IP₃R-mediated Ca²⁺ signaling controls B cell proliferation through metabolic reprogramming

Highlights
- IP₃R-Ca²⁺ is pivotal for B cell activation by regulating metabolic reprogramming
- IP₃R-Ca²⁺ governs glucose uptake, glycolytic gene expression, and mitochondrial remodeling
- IP₃R-Ca²⁺ targets the calcineurin-MEF2C-Myc pathway in driving metabolic adaptations

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IP₃R-mediated Ca²⁺ signaling controls B cell proliferation through metabolic reprogramming

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SUMMARY

Emerging evidence shows that metabolic regulation may be a critical mechanism in B cell activation and function. As targets of several most widely used immunosuppressants, Ca²⁺ signaling and calcineurin may play an important role in regulating B cell metabolism. Here, we demonstrate that IP₃R-mediated Ca²⁺ signaling and calcineurin regulate B cell proliferation and survival by activating metabolic reprogramming in response to B cell receptor (BCR) stimulation. Both IP₃R-triple-knockout (IP₃R-TKO) and calcineurin inhibition dramatically suppress the metabolic switch in oxidative phosphorylation and glycolysis of stimulated B cells through regulation of glucose uptake, glycolytic enzyme expression, and mitochondrial remodeling, leading to impaired cell-cycle entry and survival. In addition, IP₃R-Ca²⁺ acts as a master regulator of the calcineurin-MEF2C-Myc pathway in driving B cell metabolic adaptations. As genetic defects of IP₃Rs were recently identified as a new class of inborn errors of immunity, these results have important implications for understanding the pathogenesis of such diseases.

INTRODUCTION

Appropriate activation of B cells is important for maintaining immune homeostasis free from immunodeficiency and autoimmune diseases (Baba and Kurosaki, 2016; LeBien and Tedder, 2008). Recent studies have shown that metabolic programming may be a critical mechanism involved in B cell activation and function (Caro-Maldonado et al., 2014; Dufort et al., 2014; Jellusova, 2020). In comparison with mature naive B cells, which remain in a quiescent state with low metabolic requirements, activated B cells upregulate both glycolysis and oxidative phosphorylation in a balanced fashion (Caro-Maldonado et al., 2014), whereas interfering with metabolic pathways via inhibiting glycolysis (Jayachandran et al., 2018) or mitochondrial respiration (Diaz-Munoz et al., 2015) has been shown to disturb B cell proliferation and immune responses, suggesting that metabolic programming may be important for B cell activation. In addition, metabolism is not only required for meeting energy demands but has also been integrated into the signaling cascades of many intracellular messengers including ATP, NADP⁺ -NADPH, and reactive oxygen species (ROS), reflecting changes in extracellular environment or intracellular metabolic status and dictating cellular fate (Wang and Green, 2012).

One of the central downstream signaling events in response to BCR ligation is intracellular Ca²⁺ elevation, which is generated by Ca²⁺ release from the ER Ca²⁺ store and a subsequent store-operated Ca²⁺ entry (SOCE) following the depletion of ER Ca²⁺ stores (Baba and Kurosaki, 2011). Inositol 1,4,5-trisphosphate receptors (IP₃Rs) are the main ER Ca²⁺ release channels with three subtypes in B cells, which we previously demonstrated are essential for BCR-mediated Ca²⁺ mobilization and B cell development (Tang et al., 2017). Loss of all three subtypes of IP₃Rs leads to completely abolished Ca²⁺ release from ER Ca²⁺ stores, as well as subsequent SOCE, and reduces recirculating mature B cell numbers and alters antibody production (Tang et al., 2017). Notably, heterozygous variants in IP₃R have recently been identified in patients with combined immunodeficiency (Neumann et al., 2021), where disrupted calcium homeostasis and abnormal proliferation and activation of B and T cells were observed. This finding demonstrates that human lymphocytes are indeed sensitive to defects in the primary ER Ca²⁺ release system; however, underlying mechanisms that are critical for treatment and intervention have not yet been clearly identified.

Increasing intracellular Ca²⁺ concentrations subsequently activate the serine-threonine phosphatase calcineurin, which participates in a number of Ca²⁺-dependent signal transduction pathways, particularly in
regulating the transcription factor NFAT during T cell activation (Vaeth and Feske, 2018). Notably, two of the most widely used immunosuppressive drugs, cyclosporine A (CsA) and FK506, target calcineurin and have been shown to selectively abrogate Ca\(^{2+}\)-regulated activation pathways in both B cells and T cells (Klaus et al., 1994), but we still lack a thorough and complete understanding of their mechanism of action. Whether this involves IP\(_3\)R-Ca\(^{2+}\) signals that modulate metabolic adaptations during B cell activation and proliferation is largely unexplored.

In the present study, we use B cell-specific IP\(_3\)R-TKO mice to show that IP\(_3\)R-mediated Ca\(^{2+}\) signaling and calcineurin are essential for B cell proliferation by triggering metabolic reprogramming in response to BCR stimulation. IP\(_3\)R-Ca\(^{2+}\) and calcineurin control B cell metabolic reprogramming through regulating glucose uptake, glycolytic enzyme expression, as well as mitochondrial remodeling, likely involving the regulation of c-Myc, which is dependent on the calcineurin-MEF2C pathway. We present a paradigm whereby loss of IP\(_3\)Rs and calcineurin inhibition leads to defective B cell proliferation and function upon antigen infection as a result of the inactivation of metabolic reprogramming, underpinning IP\(_3\)R-Ca\(^{2+}\) as an important metabolic trigger for B cell activation and proliferation, which may provide novel insights into the etiology of immune-related diseases.

**RESULTS**

**Metabolic reprogramming activated by IP\(_3\)R-Ca\(^{2+}\)-calcineurin is required for BCR-induced cell cycle entry**

To first investigate whether IP\(_3\)R-mediated Ca\(^{2+}\) signaling controls BCR-mediated proliferation via calcineurin, we measured calcineurin activity in IP\(_3\)R-TKO B cells and found it was significantly impaired compared to control (Figures S1A and S1B), confirming that calcineurin is indeed downstream of IP\(_3\)R-Ca\(^{2+}\). To verify the role of IP\(_3\)R-Ca\(^{2+}\) signaling and calcineurin in controlling BCR-mediated proliferation, we performed a proliferation assay in control and IP\(_3\)R-TKO B cells with or without the treatment of calcineurin inhibitor, FK506. In accordance with our previous findings (Tang et al., 2017), BCR-mediated upregulation of the cell cycle marker Ki-67 was dramatically blocked in IP\(_3\)R-deficient B cells compared to control (Figures 1A and 1B). In addition, FK506 also completely blocked BCR-induced upregulation of Ki-67 in B cells (Figures 1A and 1B), demonstrating a defect in antigen-dependent cell-cycle entry in B cells upon the loss of IP\(_3\)R-mediated Ca\(^{2+}\) release or inhibition of calcineurin. Along with proliferation, we also found that cell survival was impaired in both IP\(_3\)R-deficient and FK506-treated B cells after BCR stimulation, as indicated by reduced percentages of live cells by the LIVE/DEAD staining (Figures S1C and S1D). In addition, deletion of all IP\(_3\)Rs or another calcineurin inhibitor CsA could significantly reduce the expression of antiapoptotic genes, Bcl2l1a and Bcl2l1 (Figure S1E), suggesting that IP\(_3\)R-Ca\(^{2+}\) release and calcineurin also play an important role in regulating B cell survival. We further investigated whether BCR expression was affected in the loss IP\(_3\)Rs. Interestingly, we found a significantly elevated rather than reduced IgM expression in IP\(_3\)R-TKO B cells compared to control (Figure S1F), suggesting that the defect in BCR-induced proliferation and survival in IP\(_3\)R-TKO B cells was not due to impaired BCR expression.

Cells have to pass a metabolic checkpoint and meet nutrient sufficiency before progressing through G1 and committing to mitosis (Foster et al., 2010). Glycolysis is the main source of anabolic metabolism after B cell activation, for which the primary source of energy and carbon is glucose (Jellusova, 2020). Consistently, 2-deoxy-D-glucose (2-DG), a glucose analog that suppresses glycolysis (Wick et al., 1957), could dramatically inhibit both cell cycle entry (Figures 1C and 1D) and survival (Figures S1G and S1H) of BCR-activated B cells, confirming that glucose uptake and utilization is required for cell-cycle commitment and survival of B cells. To test whether IP\(_3\)R and calcineurin contribute to this metabolic switch of B cells, we examined oxidative phosphorylation (OXPHOS) and aerobic glycolysis of B cells using the Seahorse Extracellular Flux analyzer. Mitochondrial oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were first measured in B cells following 6 h of BCR stimulation. At this time point, B cells have not entered the cell cycle, and therefore metabolic changes are not caused by potential secondary effects of proliferation (Caro-Maldonado et al., 2014). In comparison to unstimulated B cells, BCR stimulation indeed significantly increased both basal and maximal OCR (Figures 1E and 1F). However, BCR stimulation-induced increases in basal and maximal OCR were completely abolished in IP\(_3\)R-TKO B cells (Figures 1E and 1F). In addition, BCR stimulation induced a dramatic increase in basal ECAR of control B cells, which was also impaired in IP\(_3\)R-TKO B cells (Figures 1G and 1H). Combined analysis of the respiratory capacity and glycolytic function of stimulated B cells clearly separated control cells from IP\(_3\)R-TKO B cells, the latter of which resembled more closely to resting cells (Figure 1I). In addition, calcineurin inhibitor FK506 or CsA also
exhibited a similar inhibitory effect on such activation-induced increases in OCR and ECAR in B cells (Figures 1J–1L). Taken together, these results clearly demonstrate that IP3R-Ca2+ and calcineurin are required for the metabolic switch of B cells as a precondition for cell-cycle entry and survival.

IP3Rs and calcineurin modulate glucose uptake and glycolytic enzyme expression

One essential pathway to increase glycolysis is through increased glucose uptake. Given that 2-DG could dramatically inhibit both cell cycle entry (Figures 1C and 1D) and cell survival in BCR-activated B cells (Figures S1G and S1H), we hypothesized that IP3R and calcineurin might directly control glucose uptake in B cells. To test this, we first evaluated the ability of glucose uptake in B cells using the fluorescent glucose analogue 2-NBDG and found that BCR stimulation induced a 3-fold increase of glucose uptake in control B cells, which was almost absent in IP3R-TKO or CsA-treated B cells (Figure 2A), suggesting that IP3R-Ca2+ release and calcineurin activation are essential for glucose uptake in activated B cells.

GLUT1 plays an important role in controlling glucose uptake in various types of cells including B cells, with induced expression upon BCR stimulation (Akkaya et al., 2018; Caro-Maldonado et al., 2014). Indeed, we also found that GLUT1 expression was significantly upregulated in BCR-stimulated control B cells when compared with unstimulated cells (Figure 2B). In addition, expression of GLUT3, another important player in regulating glucose uptake (Akkaya et al., 2018), was also significantly elevated in response to BCR stimulation (Figure 2C). Consistent with the defects of glucose uptake observed in IP3R-TKO and CsA-treated B cells, upregulation of GLUT1 and GLUT3 expression upon BCR stimulation was also significantly reduced by IP3R deletion and calcineurin inhibition (Figures 2B and 2C), which is in agreement with defects in...
glucose uptake and cell growth (Figures 2A and S2A). Because glycolysis is the major pathway for glucose metabolism in which glucose will convert to pyruvate (under aerobic condition) or lactate (anaerobic) through a series of enzyme-catalyzed reactions (Donnelly and Finlay, 2015), we investigated whether deletion of IP3Rs and calcineurin inhibition could also affect the expression of the glycolytic enzymes. We found BCR stimulation significantly upregulated the expression of hexokinase ($Hk2$), aldolase A ($Aldoa$), phosphoglycerate mutase 1 ($Pgam1$), pyruvate kinase M2 ($Pkm2$), and lactate dehydrogenase A ($Ldha$) in control B cells (Figure S2B), whereas such increases were severely impaired in IP3R-TKO or CsA-treated B cells (Figure S2B). Therefore, these results indicate that IP3R and calcineurin modulate glucose uptake and regulate glucose uptake and cell growth.
the induction of gene expression of both glucose transporters and glycolytic enzymes in B cells upon BCR stimulation to control glucose uptake.

**IP₃Rs and calcineurin are required for BCR-induced increases in mitochondrial mass and mitochondrial enzyme expression**

B cell metabolic reprogramming is a balanced process involving increased expression of both glycolytic and mitochondrial components (Jellusova and Rickert, 2017). Given that B cell stimulation enhanced mitochondrial respiration, which was abolished in both IP₃R-deficient and CsA-treated B cells (Figures 1E and 1J), we next investigated the role of IP₃R-mediated Ca²⁺ signaling and calcineurin in mitochondrial metabolism of B cells. Because mitochondrial mass is correlated with cell metabolic activity (Caro-Maldonado et al., 2014), we quantified mitochondrial mass by Mitotracker Green staining. Consistent with previous reports (Akkaya et al., 2018; Caro-Maldonado et al., 2014), B cell activation led to a significant elevation in mitochondrial mass as evidenced by increased fluorescence with Mitotracker Green upon BCR stimulation in control B cells (Figure 2D). However, no such increase could be observed in IP₃R-TKO B cells or B cells treated with CsA (Figure 2D). In addition, the flow cytometry analysis of mitochondrial protein TOM20 and VDAC1 also showed the same trends among control, IP₃R-TKO, and CsA-treated B cells (Figures 2E and 2F), further confirming that IP₃R-mediated Ca²⁺ signaling and calcineurin are essential for BCR-induced increase in mitochondrial mass.

We then analyzed the expression of mitochondrial electron transport chain (ETC) complexes. Interestingly, BCR stimulation increased the protein levels of ATP5A (complex V) and UQCRC2 (complex III) in control B cells, whereas NDUF8B (complexes I), SDHB (complex II), and MTCO1 (complex IV) remained unchanged (Figures S2C and S2D). Such increases in ATP5A and UQCRC2 expression upon BCR stimulation was also accompanied with upregulation of PPRC1 mRNA levels (Figures S2C–S2E), one of the PGC-1 family of transcriptional co-regulators that has been reported to regulate the expression of mitochondrial ETC complexes and mitochondrial biogenesis (Scarpulla, 2011). Notably, increased expression of ATP5A, UQCRC2, or PPRC1 was significantly impaired in IP₃R-TKO and CsA-treated B cells (Figures S2C–S2E), which was consistent with the changes in mitochondrial respiration and mitochondrial mass (Figures 1E, 1J, and 2D). Our results thus strongly suggest that IP₃R-mediated Ca²⁺ and calcineurin control mitochondrial respiration by regulating mitochondrial mass and ETC complexes upon BCR stimulation. Importantly, ionomycin treatment of IP₃R-TKO B cells could efficiently restore the defects in BCR-induced cell growth, glucose uptake, and mitochondrial mass increase (Figures S3A–S3C), validating a central role for Ca²⁺ in B cell metabolic programming.

**c-Myc is required for IP₃R and calcineurin-dependent metabolic reprogramming**

To understand the molecular mechanism responsible for the defect of metabolic switch induced by IP₃R deletion, we analyzed the transcriptional regulation of several metabolic regulators, including c-Myc (Jellusova, 2020), Irf4 (Low et al., 2019), and c-Rel (Cu et al., 2021; Li et al., 2020). We first measured the mRNA expression of these metabolic regulators; although BCR stimulation increased the mRNA levels of all these genes in control B cells, these increases were significantly attenuated in IP₃R-TKO and CsA-treated B cells (Figures 3A and S4A). We then measured the c-Myc protein expression and found a similar result among control, IP₃R-TKO, and CsA-treated B cells (Figures 3B and 3C). c-Myc has been proposed to play an essential role in regulating the metabolic reprogramming following B cell activation (Caro-Maldonado et al., 2014). We therefore speculated that impaired c-Myc induction may be responsible for the defects of metabolic reprogramming observed in IP₃R-TKO or calcineurin-inhibited B cells. By using a small molecule c-Myc inhibitor 10,058-F4, we found that c-Myc inhibition was sufficient to block the increases in glucose uptake (Figure 3D), expression of GLUT1 and GLUT3 (Figures 3E and 3F), and expression of glycolytic enzymes induced by BCR stimulation in control B cells (Figure S4B). In addition, BCR stimulation-induced increases in cell growth (Figure S4C), mitochondrial mass (Figure 3G), and expression of mitochondrial proteins (Figures 3H and 3I) and Pprc1 (Figure S4D) were all significantly inhibited by 10,058-F4 in control B cells. More importantly, we found that BCR-induced expression of Irf4 and Rel was also dramatically impaired by 10,058-F4 (Figure S4E). Taken together, these results suggest that c-Myc could play a central role in controlling metabolic reprogramming in B cells upon BCR stimulation in an IP₃R and calcineurin-dependent manner.

**IP₃R-dependent MEF2C expression modulates c-Myc induction**

Given the established involvement of the PI3K-AKT-mTOR signaling pathway in controlling metabolic reprogramming as well as c-Myc expression (Berry et al., 2020; Vaeth et al., 2017), we next investigated...
whether impaired induction of c-Myc expression was caused by inactivation of the PI3K-AKT-mTOR pathway because of the loss of IP3Rs. Surprisingly, we observed an increased activation of the PI3K-AKT-mTOR pathway in IP3R-TKO B cells after BCR stimulation as evidenced by elevated phosphorylation of AKT and the ribosomal S6 protein (Figure 4A), indicating that PI3K-AKT-mTOR pathway may not be directly involved in induction of c-Myc expression in stimulated B cells.

Calcineurin-regulated transcription factor MEF2C has been previously reported to regulate B cell proliferation and survival in response to BCR stimulation (Blaeser et al., 2000; Wilker et al., 2008), and enforced expression of MEF2C in transitional B cells leads to increased c-Myc expression after BCR cross-linking (Andrews et al., 2012). To investigate whether IP3R and calcineurin may regulate c-Myc expression through MEF2C-dependent pathways, we first performed ChIP-qPCR analysis and found that MEF2C binds to the promoter region of Myc gene (Figure 4B), suggesting a direct regulation of Myc expression by MEF2C. Intriguingly, MEF2C protein expression was significantly decreased in IP3R-TKO B cells compared to control B cells (Figures 4Ca and 4D). Consistent with this, the amount of ChIP-pTed Myc DNA by MEF2C was also significantly reduced in IP3R-TKO B cells (Figure 4B). We then investigated whether reduced expression of MEF2C protein observed in
IP3R-TKO B cells was because of increased protein degradation or decreased transcription. Inhibition of proteasome by MG132 could lead to an increase of MEF2C protein in both control and IP3R-TKO B cells (Figure 4E) but was still not sufficient to reverse the decline of MEF2C protein in IP3R-TKO B cells (Figure 4E), implying that decreased MEF2C expression were not caused by protein degradation at the loss of IP3Rs. Conversely, we found that Mef2c mRNA was decreased by \( \frac{1}{2} \) in IP3R-TKO B cells compared with control cells (Figure 4F), suggesting decreased transcription of MEF2C. In addition, ChIP-qPCR analysis also revealed that MEF2C could directly bind to its own promoter, but the interaction was significantly reduced in IP3R-TKO B cells (Figure 4G), which is consistent with a previous study reporting that MEF2C could directly regulate its own transcription (Kong et al., 2016). Taken together, our results suggest that IP3R deficiency may have led to reduced MEF2C expression, which further impaired c-Myc and its downstream targets to disrupt metabolic reprogramming in B cells upon BCR stimulation.

**DISCUSSION**

B cells are exposed to changing environments and are transiently switching between quiescent stages and stages of rapid proliferation or increased protein secretion (Egawa and Bhattacharya, 2019). Consequently,
that IP3R-deficient B cells are similar to transitional B cells, which undergo apoptosis rather than survive. B cells compared to naive mature B cells (Andrews et al., 2012). This is in agreement with our finding that IP3R-deficiency in humans (Neumann et al., 2021). A reduction of IP3R subtype 3 expression at the mRNA and protein level was found in the patient’s mononuclear cells. Though abnormal proliferation and activation responses following BCR ligation in patient cells was observed, the underlying mechanism was not interrogated. Thus, our findings that loss of IP3R and/or inhibition of calcineurin leads to the inactivation of metabolic reprogramming of B cells, which impairs proliferation and survival in response to BCR stimulation, thus provide a likely explanation to the immunodeficiency and immune dysregulation observed in IP3R-defective patients and identify IP3R-Ca2+ mediated B cell metabolic pathway as diagnostic targets for patients with specific inborn errors of immunity. Whether activation of metabolic reprogramming through targeting the IP3R-calcineurin-MEF2C-Myc axis may help remedy the disease, is worthy of future investigation.

Apart from this, calcineurin inhibitors are widely administered in clinical medicine as immunosuppressants to prevent graft-versus-host rejection and treat certain autoimmune diseases (Guada et al., 2016); although inhibition of gene expression dependent on NFAT has been attributed as the main mechanistic paradigm (Hodge et al., 1996; Feng et al., 2001; Ranger et al., 1998), their underlying mechanism in prohibiting B cell proliferation was only partially understood. Our finding that IP3R-Ca2+ and calcineurin regulates B cell metabolic reprogramming thus reveals an alternative route by which calcineurin contributes to B cell proliferation, highlighting inhibition of the Ca2+ metabolic trigger and subsequent impairment of glucose uptake, glycolytic enzyme expression, and mitochondrial remodeling as the primary cause of immune-suppression. In addition, calcineurin inhibitors like CsA are associated with a number of side effects because of their high toxicity, such as carcinogenicity (Guada et al., 2016); therefore, there is substantial interest in developing targeted alternatives to CsA that maintain immunosuppressive function but with fewer off-target effects (Waldmeier et al., 2003). In this regard, targeting IP3R with antagonists (such as IRBIT (Gambardella et al., 2021)) to specifically block B cell metabolic reprogramming and proliferation may prove to be a more targeted approach in treating autoimmune diseases or immune rejection.

Mechanistically, we reveal that MEF2C-dependent regulation of c-Myc may be important for IP3R-mediated calcium signaling in regulating BCR-induced metabolic reprogramming. Interestingly, MEF2C has been reported to express at a low protein level and exhibit impaired DNA-binding activity in transitional B cells compared to naive mature B cells (Andrews et al., 2012). This is in agreement with our finding that IP3R-deficient B cells are similar to transitional B cells, which undergo apoptosis rather than survive and proliferate upon BCR stimulation, possibly because of reduced MEF2C expression and activity. Notably, we found that MEF2C binds to the promoter region of Myc, suggesting that IP3R-dependent MEF2C expression modulates c-Myc induction. This is supported by previous studies that found: 1) enforced expression of MEF2C in transitional B cells enhances cell proliferation, survival, and expression of Myc transcripts (Andrews et al., 2012), and 2) MEF2C controls MYC expression by regulating the activity of MYC Epstein-Barr virus super-enhancers (ESEs) in lymphoblastoid cell lines (Wang et al., 2019), corroborating a direct role of MEF2C in c-Myc expression. However, it should be noted that calcineurin may also...
affect other molecules and pathways that can have an impact on metabolism independently of c-Myc, such as the transcriptional factor EB (TFEB), a known substrate of calcineurin and a master regulator of lysosomal biogenesis and autophagy (Medina et al., 2015). Alternatively, Ca²⁺ has been shown to directly impact cellular energy metabolism in skeletal muscle through modulating the mitochondrial ETC and the F₁F₀ ATP synthase (Glancy et al., 2013).

Collectively, our study revealed IP₃R-mediated Ca²⁺ signaling as one of the earliest triggering events in B cell activation and metabolic reprogramming by coordinated regulation of glucose uptake, glycolytic enzyme expression, and mitochondrial remodeling, likely through the calcineurin-MEF2C-Myc pathway, which may provide mechanistic insights into the disease etiology of immunodeficiency patients with IP₃R mutations and the pharmacological mechanism of calcineurin inhibitors.

Limitations of the study
Although we found that IP₃R-mediated Ca²⁺ signaling is essential for B cell activation and proliferation through regulating metabolic reprogramming, our results also showed that at least in some instances (for example cell proliferation), anti-IgM in IP₃R-TKO B cells still produced somewhat of an effect, which may implicate a (minor) role for Ca²⁺-independent effects or Ca²⁺-dependent effects mediated through other channels besides IP₃Rs. Another limitation of this study is that we have not tested other types of BCR stimulation, such as IgD, which based on past literature may produce effects different than that of anti-IgM.

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SUPPLEMENTAL INFORMATION
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AUTHOR CONTRIBUTIONS
H.T., Y.L., S.W., J.J., X.Z., C.H., Y.L., L.H., Y.G., and L.S. performed the experiments. H.T., J.L., and K.O. designed the research. H.T., Y.L., Y.B., X.F., L.S., C.W., and K.O. wrote the manuscript.

DECLARATION OF INTERESTS
The authors declare no competing interests.

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## STAR METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| anti-rabbit IP3R1   | Our lab | Ouyang et al., 2014 |
| anti-rabbit IP3R2   | Our lab | Ouyang et al., 2014 |
| anti-mouse IP3R3    | BD Biosciences | Cat# 610312, RRID:AB_397704 |
| anti-rabbit MEF2C   | Abcam  | Cat# ab211493, RRID:AB_2864417 |
| anti-rabbit Akt (pan) (C67E7) | Cell Signaling Technologies | Cat# 4691, RRID:AB_915783 |
| anti-rabbit phospho-Akt (Ser473) (D9E) | Cell Signaling Technologies | Cat# 4060, RRID:AB_2315049 |
| anti-rabbit phospho-S6 (Ser235/236) (D57.2.2E) | Cell Signaling Technologies | Cat# 4858, RRID:AB_916156 |
| anti-mouse β-actin (C4) | Santa cruz | Cat# sc-47778 HRP, RRID:AB_2714189 |
| Total Oxphos Rodent WB antibody cocktail | Abcam | Cat# ab110413, RRID:AB_2629281 |
| anti-rabbit GLUT1 [EPR3915] | Abcam | Cat# ab115730, RRID:AB_10903230 |
| anti-rabbit GLUT1 (FITC) | Abcam | ab136180 |
| anti-rabbit Ki-67 (D3B5) | Cell Signaling Technologies | Cat# 9129, RRID:AB_2687446 |
| anti-mouse VDAC1 [20B12AF2] (Alexa Fluor®488) | Abcam | Cat# ab186735, RRID:AB_2889972 |
| anti-mouse IgM (RMM-1) (FITC) | Biolegend | Cat# 406505, RRID:AB_315055 |
| Alexa-647 conjugated anti-rabbit IgG secondary antibody | Invitrogen | Cat# A-21245, RRID:AB_2534813 |
| anti-rabbit c-Myc (D84C12) | Cell Signaling Technologies | Cat# 5605, RRID:AB_1903938 |
| **Chemicals, peptides, and recombinant proteins** |        |            |
| AffiniPure F(ab’)2 Fragment Goat Anti-Mouse IgM, Mu Chain Specific | Jackson ImmunoResearch | 115-006-020 |
| CsA | Sigma Aldrich | 59865-13-3 |
| FK506 | Sigma Aldrich | 109581-93-3 |
| 2-Desoxy-Glucose (2-DG) | Sigma Aldrich | 154-17-6 |
| 2-NBDG | ThermoFisher Scientific | N13195 |
| Ionomycin | Sigma Aldrich | 56092-82-1 |
| Mito-Tracker Green | Beyotime | C1048 |
| LIVE/DEAD™ Fixable Dead Cell Stain | Invitrogen | L34971 |
| **Critical commercial assays** |        |            |
| Calcineurin Cellular Activity Assay Kit | Eno Life Sciences | BML-AK816-001 |
| Seahorse XF Cell Mito Stress Test Kit | Agilent Technologies | 103015-100 |
| Chromatin IP kit | Cell Signaling Technologies | 9005 |
| Transcript One-Step gDNA Removal and cDNA Synthesis SuperMix Kit | TransGen Biotech | AH311-02 |
| **Experimental models: Organisms/strains** |        |            |
| Mouse: IP3R-triple-floxed: Itpr1f/fItp2f/fItp3f/f | Our lab | N/A |
| Mouse: CD19-Cre: Cd19tm1(cre)Cgn/J | The Jackson Laboratory | 006785, RRID:IMSR_JAX:006785 |
| **Oligonucleotides** |        |            |
| Primers for qPCR | See Table S1 | N/A |

(Continued on next page)
RESOURCES AVAILABILITY

Lead contact
Further information about the protocols and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Huayuan Tang (tanghuayuan33@126.com).

Materials availability
This study did not generate new unique reagents.

Data and code availability
All data reported in this paper will be shared by the lead contact upon request.

This paper does not report original code.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals
IP3R triple-floxed (ltptr1/floorptr2/floorptr3/f) mice have been described previously (Lin et al., 2016, 2019; Ouyang et al., 2014). ltptr1/floorptr2/floorptr3/f mice were crossed with Cd19tm1(cre)Cgn/J (CD19-Cre) mice (The Jackson Laboratory). ltptr1/floorptr2/floorptr3/f Cd19cre+ mice were used as B cell-specific IP3R triple knockout (IP3R-TKO) mice. ltptr1/floorptr2/floorptr3/f Cd19cre- mice were used as control mice. All mice were on a pure C57BL/6 genetic background. Male and female mice were used between 8 and 16 weeks of age. All mice were housed under specific pathogen-free conditions with a 12/12 h day/night cycle. All animal care and use procedures in this study were approved by the Institutional Animal Care and Use Committee.

B cell isolation and culture
Single-cell suspensions were obtained from spleens of control and IP3R-TKO mice. After RBCs were depleted by hypotonic lysis, splenic B cells were purified by the negative selection of CD43+ cells with anti-CD43 magnetic beads (Invitrogen, 11422D). The purity of the B cells (B220+) was > 95% determined by FACS analysis. Splenic B cells were stimulated in RPMI 1640 medium (supplemented with 10% FBS, 2 mM L-glutamine, 50 μM 2-mercaptoethanol and 100 U/ml penicillin plus streptomycin) with 20 μg/ml anti-IgM F(ab)'2 (Jackson ImmunoResearch, 115-006-020), 1 μM CsA (Sigma Aldrich, 59865-13-3), 1.25 μM FK506 (Sigma Aldrich, 109581-93-3), 10 mM 2-DG (Sigma Aldrich, 154-17-6), 1 μM Ionomycin (Sigma Aldrich, 56092-82-1) or left unstimulated as indicated.

METHOD DETAILS

Cellular calcineurin activity assay
The splenic B cell extracts were prepared using reagents provided in the calcineurin cellular activity assay kit (Eno Life Sciences, BML-AK816-001). The assay was performed according to manual's instructions. The absorbance values from the assay were converted into amount of released phosphate following the manual.
Extracellular flux analysis

Oxygen consumption rates (OCR) and extracellular acidification rates (ECAR) were measured using an XFe24 Extracellular Flux Analyzer (Agilent). In brief, $1 \times 10^6$ B cells per well were resuspended in Seahorse medium (Agilent) and plated in 24-well Seahorse plates (Agilent) coated with poly-L-lysine (Sigma). Cells were maintained at 37°C in a non-CO$_2$ incubator for at least 1 h before the assay. For the mitochondrial stress test, cells were treated sequentially with 1 μM oligomycin (an inhibitor of the ATP synthase), 10 μM protonophore Carbonyl cyanide-4- (trifluoromethoxy) phenylhydrazone (FCCP) and 1 μM rotenone plus 1 μM antimycin A (inhibitors of complex I and III of the respiratory chain, respectively). The basal oxygen consumption rate (OCR) was calculated by subtracting the OCR after rotenone and antimycin A treatment from the OCR measured after addition of FCCP. The maximal OCR was calculated by subtracting the OCR after rotenone and antimycin A treatment from the OCR measured after addition of FCCP.

Real-time PCR

Total RNA was extracted using TRIzol reagent (Invitrogen) or RNeasy Mini Kit (Qiagen), and cDNA was synthesized using TransStart One-Step gDNA Removal and cDNA Synthesis SuperMix Kit (TransGen Biotech) as previously described (Chen et al., 2021b). Quantitative real-time PCR (RT-PCR) was performed using TransStart Tip Green qPCR SuperMix (TransGen Biotech) according to the manufacturer’s instructions. The primer sequences for Bcl2la1, Bcl2l1, Myc, Rel, Irf4, Pprc1, Hk2, Aldoa, Pgam1, Pkm2, Ldha, Methc and 18s rRNA are presented in Table S1. Relative transcript abundance was normalized to 18S ribosomal RNA (18S rRNA). Each sample was run at least in duplicate.

Immunoblotting

Protein analysis was performed as previously described (Chen et al., 2021a). In brief, total cell lysates were prepared in lysis buffer containing 8 M urea, 2 M thiourea, 3% SDS, 75 mM DTT, 0.05 M Tris-HCl [pH 6.8], and 0.03% bromophenol blue. Lysates from equal numbers of cells for each condition were subjected to SDS-PAGE and analyzed by Western blotting using the following antibodies: anti-IP3R1 (Ouyang et al., 2014), anti-IP3R2 (Ouyang et al., 2014), anti-IP3R3 (BD Biosciences, 610312), anti-MEF2C (Abcam, ab211493), anti-c-Myc (Cell Signaling Technologies, 5605), anti-panAkt (Cell Signaling Technologies, 4691), anti-Akt p-Ser473 (Cell Signaling Technologies, 4060), anti-S6 p-Ser235/236 (Cell Signaling Technologies, 4858), anti-β-actin (Santa cruz, sc-7778), and Total Oxphos Rodent WB antibody cocktail (Abcam, ab110413). Proteins were visualized using an HPR-conjugated anti-mouse or anti-rabbit secondary antibody (Cell Signaling Technologies) and chemiluminescent ECL reagent (Thermo Fisher). Densitometric quantification was performed using ImageJ.

Flow cytometry

Cells were washed in ice-cold PBS containing 1% FBS before blocking with anti-FcgRII/FcgRIII antibodies (2.4G2, eBioscience). Staining of surface molecules with fluorescently labeled antibodies was performed at room temperature for 20 min in the dark. GLUT1 or GLUT3 expression was detected in cells fixed and permeabilized with the IC Staining Buffer Kit (BioLegend) using a polyclonal rabbit anti-GLUT1 antibody (Abcam, ab115730) together with an Alexa-647 conjugated anti-rabbit IgG secondary antibody (Invitrogen) or a polyclonal, FITC-conjugated anti-GLUT3 antibody (Abcam, ab136180). Samples were acquired on a LSRII flow cytometer using FACS Diva software (BD Biosciences) and further analyzed with FlowJo software (Tree Star).

Glucose uptake measurement

Glucose uptake was analyzed directly using the fluorescent glucose analog 2-NBDG (ThermoFisher Scientific). Stimulated and unstimulated cells were incubated in glucose-free RPMI medium containing 100 μM 2-NBDG for 2 hr at 37°C and the amount of 2-NBDG taken up by viable B cells was assessed by flow cytometry after staining with Propidium iodide (PI).

ChIP assay

ChIP assay was performed using a Chromatin IP kit (Cell Signaling Technologies, 9005) according to the manual. Protein-DNA complex was immunoprecipitated with antibodies against MEF2C (Abcam, ab211493) and normal rabbit IgG (Cell Signaling Technologies, 2729). The purified DNA was quantified by qPCR to detect the enrichment of Myc promoter (promoter-1, forward: 5’-TAGACCGCAGA GACTCCT-3’, reverse: 5’-CCTGCGCA GTCCAGTAAAGT-3’, promoter-2, forward: 5’-AAATCCGAGAGC
CACAACCC-3', reverse: 5’-CCTAGTCTCCTTTGCTGC-3') and Mef2c promoter (promoter-1, forward: 5’-TGGAGAGGAATAGGCGTGGA-3'; reverse: 5’-AGTCCAGCCTGTGTTCTG-3'; promoter-2, forward: 5’-ATAGCCTGGCTCTCAGTCTG-3'; reverse: 5’-TGGATTACTCAGCCCTGTT-3') by normalized to the total input control.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

All data are represented as mean ± SEM. All data were assessed for normality using probability plots and the Shapiro-Wilk test for normality. The unpaired Student’s t-test (parametric) or the Mann–Whitney test (non-parametric, Figures 1B and 1D) was used to assess the p value when comparing two groups. All data were analyzed using GraphPad Prism 8.0; A two-sided p value < 0.05 was considered statistically significant. *p < 0.05, **p < 0.01, ***p < 0.001. The number of biological replicates is indicated in all of the figures and the statistical methods are noted in the figure legends and Methods.