing of *Mief1* and *Mief2* transcripts encoding the mitochondrial remodeling factors MiD49/51. MiD49/51 helps recruit essential factors like Drp1 to mitochondria to regulate mitochondrial fission (*39*). Changes in splicing of metabolic genes results in mitochondrial dysfunction, as evidenced by increased OCR in SRPK3-deficient cells. Bioenergetically disrupted mitochondria require higher levels of ATP for maintaining organelle integrity, which increases the basal oxygen consumption rate (*40, 44, 67*). These patterns of high basal OCR and increased glycolysis resemble those in aging cells, which may indicate that the loss of SRPK proteins (*e.g.* SRPK1) contributes to metabolic shifts observed in aging (*68*).

In summary, SRPK3 is essential for multiple functions in B cells. Overall, we propose that SRPK3 is an integral component of splicing pathways in multiple peripheral B cell compartments. Moreover, SRPK3 likely has similar functions in human B cells. A pediatric male patient carrying the mutation I446S in his single *SRPK3* allele is reported to have hypogammaglobulinemia, poor responses to vaccines, and frequent ear/sinus and viral infections in addition to expected muscle weakness (MyGene2; *31*), while his mother is 'nearly asymptomatic'. These reports suggest important sex-specific differences resulting from mutations in *SRPK3.* Finally, while SRPK1 and SRPK2 have been studied in many cancers (*69*), the roles of SRPK3 and its importance as a biomarker have not been delineated in human cancer.

Additional studies are needed to expand our understanding of SRPK3 functions in normal immunity and effects of its dysregulation in human immunodeficiencies and cancer.

Materials and Methods:

Study Design

The overall goal of these studies was to understand SRPK3's biological roles in B lymphocyte development and function. This was accomplished by specifically ablating *Srpk3* genes using Cre-Lox technology in B lineage cells *in vivo* and monitoring B lymphocyte development, activation and function as a regulator of pre-mRNA splicing. The observed molecular and cellular changes in B cell function due to the loss of SRPK3 was the basis for conclusions drawn.

Animals

All mice were maintained on the C57BL/6N genetic background. The *Srpk3-floxed* mice were obtained from KOMP Repository (Davis, CA). The *Cd79a-Cre*$^{Tg/+}$ mice (*21*) were kindly provided by John Cambier (University of Colorado, Aurora). Genotyping of *Srpk3* and the *Cd79a-Cre* transgene was performed using tail DNA, PCR and primers as described previously (*70*) (Table S1). All experiments used age-matched male or female test and control littermate mice (6-8 week old). *WT* littermate controls included mice of the following genotypes: *Srpk3*$^{fl/fl}$*Cd79a*$^{+/+}$, *Srpk3*$^{fl/y}$*Cd79a*$^{+/+}$, *Srpk3*$^{WT/WT}$*Cd79a-Cre*$^{Tg/+}$, *Srpk3*$^{WT/WT}$*Cd79a-Cre*$^{+/+}$, *Srpk3*$^{WT/y}$*Cd79a-Cre*$^{Tg/+}$ and *Srpk3*$^{WT/y}$*Cd79a-Cre*$^{+/+}$. Mice were bred and housed in the Biological Resources Center at National Jewish Health. All experiments were performed under a protocol (AS2558-04-20) approved by the Institutional Animal Care and Use Committee at

National Jewish Health. Further information on the materials and methods used can be found in the Supplementary Materials.

SUPPLEMENTARY MATERIALS

Material and Methods

Fig. S1. Efficient deletion of floxed *Srpk3* alleles in bone marrow and splenic B cells.

Fig. S2. Normal splenic B cell development in mice that lack SRPK3.

Fig. S3. Kappa and lambda light chain expression on splenic B cell subsets in *Srpk3-cKO* mice.

Fig. S4. Intracellular kappa expression in bone marrow pre-B cells.

Fig. S5. Purity of FACS sorted immature and mature bone marrow B cells and relative variation between RNA-seq replicates.

Fig. S6. Elevated IgG1 immunoglobulin levels in *Srpk3-cKO* naive mice.

Fig. S7. T lymphocyte-independent type-1 and T lymphocyte-dependent humoral immune responses in *Srpk3-cKO* mice.

Fig. S8. NP-Ficoll immunized mice are able to undergo class switch recombination.

Fig. S9. Decreased frequencies of IL-5Rα expressing and elevated frequencies of IgD expressing marginal zone B cells in *Srpk3-cKO* naïve mice.

Fig. S10. *Srpk3-cKO* marginal zone B cells are capable of proliferating in response to simulation in vitro.

Fig. S11. *Srpk3-cKO* marginal zone B cells can differentiate into plasma cells in vitro.

Fig. S12. In vitro stimulated *Srpk3-cKO* marginal zone B cells can undergo class switch recombination.

Fig. S13. Purity of FACS sorted marginal zone B cells and relative variation between RNA-seq replicates across conditions.

Fig. S14. SRPK3 is necessary for regulating alternative splicing patterns in naïve and 72-hour stimulated marginal zone B cells.

Data file S1. Tpms values for immature and mature bone marrow B cell populations between *WT* and *Srpk3*-deficient samples. Raw data.

Data file S2. Significant alternative splicing events between *WT* and *Srpk3*-deficient immature and mature bone marrow B cells. Raw data.

Data file S3. Tpms of naïve, 8 hour stimulated and 72 hour stimulated *WT* and *Srpk3*-deficient marginal zone B cell samples. Raw data.

Data file S4. Significant alternative splicing events in naïve, 8-hour stimulated and 72-hour stimulated *WT* and *Srpk3*-deficient MZ B cells. Raw data.

Table S1. PCR Primer List

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References and Notes:

1. A. Schaub, E. Glasmacher, Splicing in immune cells-mechanistic insights and emerging topics. *Int Immunol* **29**, 173-181 (2017).
2. M. Yabas, H. Elliott, G. F. Hoyne, The Role of Alternative Splicing in the Control of Immune Homeostasis and Cellular Differentiation. *Int J Mol Sci* **17**, (2015).
3. S. R. Bruce, B-cell and plasma-cell splicing differences: A potential role in regulated immunoglobulin RNA processing. *Rna* **9**, 1264-1273 (2003).
4. A. M. Nelson, N. T. Carew, S. M. Smith, C. Milcarek, RNA Splicing in the Transition from B Cells to Antibody-Secreting Cells: The Influences of ELL2, Small Nuclear RNA, and Endoplasmic Reticulum Stress. *J Immunol* **201**, 3073-3083 (2018).
5. W. P. Galej, Structural studies of the spliceosome: past, present and future perspectives. *Biochem Soc Trans* **46**, 1407-1422 (2018).
6. S. D. Wagner, J. A. Berglund, Alternative pre-mRNA splicing. *Methods Mol Biol* **1126**, 45-54 (2014).
7. J. F. Gui, W. S. Lane, X. D. Fu, A serine kinase regulates intracellular localization of splicing factors in the cell cycle. *Nature* **369**, 678-682 (1994).
8. X. Y. Zhong, J. H. Ding, J. A. Adams, G. Ghosh, X. D. Fu, Regulation of SR protein phosphorylation and alternative splicing by modulating kinetic interactions of SRPK1 with molecular chaperones. *Genes Dev* **23**, 482-495 (2009).
9. T. Giannakouros, E. Nikolakaki, I. Mylonis, E. Georgatsou, Serine-arginine protein kinases: a small protein kinase family with a large cellular presence. *FEBS J* **278**, 570-586 (2011).

10. M. Varjosalo, S. Keskitalo, A. Van Drogen, H. Nurkkala, A. Vichalkovski, R. Aebersold, M. Gstaiger, The protein interaction landscape of the human CMGC kinase group. *Cell Rep* **3**, 1306-1320 (2013).

11. J. F. Gui, H. Tronchere, S. D. Chandler, X. D. Fu, Purification and characterization of a kinase specific for the serine- and arginine-rich pre-mRNA splicing factors. *Proc Natl Acad Sci U S A* **91**, 10824-10828 (1994).

12. H. Y. Wang, W. Lin, J. A. Dyck, J. M. Yeakley, Z. Songyang, L. C. Cantley, X. D. Fu, SRPK2: a differentially expressed SR protein-specific kinase involved in mediating the interaction and localization of pre-mRNA splicing factors in mammalian cells. *J Cell Biol* **140**, 737-750 (1998).

13. J. Koizumi, Y. Okamoto, H. Onogi, A. Mayeda, A. R. Krainer, M. Hagiwara, The subcellular localization of SF2/ASF is regulated by direct interaction with SR protein kinases (SRPKs). *J Biol Chem* **274**, 11125-11131 (1999).

14. Z. Zhou, J. Qiu, W. Liu, Y. Zhou, R. M. Plocinik, H. Li, Q. Hu, G. Ghosh, J. A. Adams, M. G. Rosenfeld, X. D. Fu, The Akt-SRPK-SR axis constitutes a major pathway in transducing EGF signaling to regulate alternative splicing in the nucleus. *Mol Cell* **47**, 422-433 (2012).

15. D. Tsianou, E. Nikolakaki, A. Tzitzira, S. Bonanou, T. Giannakouros, E. Georgatsou, The enzymatic activity of SR protein kinases 1 and 1a is negatively affected by interaction with scaffold attachment factors B1 and 2. *FEBS J* **276**, 5212-5227 (2009).

16. S. K. Petersen-Mahrt, C. Estmer, C. Ohrmalm, D. A. Matthews, W. C. Russell, G. Akusjarvi, The splicing factor-associated protein, p32, regulates RNA splicing by inhibiting ASF/SF2 RNA binding and phosphorylation. *EMBO J* **18**, 1014-1024 (1999).

17. O. Nakagawa, M. Arnold, M. Nakagawa, H. Hamada, J. M. Shelton, H. Kusano, T. M. Harris, G. Childs, K. P. Campbell, J. A. Richardson, I. Nishino, E. N. Olson, Centronuclear myopathy in mice lacking a novel muscle-specific protein kinase transcriptionally regulated by MEF2. *Genes Dev* **19**, 2066-2077 (2005).

18. P. R. Wilker, M. Kohyama, M. M. Sandau, J. C. Albring, O. Nakagawa, J. J. Schwarz, K. M. Murphy, Transcription factor Mef2c is required for B cell proliferation and survival after antigen receptor stimulation. *Nat Immunol* **9**, 603-612 (2008).

19. I. D. R. Revilla, I. Bilic, B. Vilagos, H. Tagoh, A. Ebert, I. M. Tamir, L. Smeenk, J. Trupke, A. Sommer, M. Jaritz, M. Busslinger, The B-cell identity factor Pax5 regulates distinct transcriptional programmes in early and late B lymphopoiesis. *EMBO J* **31**, 3130-3146 (2012).

20. I. Debnath, K. M. Roundy, P. D. Pioli, J. J. Weis, J. H. Weis, Bone marrow-induced Mef2c deficiency delays B-cell development and alters the expression of key B-cell regulatory proteins. *Int Immunol* **25**, 99-115 (2013).

21. E. Hobeika, S. Thiemann, B. Storch, H. Jumaa, P. J. Nielsen, R. Pelanda, M. Reth, Testing gene function early in the B cell lineage in mb1-cre mice. *Proc Natl Acad Sci U S A* **103**, 13789-13794 (2006).

22. R. Pelanda, E. Hobeika, T. Kurokawa, Y. Zhang, S. Kuppig, M. Reth, Cre recombinase-controlled expression of the mb-1 allele. *Genesis* **32**, 154-157 (2002).

23. R. R. Hardy, P. W. Kincade, K. Dorshkind, The protean nature of cells in the B lymphocyte lineage. *Immunity* **26**, 703-714 (2007).

24. J. Seita, D. Sahoo, D. J. Rossi, D. Bhattacharya, T. Serwold, M. A. Inlay, L. I. Ehrlich, J. W. Fathman, D. L. Dill, I. L. Weissman, Gene Expression Commons: an open platform for absolute gene expression profiling. *PLoS One* **7**, e40321 (2012).

25. A. Cariappa, C. Chase, H. Liu, P. Russell, S. Pillai, Naive recirculating B cells mature simultaneously in the spleen and bone marrow. *Blood* **109**, 2339-2345 (2007).

26. G. Huang, G. Ge, D. Wang, B. Gopalakrishnan, D. H. Butz, R. J. Colman, A. Nagy, D. S. Greenspan, alpha3(V) collagen is critical for glucose homeostasis in mice due to effects in pancreatic islets and peripheral tissues. *J Clin Invest* **121**, 769-783 (2011).

27. M. N. Svensson, K. M. Andersson, C. Wasen, M. C. Erlandsson, M. Nurkkala-Karlsson, I. M. Jonsson, M. Brisslert, M. Bemark, M. I. Bokarewa, Murine germinal center B cells require functional Fms-like tyrosine kinase 3 signaling for IgG1 class-switch recombination. *Proc Natl Acad Sci U S A* **112**, E6644-6653 (2015).

28. A. Y. Kreins, M. J. Ciancanelli, S. Okada, X. F. Kong, N. Ramirez-Alejo, S. S. Kilic, J. El Baghdadi, S. Nonoyama, S. A. Mahdaviani, F. Ailal, A. Bousfiha, D. Mansouri, E. Nievas, C. S. Ma, G. Rao, A. Bernasconi, H. Sun Kuehn, J. Niemela, J. Stoddard, P. Deveau, A. Cobat, S. El Azbaoui, A. Sabri, C. K. Lim, M. Sundin, D. T. Avery, R. Halwani, A. V. Grant, B. Boisson, D. Bogunovic, Y. Itan, M. Moncada-Velez, R. Martinez-Barricarte, M. Migaud, C. Deswarte, L. Alsina, D. Kotlarz, C. Klein, I. Muller-Fleckenstein, B. Fleckenstein, V. Cormier-Daire, S. Rose-John, C. Picard, L. Hammarstrom, A. Puel, S. Al-Muhsen, L. Abel, D. Chaussabel, S. D. Rosenzweig, Y. Minegishi, S. G. Tangye, J. Bustamante, J. L. Casanova, S. Boisson-Dupuis, Human TYK2 deficiency: Mycobacterial and viral infections without hyper-IgE syndrome. *J Exp Med* **212**, 1641-1662 (2015).

29. R. Potla, T. Koeck, J. Wegrzyn, S. Cherukuri, K. Shimoda, D. P. Baker, J. Wolfman, S. M. Planchon, C. Esposito, B. Hoit, J. Dulak, A. Wolfman, D. Stuehr, A. C. Larner, Tyk2 tyrosine kinase expression is required for the maintenance of mitochondrial respiration in primary pro-B lymphocytes. *Mol Cell Biol* **26**, 8562-8571 (2006).

30. C. L. Nguyen, R. Possemato, E. L. Bauerlein, A. Xie, R. Scully, W. C. Hahn, Nek4 regulates entry into replicative senescence and the response to DNA damage in human fibroblasts. *Mol Cell Biol* **32**, 3963-3977 (2012).

31. J. Xin, A. Mark, C. Afrasiabi, G. Tsueng, M. Juchler, N. Gopal, G. S. Stupp, T. E. Putman, B. J. Ainscough, O. L. Griffith, A. Torkamani, P. L. Whetzel, C. J. Mungall, S. D. Mooney, A. I. Su, C. Wu, High-performance web services for querying gene and variant annotation. *Genome Biol* **17**, 91 (2016).

32. J. Wang, R. Al-Ouran, Y. Hu, S. Y. Kim, Y. W. Wan, M. F. Wangler, S. Yamamoto, H. T. Chao, A. Comjean, S. E. Mohr, Udn, N. Perrimon, Z. Liu, H. J. Bellen, MARRVEL: Integration of Human and Model Organism Genetic Resources to Facilitate Functional Annotation of the Human Genome. *Am J Hum Genet* **100**, 843-853 (2017).

33. F. Martin, J. F. Kearney, Marginal-zone B cells. *Nat Rev Immunol* **2**, 323-335 (2002).

34. L. M. Pecanha, C. M. Snapper, F. D. Finkelman, J. J. Mond, Dextran-conjugated anti-Ig antibodies as a model for T cell-independent type 2 antigen-mediated stimulation of Ig secretion in vitro. I. Lymphokine dependence. *J Immunol* **146**, 833-839 (1991).

35. C. M. Snapper, T. M. McIntyre, R. Mandler, L. M. Pecanha, F. D. Finkelman, A. Lees, J. J. Mond, Induction of IgG3 secretion by interferon gamma: a model for T cell-independent class switching in response to T cell-independent type 2 antigens. *J Exp Med* **175**, 1367-1371 (1992).

36. M. J. McCarron, P. W. Park, D. R. Fooksman, CD138 mediates selection of mature plasma cells by regulating their survival. *Blood* **129**, 2749-2759 (2017).

37. J. Tellier, S. L. Nutt, Standing out from the crowd: How to identify plasma cells. *Eur J Immunol* **47**, 1276-1279 (2017).

38. M. Scalise, L. Pochini, L. Console, M. A. Losso, C. Indiveri, The Human SLC1A5 (ASCT2) Amino Acid Transporter: From Function to Structure and Role in Cell Biology. *Front Cell Dev Biol* **6**, 96 (2018).

39. L. D. Osellame, A. P. Singh, D. A. Stroud, C. S. Palmer, D. Stojanovski, R. Ramachandran, M. T. Ryan, Cooperative and independent roles of the Drp1 adaptors Mff, MiD49 and MiD51 in mitochondrial fission. *J Cell Sci* **129**, 2170-2181 (2016).

40. J. A. Ronchi, T. R. Figueira, F. G. Ravagnani, H. C. Oliveira, A. E. Vercesi, R. F. Castilho, A spontaneous mutation in the nicotinamide nucleotide transhydrogenase gene of C57BL/6J mice results in mitochondrial redox abnormalities. *Free Radic Biol Med* **63**, 446-456 (2013).

41. G. Biamonti, L. Maita, A. Montecucco, The Krebs Cycle Connection: Reciprocal Influence Between Alternative Splicing Programs and Cell Metabolism. *Front Oncol* **8**, 408 (2018).

42. I. Kozlovski, Z. Siegfried, A. Amar-Schwartz, R. Karni, The role of RNA alternative splicing in regulating cancer metabolism. *Hum Genet* **136**, 1113-1127 (2017).

43. L. Liu, C. Luo, Y. Luo, L. Chen, Y. Liu, Y. Wang, J. Han, Y. Zhang, N. Wei, Z. Xie, W. Wu, G. Wu, Y. Feng, MRPL33 and its splicing regulator hnRNPK are required for mitochondria function and implicated in tumor progression. *Oncogene* **37**, 86-94 (2018).

44. B. K. Chacko, P. A. Kramer, S. Ravi, G. A. Benavides, T. Mitchell, B. P. Dranka, D. Ferrick, A. K. Singal, S. W. Ballinger, S. M. Bailey, R. W. Hardy, J. Zhang, D. Zhi, V. M. Darley-Usmar, The Bioenergetic Health Index: a new concept in mitochondrial translational research. *Clin Sci (Lond)* **127**, 367-373 (2014).

45. J. Han, J. H. Ding, C. W. Byeon, J. H. Kim, K. J. Hertel, S. Jeong, X. D. Fu, SR proteins induce alternative exon skipping through their activities on the flanking constitutive exons. *Mol Cell Biol* **31**, 793-802 (2011).

46. T. Bradley, M. E. Cook, M. Blanchette, SR proteins control a complex network of RNA-processing events. *RNA* **21**, 75-92 (2015).

47. S. T. Ju, M. E. Dorf, Preferential induction of specific lambda-isotypic antibodies in mice. *J Immunol* **133**, 1404-1409 (1984).

48. F. Melchers, Checkpoints that control B cell development. *J Clin Invest* **125**, 2203-2210 (2015).

49. G. E. Woloschak, C. J. Krco, Regulation of kappa/lambda immunoglobulin light chain expression in normal murine lymphocytes. *Mol Immunol* **24**, 751-757 (1987).

50. J. J. Bednarski, B. P. Sleckman, Lymphocyte development: integration of DNA damage response signaling. *Adv Immunol* **116**, 175-204 (2012).

51. J. J. Bednarski, R. Pandey, E. Schulte, L. S. White, B. R. Chen, G. J. Sandoval, M. Kohyama, M. Haldar, A. Nickless, A. Trott, G. Cheng, K. M. Murphy, C. H. Bassing, J. E. Payton, B. P. Sleckman, RAG-mediated DNA double-strand breaks activate a cell type-specific checkpoint to inhibit pre-B cell receptor signals. *J Exp Med* **213**, 209-223 (2016).

52. J. Hu, S. Tepsuporn, R. M. Meyers, M. Gostissa, F. W. Alt, Developmental propagation of V(D)J recombination-associated DNA breaks and translocations in mature B cells via dicentric chromosomes. *Proc Natl Acad Sci U S A* **111**, 10269-10274 (2014).

53. E. Jacque, E. Schweighoffer, V. L. Tybulewicz, S. C. Ley, BAFF activation of the ERK5 MAP kinase pathway regulates B cell survival. *J Exp Med* **212**, 883-892 (2015).

54. M. Ota, B. H. Duong, A. Torkamani, C. M. Doyle, A. L. Gavin, T. Ota, D. Nemazee, Regulation of the B cell receptor repertoire and self-reactivity by BAFF. *J Immunol* **185**, 4128-4136 (2010).

55. T. N. Iwata, J. A. Ramirez-Komo, H. Park, B. M. Iritani, Control of B lymphocyte development and functions by the mTOR signaling pathways. *Cytokine Growth Factor Rev* **35**, 47-62 (2017).

56. M. J. Gros, P. Naquet, R. R. Guinamard, Cell intrinsic TGF-beta 1 regulation of B cells. *J Immunol* **180**, 8153-8158 (2008).

57. K. E. Gunn, J. W. Brewer, Evidence that marginal zone B cells possess an enhanced secretory apparatus and exhibit superior secretory activity. *J Immunol* **177**, 3791-3798 (2006).

58. F. Martin, A. M. Oliver, J. F. Kearney, Marginal zone and B1 B cells unite in the early response against T-independent blood-borne particulate antigens. *Immunity* **14**, 617-629 (2001).

59. N. S. Greenspan, L. J. Cooper, Cooperative binding by mouse IgG3 antibodies: implications for functional affinity, effector function, and isotype restriction. *Springer Semin Immunopathol* **15**, 275-291 (1993).

60. M. C. Hsu, K. M. Toellner, C. G. Vinuesa, I. C. Maclennan, B cell clones that sustain long-term plasmablast growth in T-independent extrafollicular antibody responses. *Proc Natl Acad Sci U S A* **103**, 5905-5910 (2006).

61. N. Degauque, C. Brosseau, S. Brouard, Regulation of the Immune Response by the Inflammatory Metabolic Microenvironment in the Context of Allotransplantation. *Front Immunol* **9**, 1465 (2018).

62. M. Muschen, Metabolic gatekeepers to safeguard against autoimmunity and oncogenic B cell transformation. *Nat Rev Immunol* **19**, 337-348 (2019).

63. C. A. Doughty, B. F. Bleiman, D. J. Wagner, F. J. Dufort, J. M. Mataraza, M. F. Roberts, T. C. Chiles, Antigen receptor-mediated changes in glucose metabolism in B lymphocytes: role of phosphatidylinositol 3-kinase signaling in the glycolytic control of growth. *Blood* **107**, 4458-4465 (2006).

64. L. R. Waters, F. M. Ahsan, D. M. Wolf, O. Shirihai, M. A. Teitell, Initial B Cell Activation Induces Metabolic Reprogramming and Mitochondrial Remodeling. *iScience* **5**, 99-109 (2018).

65. E. Krzywinska, C. Stockmann, Hypoxia, Metabolism and Immune Cell Function. *Biomedicines* **6**, (2018).

66. P. A. Gameiro, L. A. Laviolette, J. K. Kelleher, O. Iliopoulos, G. Stephanopoulos, Cofactor balance by nicotinamide nucleotide transhydrogenase (NNT) coordinates reductive carboxylation and glucose catabolism in the tricarboxylic acid (TCA) cycle. *J Biol Chem* **288**, 12967-12977 (2013).

67. M. D. Brand, D. G. Nicholls, Assessing mitochondrial dysfunction in cells. *Biochem J* **435**, 297-312 (2011).

68. J. M. Son, E. H. Sarsour, A. Kakkerla Balaraju, J. Fussell, A. L. Kalen, B. A. Wagner, G. R. Buettner, P. C. Goswami, Mitofusin 1 and optic atrophy 1 shift metabolism to mitochondrial respiration during aging. *Aging Cell* **16**, 1136-1145 (2017).

69. D. P. Corkery, A. C. Holly, S. Lahsaee, G. Dellaire, Connecting the speckles: Splicing kinases and their role in tumorigenesis and treatment response. *Nucleus* **6**, 279-288 (2015).

70. K. Lukin, S. Fields, D. Lopez, M. Cherrier, K. Ternyak, J. Ramirez, A. J. Feeney, J. Hagman, Compound haploinsufficiencies of Ebf1 and Runx1 genes impede B cell lineage progression. *Proc Natl Acad Sci U S A* **107**, 7869-7874 (2010).

71. C. L. Swanson, T. J. Wilson, P. Strauch, M. Colonna, R. Pelanda, R. M. Torres, Type I IFN enhances follicular B cell contribution to the T cell-independent antibody response. *J Exp Med* **207**, 1485-1500 (2010).

72. R. Patro, G. Duggal, M. I. Love, R. A. Irizarry, C. Kingsford, Salmon provides fast and bias-aware quantification of transcript expression. *Nat Methods* **14**, 417-419 (2017).

73. C. Soneson, M. I. Love, M. D. Robinson, Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. *F1000Res* **4**, 1521 (2015).

74. M. I. Love, W. Huber, S. Anders, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* **15**, 550 (2014).

75. A. Dobin, C. A. Davis, F. Schlesinger, J. Drenkow, C. Zaleski, S. Jha, P. Batut, M. Chaisson, T. R. Gingeras, STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15-21 (2013).

76. S. Shen, J. W. Park, Z. X. Lu, L. Lin, M. D. Henry, Y. N. Wu, Q. Zhou, Y. Xing, rMATS: robust and flexible detection of differential alternative splicing from replicate RNA-Seq data. *Proc Natl Acad Sci U S A* **111**, E5593-5601 (2014).

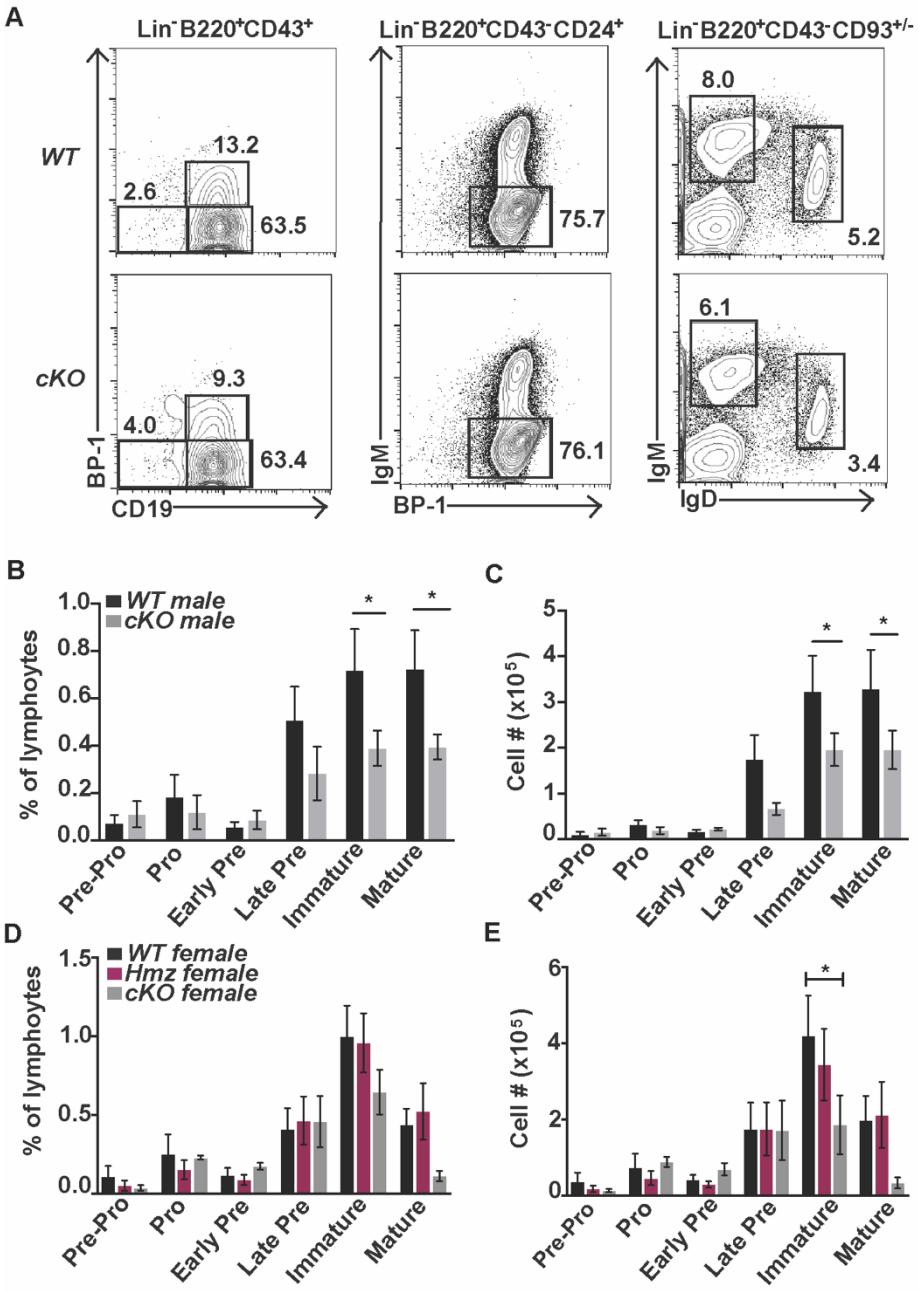

Fig. 1. Decreased immature and mature bone marrow B cell populations in *Srpk3-cKO* mice. Quantitation of bone marrow B cell subsets in mice. (**A**) Phenotype, (**B** and **D**) frequencies and (**C** and **E**) cell numbers of various bone marrow B cell populations in male *WT* (n=7-9) or *Srpk3-cKO* (n=7-9) mice (**B** and **C**) and female *WT* (n=3-7), *Srpk3-hemizygous* (*Hmz,*

n= 6-8) and *Srpk3-cKO null* (n=3-5) (**D** and **E**) mice. In panel A numbers indicate percent of phenotypic fraction. Asterisks indicate statistical significance compared to *WT* littermate controls using 2-way ANOVA. *P<0.05. Graphs represent arithmetic mean ± SEM. All data are representative of at least three independent experiments.

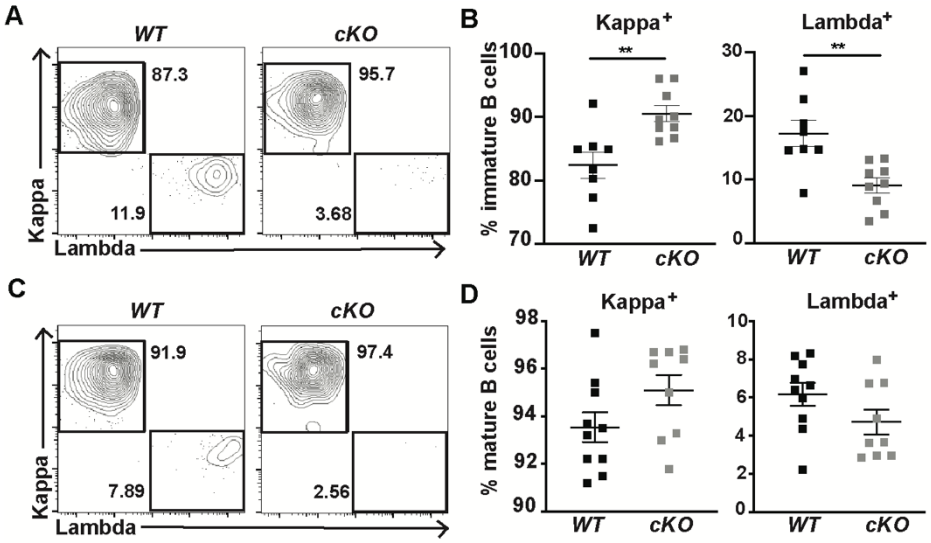

Fig. 2. Skewed kappa and lambda light chain expression on immature bone marrow B cells in *Srpk3-cKO* mice. (**A**) Phenotype and (**B**) frequencies of kappa and lambda light chain expression on immature bone marrow B cells ($CD19^{+}Lin^{-}B220^{+}IgM^{+}$) from *WT* (n=8) and *Srpk3-cKO* (n=9) male mice. (**C**) Phenotype and (**D**) frequencies of kappa and lambda light chain expression on mature bone marrow B cells ($CD19^{+}Lin^{-}B220^{hi}IgM^{+}$) from *WT* (n=10) and *Srpk3-cKO* (n=9) male mice. Each dot represents an individual mouse. Asterisks indicate statistical significance compared to *WT* littermate controls using Mann-Whitney Rank test. **P<0.005. Graphs represent arithmetic mean ± SEM. All data are representative of at least three independent experiments.

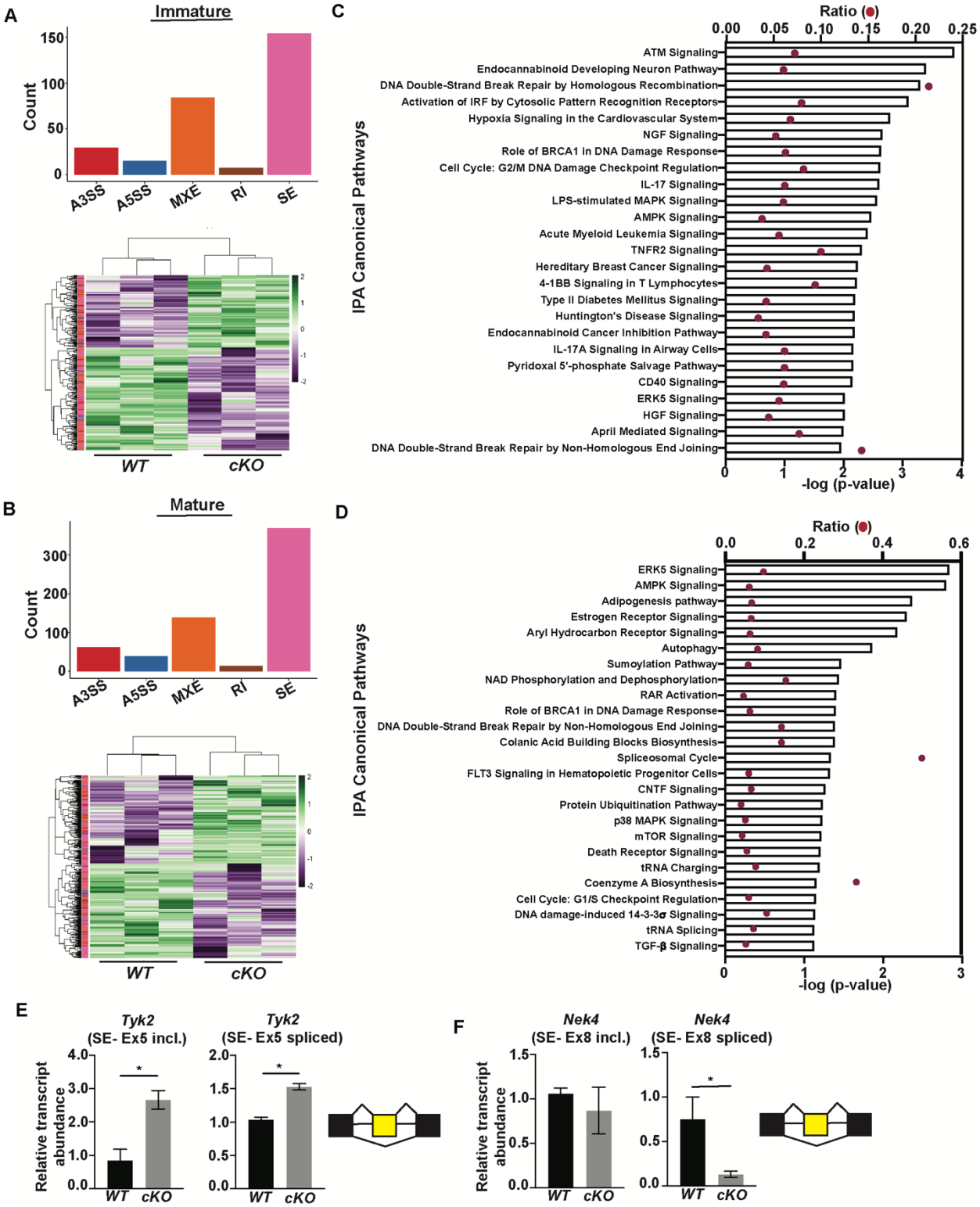

Fig. 3. SRPK3 regulates alternative splicing patterns in immature and mature bone

marrow B cells. (**A**) Significantly altered splicing event types in purified immature bone marrow B cells between *WT* (n=3) and *Srpk3-cKO* (n=3) male mice. AS event frequencies are displayed for alternative 3' splice sites (A3SS), alternative 5' splice sites (A5SS), mutually exclusive exons (MXE), retained introns (RI), and skipped exons (SE). (**B**) Significantly altered splicing event types in purified mature bone marrow B cells between *WT* (n=3) and *Srpk3-cKO* (n=3) male mice. Immature and mature bone marrow B cell samples were isolated from the same mice. Heatmaps show ψ values. Color (purple to green) represents z-score of ψ values across rows. $\Delta\psi \geq 0.1$, FDR < 0.05. No overlap in ψ between replicates across conditions. AS events in individual transcripts are color coded at left using same colors as in (**A**). (**C**) IPA enrichment for the top 30 most significantly enriched canonical pathways in the immature bone marrow B cell RNA-seq splicing dataset. (**D**) IPA enrichment for the top 25 most significantly enriched canonical pathways in the mature bone marrow B cell RNA-seq splicing dataset. All data are representative of at least three independent experiments. (**E**) Validation (qRT-PCR) of *Tyk2* differential splicing. (**F**) Validation (qRT-PCR) of *Nek4* differential splicing.

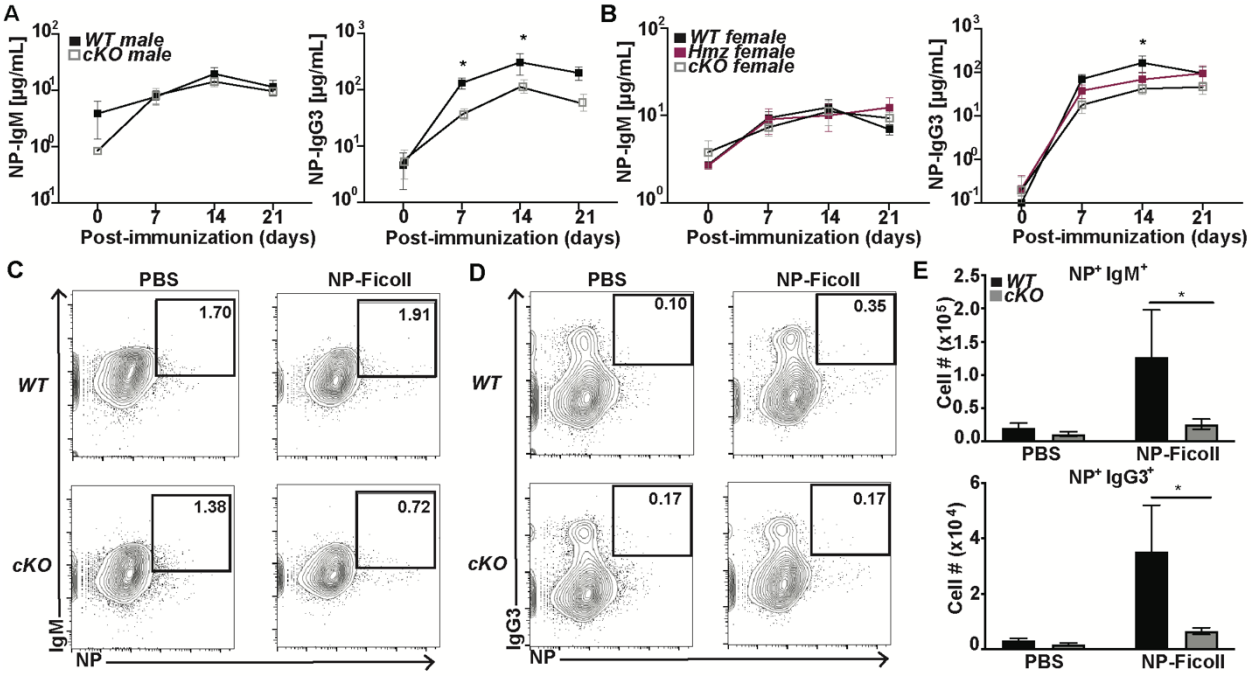

Fig. 4. SRPK3 is indispensable for T lymphocyte-independent type-2 humoral immune responses in vivo. (**A**) NP-specific ELISAs of IgM and IgG3 titers post-immunization with NP-Ficoll in male *WT* (n=7-11) and *Srpk3-cKO* (n=7) mice. (**B**) NP-specific ELISAs of IgM and IgG3 titers post-immunization with NP-Ficoll in female *WT* (n=6-8), *Srpk3-Hmz* (n= 6-7) and *Srpk3-cKO* (n=4-6) mice. All points at d14 are significant (*P<0.05)(**C** and **D**) Flow cytometry and (**E**) cell numbers of NP-specific IgM or IgG3 splenic B cells ($CD19^{+}Lin^{-}$) in unimmunized (PBS) or immunized (NP-Ficoll) male *WT* (n=5) and *Srpk3-cKO* (n=5-7) mice. Mice were harvested 4 days post-immunization for analysis. Asterisks indicate statistical significance compared to *WT* littermate controls using 2-way ANOVA. *P<0.05. Statistical significance is between *WT* and heterozygous females, as well as, *WT* and *Srpk3-cKO* females in (**B**). Graphs represent arithmetic mean ± SEM. All data are representative of at least three independent experiments.

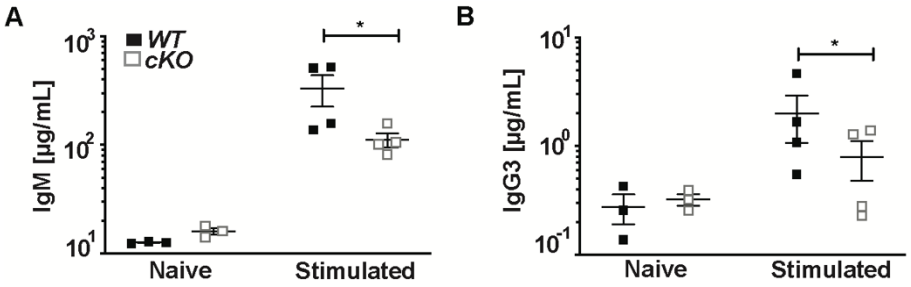

Fig. 5. *Srpk3-cKO* marginal zone B cells fail to respond to T lymphocyte-independent type-2 antigen in vitro. (**A**) ELISA detection of IgM antibody from culture supernatant after 6-days of in vitro stimulation with α-δ-dex, IL-5 and IFNg of purified marginal zone B cells from *WT* (n=3-4) and *Srpk3-cKO* (n=3-4) male mice. (**B**) ELISA detection of IgG3 antibody from culture supernatant after 6-days of in vitro stimulation as in A of purified marginal zone B cells from *WT* (n=3-4) and *Srpk3-cKO* (n=3-4) male mice. Each dot represents an individual mouse. Asterisks indicate statistical significance compared to *WT* littermate controls using 2-way ANOVA. *P<0.05. Graphs represent arithmetic mean ± SEM. Data are representative of at least three independent experiments.

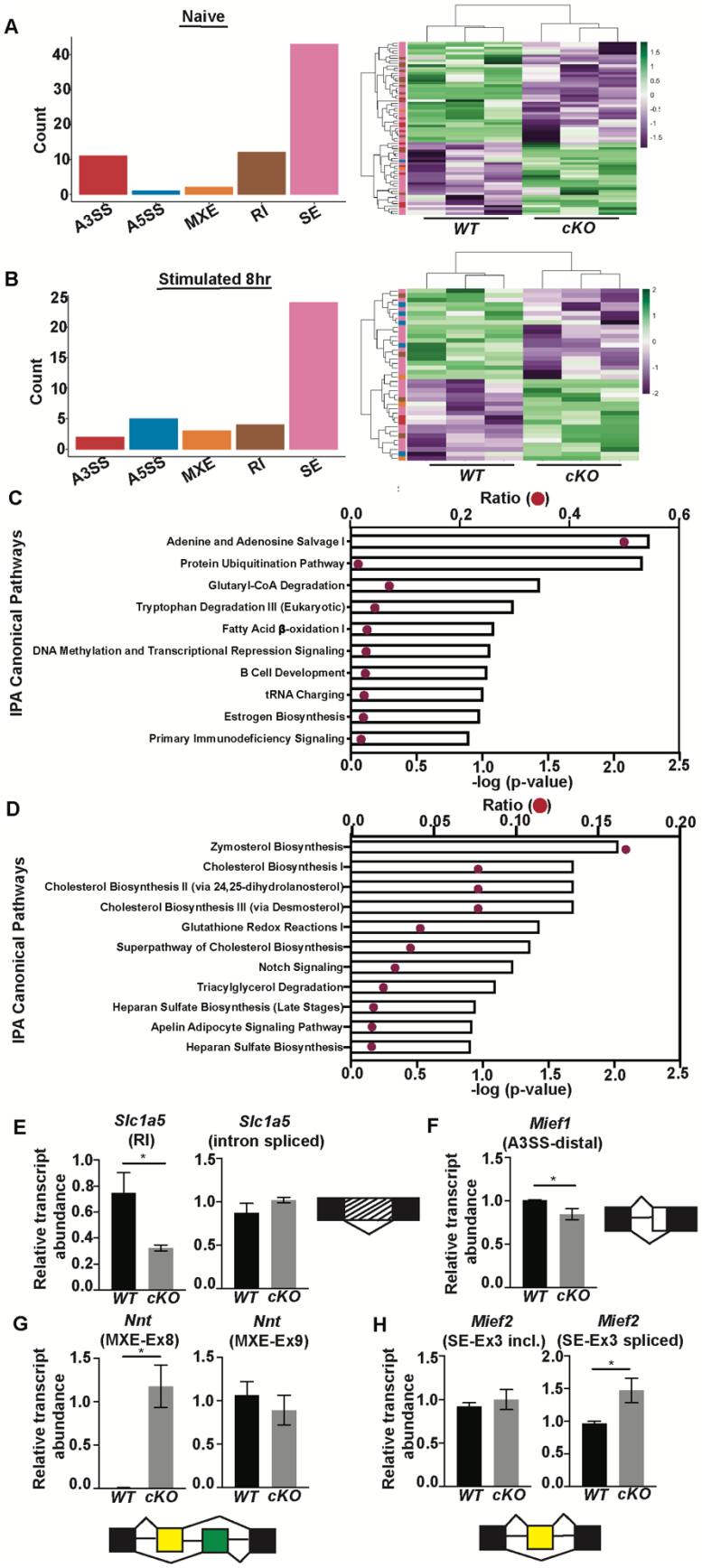
A
Naive
Count
A3SS
A5SS
MXE
RI
SE
WT
cKO
B
Stimulated 8hr
C
Ratio
IPA Canonical Pathways
Adenine and Adenosine Salvage I
Protein Ubiquitination Pathway
Glutaryl-CoA Degradation
Tryptophan Degradation III (Eukaryotic)
Fatty Acid β-oxidation I
DNA Methylation and Transcriptional Repression Signaling
B Cell Development
tRNA Charging
Estrogen Biosynthesis
Primary Immunodeficiency Signaling
-log (p-value)
D
Zymosterol Biosynthesis
Cholesterol Biosynthesis I
Cholesterol Biosynthesis II (via 24,25-dihydrolanosterol)
Cholesterol Biosynthesis III (via Desmosterol)
Glutathione Redox Reactions I
Superpathway of Cholesterol Biosynthesis
Notch Signaling
Triacylglycerol Degradation
Heparan Sulfate Biosynthesis (Late Stages)
Apelin Adipocyte Signaling Pathway
Heparan Sulfate Biosynthesis
E
Slc1a5 (RI)
Slc1a5 (intron spliced)
F
Mief1 (A3SS-distal)
Relative transcript abundance
G
Nnt (MXE-Ex8)
Nnt (MXE-Ex9)
H
Mief2 (SE-Ex3 incl.)
Mief2 (SE-Ex3 spliced)

Fig. 6. SRPK3 modulates alternative splicing patterns in marginal zone B cells. (**A**) Significantly altered splicing event types in naïve marginal zone B cells purified from *WT* (n=3) and *Srpk3-cKO* (n=3) male mice. AS event frequencies are displayed for alternative 3' splice sites (A3SS), alternative 5' splice sites (A5SS), mutually exclusive exons (MXE), retained introns (RI), and skipped exons (SE). (**B**) Significantly altered splicing event types in 8-hour stimulated marginal zone B cells purified from *WT* (n=3) and *Srpk3-cKO* (n=3) male mice. Same mice were used to split purified marginal zone B cells between conditions. Heatmaps show ψ values. Color (purple to green) represents z-score of ψ values across rows. $\Delta\psi \geq 0.1$, FDR < 0.05. No overlap in ψ between replicates across conditions. AS events in individual transcripts are color coded at left using same colors as in (**A**). (**C**) IPA enrichment for the top 10 most significantly enriched canonical pathways in the naïve marginal zone B cell RNA-seq splicing dataset. (**D**) IPA enrichment for the top 11 most significantly enriched canonical pathways in the 8-hour stimulated marginal zone B cell RNA-seq splicing dataset. (**E**) Validation (qRT-PCR) of *Slc1a5* differential splicing. (**F**) Validation (qRT-PCR) of *Mief1* differential splicing. (**G**) Validation (qRT-PCR) of *Nnt* differential splicing. (**H**) Validation (qRT-PCR) of *Mief2* differential splicing. Asterisks indicate statistical significance compared to *WT* littermate controls using unpaired, two-tailed Student's t-test. *P<0.05. Graphs represent arithmetic mean ± SEM. All data are representative of at least three independent experiments.

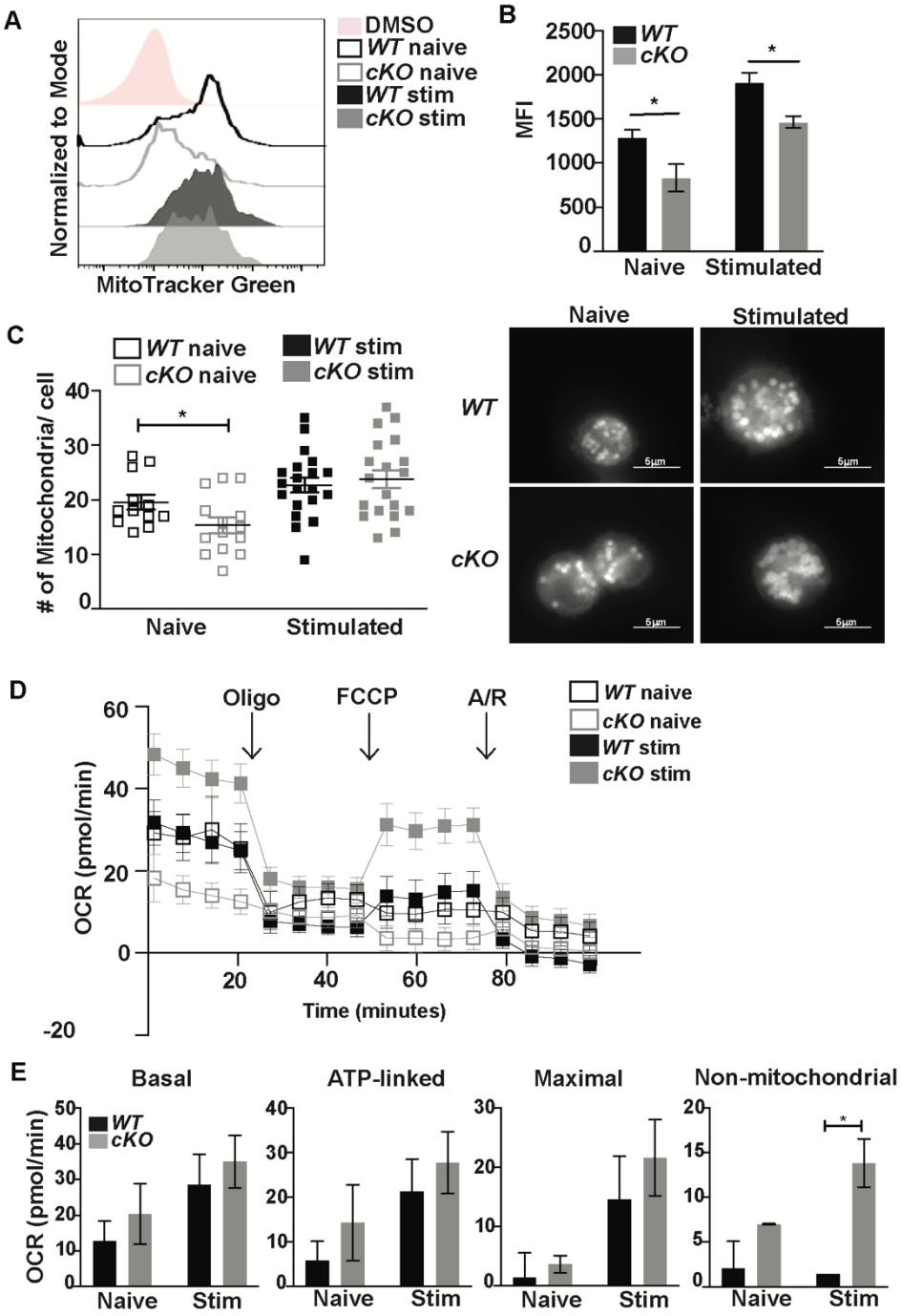

Fig. 7. Dysregulation of mitochondrial dynamics and metabolism in *Srpk3-cKO* splenic B cells. (**A**) Flow cytometric analyses and (**B**) mean fluorescence intensity (MFI) of Mitotracker Green in naïve and 24-hour stimulated (with α-δ-dex, IL-5 and IFNg) marginal zone B cells purified from *WT* (n=3) and *Srpk3-cKO* (n=3) male mice. (**C**) Number of mitochondria per cell

in naïve and 24-hour stimulated marginal zone B cells purified from *WT* (n=2) and *Srpk3-cKO* (n=2) male mice. Each dot represents an individual cell. Representative image of stained mitochondria (Mitotracker Red) in naïve or stimulated (stim) *WT* and *Srpk3-cKO* marginal zone B cells. 5μm scale. (**D**) OCR measurements between purified naïve or 5-hour stimulated *WT* and *Srpk3-cKO* splenic B cells. (**E**) OCR of basal respiration, ATP-linked respiration, maximal respiration and non-mitochondrial respiration between purified naïve or 5-hour stimulated *WT* and *Srpk3-cKO* splenic B cells. Asterisks indicate statistical significance compared to *WT* littermate controls using either 1-way ANOVA or 2-way ANOVA. *$P<0.05$. Graphs represent arithmetic mean ± SEM. All data are representative of at least three independent experiments.