HHS Public Access

Author manuscript

J Immunol. Author manuscript; available in PMC 2021 October 15.

Published in final edited form as:

J Immunol. 2020 October 15; 205(8): 2100–2108. doi:10.4049/jimmunol.2000584.

PIK3IP1 promotes extrafollicular class switching in T-dependent immune responses

Kristina Ottens[*], **Jalyn Schneider**[*], **Lawrence P. Kane**[†], **Anne B. Satterthwaite**[*,‡]

[*]Department of Internal Medicine, UT Southwestern Medical Center, Dallas, TX

[†]Department of Immunology, University of Pittsburgh School of Medicine, Pittsburgh, PA

[‡]Department of Immunology, UT Southwestern Medical Center, Dallas, TX.

Abstract

PI3 kinase (PI3K) plays multiple roles throughout the life a B cell. As such, its signaling is tightly regulated. The importance of this is illustrated by the fact that both loss and gain of function mutations in PI3K can cause immunodeficiency in humans. PIK3IP1, also known as TrIP, is a transmembrane protein that has been shown to inhibit PI3K in T cells. Results from the ImmGen consortium indicate that PIK3IP1 expression fluctuates throughout B cell development in a manner inversely correlated with PI3K activity; however, its role in B cells is poorly understood. Here we define the consequences of B cell-specific deletion of PIK3IP1. B cell development, basal immunoglobulin levels, and T-independent responses were unaffected by loss of PIK3IP1. However, there was a significant delay in the production of IgG during T-dependent responses, and secondary responses were impaired. This is likely due to a role for PIK3IP1 in the extrafollicular response, since germinal center formation and affinity maturation were normal and PIK3IP1 is not appreciably expressed in germinal center B cells. Consistent with a role early in the response, PIK3IP1 was downregulated at late time points after B cell activation, in a manner dependent on PI3K. Increased activation of the PI3K pathway was observed in PIK3IP1-deficient B cells in response to engagement of both the BCR and CD40 or strong crosslinking of CD40 alone. Taken together, these observations suggest that PIK3IP1 promotes extrafollicular responses by limiting PI3K signaling during initial interactions between B and T cells.

Introduction

The ability of the immune system to respond to foreign antigens by producing antibodies is critical to combat a wide variety of infections and is the basis for many vaccines. During humoral immune responses, B cells are activated in either a T-independent or T-dependent manner, depending on the nature of the antigen (1). In T-dependent responses, B cells receive cognate help from T cells and produce antibodies in two waves. The first is derived from the extrafollicular response. These antibodies can be either IgM or IgG and are of relatively low affinity (1). The second wave of antibodies are produced by germinal center-derived plasma cells, which have undergone somatic mutation and affinity maturation. These

Corresponding Author Anne Satterthwaite, PhD, Department of Internal Medicine, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-8884, 214-648-3172 (phone), 214-648-7995 (fax), anne.satterthwaite@utsouthwestern.edu.

antibodies are thus of higher affinity and are more likely to be class switched (1). Memory B cells can also be generated during both the extrafollicular and the germinal center stages of the response (2–7). Both phases are critical for effective immunity. Extrafollicular antibodies provide a rapid response within days of infection, and low affinity, early memory cells are thought to allow for efficient future responses against pathogen variants (2–7). Germinal center-derived plasma cells and memory cells provide longer term, high affinity responses with a wider variety of effector functions (2–7).

During T-dependent responses, B cells receive signals through both the B cell antigen receptor (BCR) and CD40 (8). Both of these receptors signal through PI3 kinase (PI3K) (9–12). The product of PI3K, PIP3, binds to the pleckstrin homology domains of several critical B cell signaling molecules. This results in their localization to the plasma membrane and their subsequent activation (reviewed in (13)). PI3K is required for the initial proliferation of B cells upon antigen encounter (10, 11, 14–16), with the subsequent fate of the B cell shaped by the strength of PI3K signaling. Class switching is limited by strong PI3K signaling and enhanced when PI3K pathway activity is weak (17, 18). The strength of PI3K signaling also controls developmental fate decisions regulating the distribution of B cells among peripheral B cell subsets – follicular (FO), marginal zone (MZ), and B1a B cells (10, 11, 14–16, 18, 19).

Consistent with its critical role in B cells, signaling downstream of PI3K is tightly regulated, mainly by PTEN and SHIP-1. These inositol phosphatases dephosphorylate PIP3, the product of PI3K; PTEN to PI(4,5)P2 and SHIP-1 to PI(3,4)P2 (13). They each have numerous, non-redundant functions in B cell development and activation (18–20), highlighting the importance of maintaining an appropriate balance of PI3K signal strength. Indeed, either too little or too much signaling via the PI3K pathway leads to immunodeficiency in both mice and humans (21, 22).

Much less is understood about mechanisms that act at the level of PI3K itself to limit its activity in B cells. PIK3IP1, also known as TrIP, is a transmembrane protein that interacts with PI3K p85/p110 heterodimers (23). PIK3IP1 downregulates PI3K activity in several non-immune cell types (24–29) and in T cells (30–33). PIK3IP1-deficient T cells exhibit increased pS6/mTOR activation downstream of PI3K (31, 32), produce elevated levels of IFNγ (31, 32), and have enhanced anti-tumor activity (33). The extracellular domain of PIK3IP1 promotes its function in T cells, although a ligand has not been identified (31–33). PIK3IP1 levels are low in several types of malignancy and strategies that upregulate it help sensitize tumors to killing by PI3K inhibitors (25, 34–44), suggesting that understanding control of PIK3IP1 expression may have therapeutic value. One such tumor is of the B lineage, suggesting that PIK3IP1 may act in B cells (36, 37), although nothing is known about its role in normal B cell responses.

Here, we define the consequences of B cell-specific deletion of PIK3IP1. B cell development, basal immunoglobulin levels, and T-independent responses were unaffected by loss of PIK3IP1. However, there was a significant delay in the production of IgG during T-dependent responses, and memory responses were impaired. This is likely due to a role for PIK3IP1 in the extrafollicular response, since germinal center formation and affinity

maturation were normal and PIK3IP1 is not appreciably expressed in germinal center B cells. Consistent with it acting early in the response, PIK3IP1 was downregulated at late time points after B cell activation in manner dependent on PI3K signaling. Increased activation of the PI3K pathway was observed in PIK3IP1-deficient B cells in response to engagement of both the BCR and CD40 or strong crosslinking of CD40 alone. Taken together, these observations suggest that PIK3IP1 promotes extrafollicular responses by limiting PI3K signaling during initial interactions between B and T cells.

Materials and Methods

Mice

PIK3IP1f/f mice on the C57BL/6 background (32) were crossed to CD19-cre mice (45) to delete PIK3IP1 specifically in B cells. Foxo3−/− (46) and control mice used in Figure 1c were on the FVB background. Mice were age matched and littermate controls were used whenever possible. All animal experiments were approved by the UT Southwestern Institutional Animal Care and Use Committee.

Flow cytometry

Bone marrow cells, splenocytes, and peritoneal wash cells were depleted of red blood cells and stained with various combinations of the following antibodies. Bone marrow: anti-CD43 FITC (BD Biosciences), anti-IgM PE (BD Biosciences), anti-B220 PerCP-Cy5.5 (Tonbo Biosciences), and anti-CD93 APC (Invitrogen). Spleen: anti-CD21 FITC (BD Biosciences), anti-CD23 PE (BD Biosciences), anti-CD95 PE (BD Biosciences), anti-IgM PerCP-Cy5.5 (BD Biosciences), B220 PerCP-Cy5.5, anti-B220 APC (Tonbo Biosciences), or anti-GL7 APC (BD Biosciences). Peritoneal wash: anti-CD11b FITC (BD Biosciences), anti-CD5 PE (Tonbo Biosciences), anti-IgM PerCP-Cy5.5, anti-B220 APC. Cultured B cells were stained with anti-CD138 PE (BD Biosciences), anti-B220 PerCP-Cy5.5, and anti-IgG1 biotin (BD Biosciences) plus streptavidin APC (Tonbo Biosciences). Samples were run on a FACS Calibur (BD) and analyzed with Flowjo (Treestar).

B cell purification

Splenocytes were depleted of red blood cells and B cells purified by negative selection using anti-CD43 beads and LD columns (Miltenyi Biotech) according to the manufacturer’s instructions.

Real time PCR

Purified splenic B cells were harvested immediately or stimulated with media alone (RPMI 1640 + 10 % FBS + L-glut + pen/strep + β-ME) or 10 ug/ml anti-IgM F(ab)’2 fragments (Jackson Immunoresearch) for 1, 6, or 17 hours. In some experiments cells were pretreated for 15 minutes with vehicle (DMSO) or 10 uM Ly294002 (Sigma-Aldrich) prior to stimulation with anti-IgM. Total RNA prepared using an RNeasy Mini kit (Qiagen), and cDNA subsequently generated with a High Capacity cDNA Reverse Transcription kit (Thermofisher). PIK3IP1 levels were measured by real time quantitative PCR using Taqman reagents (Thermofisher) for PIK3IP1 and the internal control GAPDH and a Biorad CFX96 Real-Time System. Results were normalized to GAPDH using the delta-Ct method.

Western blots

Purified splenic B cells were stimulated at 37°C for varying times from 1 minute to 1 hour with media alone (RPMI 1640 + 10 % FBS + L-glut + pen/strep + β-ME), 10 ug/ml anti-IgM F(ab)'2 fragments (Jackson Immunoresearch), 10 ug/ml anti-CD40 (IC10, Invitrogen), 10 ug/ml anti-IgM F(ab)'2 fragments plus 10 ug/ml anti-CD40, or 0.1, 0.3, or 1 ug/ml CD40L (R&D Systems). Cells were lysed in 2x Laemmli sample buffer (Biorad) and equal cell equivalents subjected to SDS page using a 4–15% gradient gel (Biorad). Gels were transferred to nitrocellulose (GE Healthcare) and blocked in 5% milk. Blots were probed overnight at 4°C with rabbit monoclonal antibodies against pAkt S473 (Cell Signaling Technology), pS6 (Cell Signaling Technology), and β-actin (Cell Signaling Technology) and subsequently for 2 hrs at room temperature with goat anti-rabbit HRP (Biorad). Blots were washed three times in TBST after each antibody incubation. Bands were detected with Clarity ECL reagent (Biorad) and imaged and quantified with a Chemidoc Imaging system (Biorad) and Image Lab software (Biorad).

In vitro class switching

Purified splenic B cells were incubated for 72 hours at 37°C in media alone (RPMI 1640 + 10 % FBS + L-glut + pen/strep + β-ME), 5 ug/ml LPS (Sigma) + 50 ng/ml IL-4 (R&D Systems), or 10 ug/ml anti-CD40 (IC10, Invitrogen) + 50 ng/ml IL-4 (R&D Systems). Cells were then stained with antibodies against B220, CD138, and IgG1 and analyzed by flow cytometry (see above).

Immunizations

To study T-independent responses, mice were prebled, immunized with 100ug/mL NP-Ficoll (Biosearch Technologies) in PBS, and bled 7 and 14 days later. For T-dependent responses, mice were prebled, immunized with 500 ug/mL NP-KLH (Biosearch Technologies) in alum (ThermoFisher Scientific), and bled 7, 14, and 28 days later. After the 28 day bleed, mice were boosted with 1mg/mL NP-KLH (Biosearch Technologies) in PBS and bled one week later. For germinal center studies, mice were immunized with 2.5×10^8 sheep red blood cells (SRBCs) (Innovative Research) or PBS and germinal centers measured by flow cytometry 5 days later (see above).

ELISAs

Total Ig: Serum from unimmunized mice was analyzed for total IgM, IgG1, IgG2c, IgG3, and IgA levels using the SBA Clonotyping System (Southern Biotech) according to the manufacturer's instructions. *anti-NP Ig*: 1:100, 1:400, or 1:1600 dilutions of serum in phosphate buffered saline (PBS) + 0.1% BSA + 0.05% Tween-20 were incubated on Corning® Costar® 96-well flat bottomed plates (Thomas Scientific Holdings LLC) that were previously coated with 25 ug/ml NP>25-BSA or NP8-BSA (Biosearch Technlogies). After washing with PBS + 0.1%BSA + 0.05% Tween-20, wells were incubated with 2 ug/ml alkaline phosphatase labeled anti-IgM, IgG3, or IgG (Southern Biotech). Plates were again washed with PBS + 0.1% BSA + 0.05% Tween-20 and incubated with alkaline phosphatase substrate (Biorad). In all cases, OD405 was measured using an ELx808 ELISA reader (BioTek Instruments, Inc).

Results

Results from the ImmGen Consortium (www.immgen.org) indicate that PIK3IP1 is expressed in the B lineage in an interesting pattern (Figure 1a). In the bone marrow, PIK3IP1 is expressed in a manner inversely correlated with PI3K activity: low in large pre-B cells in which PI3K signaling promotes proliferation and suppresses Rag expression (47, 48), and higher in small pre-B cells in which low PI3K activity allows for cell cycle arrest and light chain rearrangement (47, 48). In the periphery, PIK3IP1 is expressed in most B cell subsets but is particularly low in germinal center B cells (dark blue bar, Fig 1a).

We sought to determine whether PIK3IP1 expression is controlled by PI3K activity in peripheral B cells. We purified wild type splenic B cells, stimulated them with anti-IgM for various times, and measured PIK3IP1 expression by real time PCR. BCR crosslinking for one hour had minimal effect, but PIK3IP1 was downregulated dramatically after 17 hours (Figure 1b). Six hours of treatment resulted in an intermediate response, with PIK3IP1 still present but at reduced levels (Figure 1b). PIK3IP1 downregulation was prevented by LY294002, a PI3K inhibitor (Figure 1b). The transcription factor Foxo3 has been reported to promote PIK3IP1 expression in other cell types (26) and is downregulated by PI3K signaling upon BCR engagement (49). PIK3IP1 expression was normal in freshly isolated Foxo3−/− B cells. However, upon culture in media alone there was a small but significant increase in PIK3IP1 levels in wild type cells that did not occur in the absence of Foxo3 (Figure 1c). This suggests that a low level of PI3K activity present in *ex vivo* cells is lost during culture without activating stimuli, allowing for an increase in Foxo3-mediated transcription of PIK3IP1. Consistent with this, treatment of wild type cells with LY294002 in the absence of BCR stimulation, which should increase Foxo3 activity, resulted in a 30–40% increase in PIK3IP1 levels compared to DMSO treatment (Figure 1d). BCR stimulation downregulated PIK3IP1 normally in Foxo3−/− B cells, consistent with the known loss of Foxo3 activity and expression in response to this stimulus (49) (Figure 1c). Thus, PIK3IP1 expression is regulated by PI3K signaling in B cells.

To determine the role of PIK3IP1 in B cell development and function, we generated mice with B cell-specific PIK3IP1 deficiency by crossing PIK3IP1f/f mice (32) with CD19-cre mice. CD19-cre deletes efficiently and specifically in B cells, with no cre expression or activity observed in T cells, myeloid cells, or splenic IgM- or B220- cells (45, 50–53). Despite efficient loss of PIK3IP1 expression in CD19-cre.PIK3IP1f/f B cells (Figure 1e), B cell development occurred normally in these mice. B cell subpopulations were present at normal numbers in the bone marrow, spleen, and peritoneal cavity (Table I, Supplemental Figure 1a–c). Steady state levels of all antibody isotypes were also unaffected by the absence of PIK3IP1 (Figure 2a). Thus, PIK3IP1 is dispensable for steady state B cell development and B cell homeostasis.

PI3K signaling is known to limit class switching during humoral immune responses. To determine whether this effect is enhanced in the absence of PIK3IP1, we first immunized CD19-cre and CD19-cre.PIK3IP1f/f mice with the T-independent antigen NP-Ficoll. Both IgM and IgG3 responses were normal (Figure 2b), indicating that PIK3IP1 is not required for T-independent responses. We next tested responses to the T-dependent antigen NP-KLH.

Primary IgM responses were unaffected (Figure 3a). However, there was a significant delay in the production of anti-NP IgG (Figure 3a), with levels in CD19-Cre.PIK3IP1f/f mice not reaching those of CD19-Cre controls until day 14 post-immunization. To measure secondary responses, we boosted mice at day 28 after initial immunization and collected serum seven days later. Mice with PIK3IP1-deficient B cells failed to demonstrate a significant increase in anti-NP antibodies in response to this boost (Figure 3b). Thus, PIK3IP1 acts in B cells to promote early class switching and memory during T-dependent humoral responses.

IgG can be produced by antibody secreting cells generated during either the early extrafollicular response or the later germinal center response. The reduction in early, but not late, IgG in CD19-cre.PIK3IP1f/f mice and the low level of PIK3IP1 expression in germinal center B cells suggests that PIK3IP1 is acting to promote class switching at the extrafollicular stage rather than in germinal centers. Consistent with this model, early germinal center formation was unimpaired, as immunization with SRBCs induced an equivalent frequency of germinal center B cells in CD19-Cre (5.4% +/− 2.7, n = 4) and CD19-Cre.PIK3IP1f/fl (5.4% +/− 1.6, n = 4) mice at day 5 (Figure 4a). Germinal centers were also functional; affinity maturation was normal in CD19-cre.PIK3IP1f/f mice, as high affinity IgG was normal at day 14 (Figure 4b). There was also not a general defect in the ability of PIK3IP1-deficient B cells to undergo class switching as measured by IgG1 upregulation *in vitro* (Figure 4c, Supplemental Figure 1d).

During a T-dependent immune response B cells receive signals via both the BCR (from antigen) and CD40 (from T cells). As such, standard *in vitro* class switching assays do not necessarily reflect the physiological scenario. We thus tested the ability of PIK3IP1 to modulate responses to the BCR and CD40. Two important signaling events that depend on PI3K signaling downstream of these receptors are the phosphorylation of Akt (9, 10, 13, 54, 55) and the phosphorylation of S6 (56–58). B cells expressing a constitutively active form of the p110δ subunit of PI3K demonstrate increased phosphorylated S6 (pS6) in both mice and humans (59–61). Furthermore, in T cells anti-CD3/anti-CD28 induced pS6 depends on PI3K (32) and is inhibited by PIK3IP1 (31, 32). Surprisingly, loss of PIK3IP1 did not result in increased Akt phosphorylation in response to BCR crosslinking or anti-CD40 alone (Figure 5a,b). However, PIK3IP1 deficient B cells demonstrated increased pS6 relative to control cells when stimulated with both anti-IgM and anti-CD40 (Figure 5c). *In vivo*, B cells encounter CD40L in a trimeric form which crosslinks CD40 more efficiently than anti-CD40. We found that treatment with CD40L also resulted in increased pS6 in the absence of PIK3IP1 relative to control cells (Figure 5d). Thus, PIK3IP1 limits PI3K pathway activity in B cells under conditions reflective of those encountered by B cells during B/T cognate interactions. This explains the reduced production of IgG early in T-dependent responses *in vivo*, since excessive PI3K signaling limits class switching (17).

Discussion

Here we have defined a role for PIK3IP1 in B cell functional responses. Our data support a model in which PIK3IP1 normally promotes class switching during the early extrafollicular phase of T-dependent responses by limiting PI3K signaling in response to antigen plus CD40 engagement. In contrast, germinal center formation, affinity maturation, and the late

phase of class switching are unaffected in the absence of B cell expressed PIK3IP1, likely because of the low level of PIK3IP1 expression in germinal center B cells. Interestingly, PTEN, another negative regulator of PI3K signaling, is elevated in germinal center B cells (62). This suggests that different regulators of PI3K contribute to different phases of the humoral immune response.

Future studies of signaling in vivo during a T-dependent immune response would provide further support for this model. This could be achieved using an NP-specific BCR transgene (63) to increase the frequency of responding cells in both control and B cell specific PIK3IP1 knockout mice. Activation of PI3K pathway components could then be measured flow cytometry or immunofluorescence in antigen specific extrafollicular and germinal center B cells. Manipulations that eliminate germinal center responses but preserve T-dependent extrafollicular responses, such as deletion of Bcl6 in B cells (6), would also be useful to further define the role of PIK3IP1 in signaling and functional responses *in vivo*.

It has recently been shown that B cell activation by B cell receptor crosslinking results in activation induced cell death if a second signal such as CD40 engagement is not received within approximately nine hours of initial antigen encounter (64). Thus, there appears to be a limited time window during which B cells can receive cognate help from T cells for an effective immune response (64). Our results suggest that there may be a similar window for efficient CD40-induced class switching during the extrafollicular response. PIK3IP1 promotes class switching but is downregulated at late time points after BCR engagement. Thus, if B cells do not engage T cell help early enough after seeing antigen, their ability to undergo class switching would be decreased due to reduced PI3KIP1 expression.

Cognate T cell help might also promote PIK3IP1 function. PIK3IP1 is a cell surface molecule, and its extracellular domain is required for its function in T cells (31–33). A ligand for PIK3IP1 has been postulated to be expressed on T cells, and the PIK3IP1 extracellular domain can oligomerize (31–33). Thus, it is possible that the ability of PIK3IP1 to promote T-dependent class switching is enhanced by engagement of a ligand (PIK3IP1 itself or another molecule) on T cells during initial cognate interactions. The DCIR2+ subpopulation of dendritic cells are another potential source of a ligand for PIK3IP1. When antigen is targeted to these dendritic cells, they interact with B cells to drive a T-dependent, extrafollicular, class switched response in the absence of germinal center formation (65). This is similar to the response that is promoted by PIK3IP1 expression in B cells. Further studies of the interaction between PIK3IP1 in B cells and its potential ligands on other cell types will shed light on how the context in which B cells are activated shapes their subsequent fate.

Extrafollicular, T-dependent antibody and memory responses are important in clinically relevant situations. Extrafollicular responses contribute to the production of autoantibodies in the MRL.lpr mouse model of lupus (66–70) and in systemic lupus erythematosus patients (70–73). The bacteria Salmonella typhimirium elicits a predominantly T-dependent, extrafollicular antibody response, with germinal centers forming only very late after infection (74–77). Germinal center-independent memory B cells that arise early in T-dependent responses are also of great importance. These lower affinity memory cells likely

allow for a secondary response to a broad range of pathogen variants, while germinal center derived high affinity memory cells are more tailored towards responding to reinfection with the original challenge (4, 6, 7, 78). Early IgM memory cells are known to be important in several mouse models of infectious disease, including infection with *E. muris*, a tick-borne intracellular bacteria (79, 80), and malaria (81, 82). Mice with specific defects in extrafollicular responses, such as those lacking PIK3IP1 in B cells described here, may serve as a useful model to study these diseases.

Here we add PIK3IP1 to the list of negative regulators of PI3K signaling that have unique functions in B cells, further emphasizing the need to keep PI3K activity “just right” a la Goldilocks and the Three Bears (21, 22). While PIK3IP1 interacts with the PI3K p85/p110 heterodimer to limit is ability to signal (23), PTEN and SHIP-1 dephosphorylate the product of PI3K, PIP3, and thus act further downstream (13). Thus, there may be situations in which excess PIP3 produced in the absence of PIK3IP1 is dephosphorylated by PTEN and/or SHIP-1. As such, additional roles for PIK3IP1 in B cells beyond those illustrated here may be revealed in the absence of PTEN or SHIP-1. These could have particular relevance in autoimmune disease or B cell malignancy, which PTEN and SHIP-1 are known to limit (83, 84).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH Grant AI126051. A.B.S. is a Southwestern Medical Foundation Scholar in Biomedical Research and holds the Peggy Chavellier Professorship in Arthritis Research and Treatment.

Abbreviations

KLH	keyhole limpet hemocyanin
NP	4-Hydroxy-3-nitrophenylacetyl
PIK3IP1	Phosphoinositide-3-Kinase Interacting Protein 1
PIP3	Phosphatidylinositol (3,4,5)-trisphosphate
PTEN	Phosphatase and tensin homolog

References

1. Shapiro-Shelef M, and Calame K. 2005 Regulation of plasma-cell development. Nat Rev Immunol 5: 230–242. [PubMed: 15738953]
2. Tarlinton D, and Good-Jacobson K. 2013 Diversity among memory B cells: origin, consequences, and utility. Science 341: 1205–1211. [PubMed: 24031013]
3. Weisel F, and Shlomchik M. 2017 Memory B Cells of Mice and Humans. Annu Rev Immunol 35: 255–284. [PubMed: 28142324]
4. Weisel FJ, Zuccarino-Catania GV, Chikina M, and Shlomchik MJ. 2016 A Temporal Switch in the Germinal Center Determines Differential Output of Memory B and Plasma Cells. Immunity 44: 116–130. [PubMed: 26795247]

5. Inamine A, Takahashi Y, Baba N, Miyake K, Tokuhisa T, Takemori T, and Abe R. 2005 Two waves of memory B-cell generation in the primary immune response. Int Immunol 17: 581–589. [PubMed: 15824068]

6. Kaji T, Ishige A, Hikida M, Taka J, Hijikata A, Kubo M, Nagashima T, Takahashi Y, Kurosaki T, Okada M, Ohara O, Rajewsky K, and Takemori T. 2012 Distinct cellular pathways select germline-encoded and somatically mutated antibodies into immunological memory. J Exp Med 209: 2079–2097. [PubMed: 23027924]

7. Taylor JJ, Pape KA, and Jenkins MK. 2012 A germinal center-independent pathway generates unswitched memory B cells early in the primary response. J Exp Med 209: 597–606. [PubMed: 22370719]

8. Noelle RJ, Roy M, Shepherd DM, Stamenkovic I, Ledbetter JA, and Aruffo A. 1992 A 39-kDa protein on activated helper T cells binds CD40 and transduces the signal for cognate activation of B cells. Proc Natl Acad Sci U S A 89: 6550–6554. [PubMed: 1378631]

9. Andjelic S, Hsia C, Suzuki H, Kadowaki T, Koyasu S, and Liou HC. 2000 Phosphatidylinositol 3-kinase and NF-kappa B/Rel are at the divergence of CD40-mediated proliferation and survival pathways. J Immunol 165: 3860–3867. [PubMed: 11034392]

10. Clayton E, Bardi G, Bell SE, Chantry D, Downes CP, Gray A, Humphries LA, Rawlings D, Reynolds H, Vigorito E, and Turner M. 2002 A crucial role for the p110delta subunit of phosphatidylinositol 3-kinase in B cell development and activation. J Exp Med 196: 753–763. [PubMed: 12235209]

11. Fruman DA, Snapper SB, Yballe CM, Davidson L, Yu JY, Alt FW, and Cantley LC. 1999 Impaired B cell development and proliferation in absence of phosphoinositide 3-kinase p85alpha. Science 283: 393–397. [PubMed: 9888855]

12. Glassford J, Soeiro I, Skarell SM, Banerji L, Holman M, Klaus GG, Kadowaki T, Koyasu S, and Lam EW. 2003 BCR targets cyclin D2 via Btk and the p85alpha subunit of PI3-K to induce cell cycle progression in primary mouse B cells. Oncogene 22: 2248–2259. [PubMed: 12700661]

13. Marshall AJ, Niiro H, Yun TJ, and Clark EA. 2000 Regulation of B-cell activation and differentiation by the phosphatidylinositol 3-kinase and phospholipase Cgamma pathway. Immunol Rev 176: 30–46. [PubMed: 11043766]

14. Durand CA, Hartvigsen K, Fogelstrand L, Kim S, Iritani S, Vanhaesebroeck B, Witztum JL, Puri KD, and Gold MR. 2009 Phosphoinositide 3-kinase p110 delta regulates natural antibody production, marginal zone and B-1 B cell function, and autoantibody responses. Journal of immunology 183: 5673–5684.

15. Jou ST, Carpino N, Takahashi Y, Piekorz R, Chao JR, Carpino N, Wang D, and Ihle JN. 2002 Essential, nonredundant role for the phosphoinositide 3-kinase p110delta in signaling by the B-cell receptor complex. Molecular and cellular biology 22: 8580–8591. [PubMed: 12446777]

16. Okkenhaug K, Bilancio A, Farjot G, Priddle H, Sancho S, Peskett E, Pearce W, Meek SE, Salpekar A, Waterfield MD, Smith AJ, and Vanhaesebroeck B. 2002 Impaired B and T cell antigen receptor signaling in p110delta PI 3-kinase mutant mice. Science 297: 1031–1034. [PubMed: 12130661]

17. Omori SA, Cato MH, Anzelon-Mills A, Puri KD, Shapiro-Shelef M, Calame K, and Rickert RC. 2006 Regulation of class-switch recombination and plasma cell differentiation by phosphatidylinositol 3-kinase signaling. Immunity 25: 545–557. [PubMed: 17000121]

18. Suzuki A, Kaisho T, Ohishi M, Tsukio-Yamaguchi M, Tsubata T, Koni PA, Sasaki T, Mak TW, and Nakano T. 2003 Critical roles of Pten in B cell homeostasis and immunoglobulin class switch recombination. J Exp Med 197: 657–667. [PubMed: 12615906]

19. Anzelon AN, Wu H, and Rickert RC. 2003 Pten inactivation alters peripheral B lymphocyte fate and reconstitutes CD19 function. Nat Immunol 4: 287–294. [PubMed: 12563260]

20. O'Neill SK, Getahun A, Gauld SB, Merrell KT, Tamir I, Smith MJ, Dal Porto JM, Li QZ, and Cambier JC. 2011 Monophosphorylation of CD79a and CD79b ITAM motifs initiates a SHIP-1 phosphatase-mediated inhibitory signaling cascade required for B cell anergy. Immunity 35: 746–756. [PubMed: 22078222]

21. Walsh CM, and Fruman DA. 2014 Too much of a good thing: immunodeficiency due to hyperactive PI3K signaling. The Journal of clinical investigation 124: 3688–3690. [PubMed: 25133419]

22. Tangye SG, Bier J, Lau A, Nguyen T, Uzel G, and Deenick EK. 2019 Immune Dysregulation and Disease Pathogenesis due to Activating Mutations in PIK3CD-the Goldilocks' Effect. J Clin Immunol 39: 148–158. [PubMed: 30911953]

23. Zhu Z, He X, Johnson C, Stoops J, Eaker AE, Stoffer DS, Bell A, Zarnegar R, and DeFrances MC. 2007 PI3K is negatively regulated by PIK3IP1, a novel p110 interacting protein. Biochem Biophys Res Commun 358: 66–72. [PubMed: 17475214]

24. He X, Zhu Z, Johnson C, Stoops J, Eaker AE, Bowen W, and DeFrances MC. 2008 PIK3IP1, a negative regulator of PI3K, suppresses the development of hepatocellular carcinoma. Cancer Res 68: 5591–5598. [PubMed: 18632611]

25. Sun HX, Yang ZF, Tang WG, Ke AW, Liu WR, Li Y, Gao C, Hu B, Fu PY, Yu MC, Gao BW, Shi YH, Fan J, and Xu Y. 2020 MicroRNA-19a-3p regulates cell growth through modulation of the PIK3IP1-AKT pathway in hepatocellular carcinoma. J Cancer 11: 2476–2484. [PubMed: 32201518]

26. Schmidt-Strassburger U, Schips TG, Maier HJ, Kloiber K, Mannella F, Braunstein KE, Holzmann K, Ushmorov A, Liebau S, Boeckers TM, and Wirth T. 2012 Expression of constitutively active FoxO3 in murine forebrain leads to a loss of neural progenitors. FASEB J 26: 4990–5001. [PubMed: 22935140]

27. Song HK, Kim J, Lee JS, Nho KJ, Jeong HC, Kim J, Ahn Y, Park WJ, and Kim do H. 2015 Pik3ip1 modulates cardiac hypertrophy by inhibiting PI3K pathway. PloS one 10: e0122251.

28. Joshi S, Wei J, and Bishopric NH. 2016 A cardiac myocyte-restricted Lin28/let-7 regulatory axis promotes hypoxia-mediated apoptosis by inducing the AKT signaling suppressor PIK3IP1. Biochim Biophys Acta 1862: 240–251. [PubMed: 26655604]

29. Li S, Fu Y, Pang Y, Tong H, Li S, and Yan Y. 2019 GRP94 promotes muscle differentiation by inhibiting the PI3K/AKT/mTOR signaling pathway. J Cell Physiol 234: 21211–21223. [PubMed: 31025379]

30. DeFrances MC, Debelius DR, Cheng J, and Kane LP. 2012 Inhibition of T-cell activation by PIK3IP1. Eur J Immunol 42: 2754–2759. [PubMed: 22706993]

31. Johnson MO, Wolf MM, Madden MZ, Andrejeva G, Sugiura A, Contreras DC, Maseda D, Liberti MV, Paz K, Kishton RJ, Johnson ME, de Cubas AA, Wu P, Li G, Zhang Y, Newcomb DC, Wells AD, Restifo NP, Rathmell WK, Locasale JW, Davila ML, Blazar BR, and Rathmell JC. 2018 Distinct Regulation of Th17 and Th1 Cell Differentiation by Glutaminase-Dependent Metabolism. Cell 175: 1780–1795 e1719. [PubMed: 30392958]

32. Uche UU, Piccirillo AR, Kataoka S, Grebinoski SJ, D'Cruz LM, and Kane LP. 2018 PIK3IP1/TrIP restricts activation of T cells through inhibition of PI3K/Akt. J Exp Med 215: 3165–3179. [PubMed: 30429249]

33. Chen Y, Wang J, Wang X, Li X, Song J, Fang J, Liu X, Liu T, Wang D, Li Q, Wen S, Ma D, Xia J, Luo L, Zheng SG, Cui J, Zeng G, Chen L, Cheng B, and Wang Z. 2019 Pik3ip1 Is a Negative Immune Regulator that Inhibits Antitumor T-Cell Immunity. Clin Cancer Res 25: 6180–6194. [PubMed: 31350312]

34. Zheng Y, Yin L, Chen H, Yang S, Pan C, Lu S, Miao M, and Jiao B. 2012 miR-376a suppresses proliferation and induces apoptosis in hepatocellular carcinoma. FEBS Lett 586: 2396–2403. [PubMed: 22684007]

35. Bitler BG, Aird KM, Garipov A, Li H, Amatangelo M, Kossenkov AV, Schultz DC, Liu Q, Shih Ie M, Conejo-Garcia JR, Speicher DW, and Zhang R. 2015 Synthetic lethality by targeting EZH2 methyltransferase activity in ARID1A-mutated cancers. Nat Med 21: 231–238. [PubMed: 25686104]

36. Chiron D, Di Liberto M, Martin P, Huang X, Sharman J, Blecua P, Mathew S, Vijay P, Eng K, Ali S, Johnson A, Chang B, Ely S, Elemento O, Mason CE, Leonard JP, and Chen-Kiang S. 2014 Cell-cycle reprogramming for PI3K inhibition overrides a relapse-specific C481S BTK mutation revealed by longitudinal functional genomics in mantle cell lymphoma. Cancer Discov 4: 1022–1035. [PubMed: 25082755]

37. Chiron D, Martin P, Di Liberto M, Huang X, Ely S, Lannutti BJ, Leonard JP, Mason CE, and Chen-Kiang S. 2013 Induction of prolonged early G1 arrest by CDK4/CDK6 inhibition reprograms lymphoma cells for durable PI3Kdelta inhibition through PIK3IP1. Cell Cycle 12: 1892–1900. [PubMed: 23676220]

38. Wong CC, Martincorena I, Rust AG, Rashid M, Alifrangis C, Alexandrov LB, Tiffen JC, Kober C, Chronic C Myeloid Disorders Working Group of the International Cancer Genome, Green AR, Massie CE, Nangalia J, Lempidaki S, Dohner H, Dohner K, Bray SJ, McDermott U, Papaemmanuil E, Campbell PJ, and Adams DJ. 2014 Inactivating CUX1 mutations promote tumorigenesis. Nat Genet 46: 33–38. [PubMed: 24316979]

39. Kitagawa M, Liao PJ, Lee KH, Wong J, Shang SC, Minami N, Sampetrean O, Saya H, Lingyun D, Prabhu N, Diam GK, Sobota R, Larsson A, Nordlund P, McCormick F, Ghosh S, Epstein DM, Dymock BW, and Lee SH. 2017 Dual blockade of the lipid kinase PIP4Ks and mitotic pathways leads to cancer-selective lethality. Nat Commun 8: 2200. [PubMed: 29259156]

40. Zhao J, and Cheng L. 2017 Long non-coding RNA CCAT1/miR-148a axis promotes osteosarcoma proliferation and migration through regulating PIK3IP1. Acta Biochim Biophys Sin (Shanghai) 49: 503–512. [PubMed: 28549102]

41. An N, Khan S, Imgruet MK, Gurbuxani SK, Konecki SN, Burgess MR, and McNerney ME. 2018 Gene dosage effect of CUX1 in a murine model disrupts HSC homeostasis and controls the severity and mortality of MDS. Blood 131: 2682–2697. [PubMed: 29592892]

42. Fukumoto T, Park PH, Wu S, Fatkhutdinov N, Karakashev S, Nacarelli T, Kossenkov AV, Speicher DW, Jean S, Zhang L, Wang TL, Shih IM, Conejo-Garcia JR, Bitler BG, and Zhang R. 2018 Repurposing Pan-HDAC Inhibitors for ARID1A-Mutated Ovarian Cancer. Cell Rep 22: 3393–3400. [PubMed: 29590609]

43. Ho CM, Lee FK, Huang SH, and Cheng WF. 2018 Everolimus following 5-aza-2-deoxycytidine is a promising therapy in paclitaxel-resistant clear cell carcinoma of the ovary. Am J Cancer Res 8: 56–69. [PubMed: 29416920]

44. Lee K, Kitagawa M, Liao PJ, Virshup DM, and Lee SH. 2020 A Ras-LSD1 axis activates PI3K signaling through PIK3IP1 suppression. Oncogenesis 9: 2. [PubMed: 31900384]

45. Rickert RC, Roes J, and Rajewsky K. 1997 B lymphocyte-specific, Cre-mediated mutagenesis in mice. Nucleic Acids Res 25: 1317–1318. [PubMed: 9092650]

46. Castrillon DH, Miao L, Kollipara R, Horner JW, and DePinho RA. 2003 Suppression of ovarian follicle activation in mice by the transcription factor Foxo3a. Science 301: 215–218. [PubMed: 12855809]

47. Herzog S, Reth M, and Jumaa H. 2009 Regulation of B-cell proliferation and differentiation by pre-B-cell receptor signalling. Nat Rev Immunol 9: 195–205. [PubMed: 19240758]

48. Clark MR, Mandal M, Ochiai K, and Singh H. 2014 Orchestrating B cell lymphopoiesis through interplay of IL-7 receptor and pre-B cell receptor signalling. Nat Rev Immunol 14: 69–80. [PubMed: 24378843]

49. Hinman RM, Nichols WA, Diaz TM, Gallardo TD, Castrillon DH, and Satterthwaite AB. 2009 Foxo3−/− mice demonstrate reduced numbers of pre-B and recirculating B cells but normal splenic B cell sub-population distribution. Int Immunol 21: 831–842. [PubMed: 19502585]

50. Schwenk F, Sauer B, Kukoc N, Hoess R, Muller W, Kocks C, Kuhn R, and Rajewsky K. 1997 Generation of Cre recombinase-specific monoclonal antibodies, able to characterize the pattern of Cre expression in cre-transgenic mouse strains. J Immunol Methods 207: 203–212. [PubMed: 9368647]

51. Ye M, Iwasaki H, Laiosa CV, Stadtfeld M, Xie H, Heck S, Clausen B, Akashi K, and Graf T. 2003 Hematopoietic stem cells expressing the myeloid lysozyme gene retain long-term, multilineage repopulation potential. Immunity 19: 689–699. [PubMed: 14614856]

52. Hobeika E, Thiemann S, Storch B, Jumaa H, Nielsen PJ, Pelanda R, and Reth M. 2006 Testing gene function early in the B cell lineage in mb1-cre mice. Proc Natl Acad Sci U S A 103: 13789–13794. [PubMed: 16940357]

53. Pao LI, Lam KP, Henderson JM, Kutok JL, Alimzhanov M, Nitschke L, Thomas ML, Neel BG, and Rajewsky K. 2007 B cell-specific deletion of protein-tyrosine phosphatase Shp1 promotes B-1a cell development and causes systemic autoimmunity. Immunity 27: 35–48. [PubMed: 17600736]

54. Gold MR, Scheid MP, Santos L, Dang-Lawson M, Roth RA, Matsuuchi L, Duronio V, and Krebs DL. 1999 The B cell antigen receptor activates the Akt (protein kinase B)/glycogen synthase

kinase-3 signaling pathway via phosphatidylinositol 3-kinase. J Immunol 163: 1894–1905. [PubMed: 10438924]

55. Aiba Y, Yamazaki T, Okada T, Gotoh K, Sanjo H, Ogata M, and Kurosaki T. 2006 BANK negatively regulates Akt activation and subsequent B cell responses. Immunity 24: 259–268. [PubMed: 16546095]
56. Donahue AC, and Fruman DA. 2003 Proliferation and survival of activated B cells requires sustained antigen receptor engagement and phosphoinositide 3-kinase activation. J Immunol 170: 5851–5860. [PubMed: 12794110]
57. So L, Yea SS, Oak JS, Lu M, Manmadhan A, Ke QH, Janes MR, Kessler LV, Kucharski JM, Li LS, Martin MB, Ren P, Jessen KA, Liu Y, Rommel C, and Fruman DA. 2013 Selective inhibition of phosphoinositide 3-kinase p110alpha preserves lymphocyte function. J Biol Chem 288: 5718–5731. [PubMed: 23275335]
58. Ando R, Shima H, Tamahara T, Sato Y, Watanabe-Matsui M, Kato H, Sax N, Motohashi H, Taguchi K, Yamamoto M, Nio M, Maeda T, Ochiai K, Muto A, and Igarashi K. 2016 The Transcription Factor Bach2 Is Phosphorylated at Multiple Sites in Murine B Cells but a Single Site Prevents Its Nuclear Localization. J Biol Chem 291: 1826–1840. [PubMed: 26620562]
59. Avery DT, Kane A, Nguyen T, Lau A, Nguyen A, Lenthall H, Payne K, Shi W, Brigden H, French E, Bier J, Hermes JR, Zahra D, Sewell WA, Butt D, Elliott M, Boztug K, Meyts I, Choo S, Hsu P, Wong M, Berglund LJ, Gray P, O'Sullivan M, Cole T, Holland SM, Ma CS, Burkhart C, Corcoran LM, Phan TG, Brink R, Uzel G, Deenick EK, and Tangye SG. 2018 Germline-activating mutations in PIK3CD compromise B cell development and function. J Exp Med 215: 2073–2095. [PubMed: 30018075]
60. Wray-Dutra MN, Al Qureshah F, Metzler G, Oukka M, James RG, and Rawlings DJ. 2018 Activated PIK3CD drives innate B cell expansion yet limits B cell-intrinsic immune responses. J Exp Med 215: 2485–2496. [PubMed: 30194267]
61. Farmer JR, Allard-Chamard H, Sun N, Ahmad M, Bertocchi A, Mahajan VS, Aicher T, Arnold J, Benson MD, Morningstar J, Barmettler S, Yuen G, Murphy SJH, Walter JE, Ghebremichael M, Shalek AK, Batista F, Gerszten R, and Pillai S. 2019 Induction of metabolic quiescence defines the transitional to follicular B cell switch. Sci Signal 12.
62. Luo W, Hawse W, Conter L, Trivedi N, Weisel F, Wikenheiser D, Cattley RT, and Shlomchik MJ. 2019 The AKT kinase signaling network is rewired by PTEN to control proximal BCR signaling in germinal center B cells. Nat Immunol 20: 736–746. [PubMed: 31011187]
63. Sonoda E, Pewzner-Jung Y, Schwers S, Taki S, Jung S, Eilat D, and Rajewsky K. 1997 B cell development under the condition of allelic inclusion. Immunity 6: 225–233. [PubMed: 9075923]
64. Akkaya M, Traba J, Roesler AS, Miozzo P, Akkaya B, Theall BP, Sohn H, Pena M, Smelkinson M, Kabat J, Dahlstrom E, Dorward DW, Skinner J, Sack MN, and Pierce SK. 2018 Second signals rescue B cells from activation-induced mitochondrial dysfunction and death. Nat Immunol 19: 871–884. [PubMed: 29988090]
65. Chappell CP, Draves KE, Giltiay NV, and Clark EA. 2012 Extrafollicular B cell activation by marginal zone dendritic cells drives T cell-dependent antibody responses. J Exp Med 209: 1825–1840. [PubMed: 22966002]
66. Herlands RA, William J, Hershberg U, and Shlomchik MJ. 2007 Anti-chromatin antibodies drive in vivo antigen-specific activation and somatic hypermutation of rheumatoid factor B cells at extrafollicular sites. Eur J Immunol 37: 3339–3351. [PubMed: 18034429]
67. Odegard JM, Marks BR, DiPlacido LD, Poholek AC, Kono DH, Dong C, Flavell RA, and Craft J. 2008 ICOS-dependent extrafollicular helper T cells elicit IgG production via IL-21 in systemic autoimmunity. J Exp Med 205: 2873–2886. [PubMed: 18981236]
68. Sweet RA, Christensen SR, Harris ML, Shupe J, Sutherland JL, and Shlomchik MJ. 2010 A new site-directed transgenic rheumatoid factor mouse model demonstrates extrafollicular class switch and plasmablast formation. Autoimmunity 43: 607–618. [PubMed: 20370572]
69. Ols ML, Cullen JL, Turqueti-Neves A, Giles J, and Shlomchik MJ. 2016 Dendritic Cells Regulate Extrafollicular Autoreactive B Cells via T Cells Expressing Fas and Fas Ligand. Immunity 45: 1052–1065. [PubMed: 27793595]

70. Suurmond J, Atisha-Fregoso Y, Barlev AN, Calderon SA, Mackay MC, Aranow C, and Diamond B. 2019 Patterns of ANA+ B cells for SLE patient stratification. JCI Insight 4.

71. Malkiel S, Barlev AN, Atisha-Fregoso Y, Suurmond J, and Diamond B. 2018 Plasma Cell Differentiation Pathways in Systemic Lupus Erythematosus. Front Immunol 9: 427. [PubMed: 29556239]

72. Jenks SA, Cashman KS, Woodruff MC, Lee FE, and Sanz I. 2019 Extrafollicular responses in humans and SLE. Immunol Rev 288: 136–148. [PubMed: 30874345]

73. Jenks SA, Cashman KS, Zumaquero E, Marigorta UM, Patel AV, Wang X, Tomar D, Woodruff MC, Simon Z, Bugrovsky R, Blalock EL, Scharer CD, Tipton CM, Wei C, Lim SS, Petri M, Niewold TB, Anolik JH, Gibson G, Lee FE, Boss JM, Lund FE, and Sanz I. 2018 Distinct Effector B Cells Induced by Unregulated Toll-like Receptor 7 Contribute to Pathogenic Responses in Systemic Lupus Erythematosus. Immunity 49: 725–739 e726. [PubMed: 30314758]

74. Cunningham AF, Gaspal F, Serre K, Mohr E, Henderson IR, Scott-Tucker A, Kenny SM, Khan M, Toellner KM, Lane PJ, and MacLennan IC. 2007 Salmonella induces a switched antibody response without germinal centers that impedes the extracellular spread of infection. J Immunol 178: 6200–6207. [PubMed: 17475847]

75. Linterman MA, Beaton L, Yu D, Ramiscal RR, Srivastava M, Hogan JJ, Verma NK, Smyth MJ, Rigby RJ, and Vinuesa CG. 2010 IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. J Exp Med 207: 353–363. [PubMed: 20142429]

76. Lee SK, Rigby RJ, Zotos D, Tsai LM, Kawamoto S, Marshall JL, Ramiscal RR, Chan TD, Gatto D, Brink R, Yu D, Fagarasan S, Tarlinton DM, Cunningham AF, and Vinuesa CG. 2011 B cell priming for extrafollicular antibody responses requires Bcl-6 expression by T cells. J Exp Med 208: 1377–1388. [PubMed: 21708925]

77. Di Niro R, Lee SJ, Vander Heiden JA, Elsner RA, Trivedi N, Bannock JM, Gupta NT, Kleinstein SH, Vigneault F, Gilbert TJ, Meffre E, McSorley SJ, and Shlomchik MJ. 2015 Salmonella Infection Drives Promiscuous B Cell Activation Followed by Extrafollicular Affinity Maturation. Immunity 43: 120–131. [PubMed: 26187411]

78. Takemori T, Kaji T, Takahashi Y, Shimoda M, and Rajewsky K. 2014 Generation of memory B cells inside and outside germinal centers. Eur J Immunol 44: 1258–1264. [PubMed: 24610726]

79. Yates JL, Racine R, McBride KM, and Winslow GM. 2013 T cell-dependent IgM memory B cells generated during bacterial infection are required for IgG responses to antigen challenge. J Immunol 191: 1240–1249. [PubMed: 23804710]

80. Papillion AM, Kenderes KJ, Yates JL, and Winslow GM. 2017 Early derivation of IgM memory cells and bone marrow plasmablasts. PLoS One 12: e0178853.

81. Krishnamurty AT, Thouvenel CD, Portugal S, Keitany GJ, Kim KS, Holder A, Crompton PD, Rawlings DJ, and Pepper M. 2016 Somatically Hypermutated Plasmodium-Specific IgM(+) Memory B Cells Are Rapid, Plastic, Early Responders upon Malaria Rechallenge. Immunity 45: 402–414. [PubMed: 27473412]

82. Borges da Silva H, Machado de Salles E, Lima-Mauro EF, Sardinha LR, Alvarez JM, and D'Imperio Lima MR. 2018 CD28 deficiency leads to accumulation of germinal-center independent IgM+ experienced B cells and to production of protective IgM during experimental malaria. PLoS One 13: e0202522.

83. Miletic AV, Anzelon-Mills AN, Mills DM, Omori SA, Pedersen IM, Shin DM, Ravetch JV, Bolland S, Morse HC 3rd, and Rickert RC. 2010 Coordinate suppression of B cell lymphoma by PTEN and SHIP phosphatases. J Exp Med 207: 2407–2420. [PubMed: 20956547]

84. Franks SE, and Cambier JC. 2018 Putting on the Brakes: Regulatory Kinases and Phosphatases Maintaining B Cell Anergy. Front Immunol 9: 665. [PubMed: 29681901]

Key points

PIK3IP1 is expressed in B cells and is downregulated late after activation.

Lack of PIK3IP1 impairs early class switching during T-dependent responses.

PIK3IP1 is not required for germinal center responses or T-independent responses.

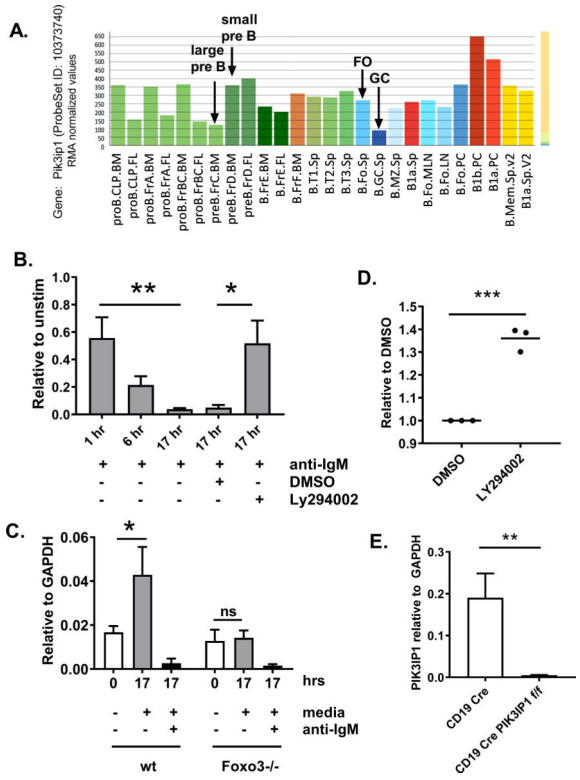

Figure 1: PIK3IP1 expression in B cells.

A) PIK3IP1 expression in B cells from www.immgen.org. B-E) PIK3IP1 levels were measured by real time PCR and normalized to GAPDH by the delta-Ct method. (B) To determine whether PIK3IP1 expression changes during the process of B cell activation, purified splenic B cells from wild type mice were stimulated with 10 ug/ml anti-IgM F(ab)'2 fragments for 1 hr, 6 hrs, or overnight (17 hrs). To define the role of PI3K in PIK3IP1 expression, cells were pretreated with vehicle (DMSO) or the PI3K inhibitor LY294002 prior to an overnight (17 hr) stimulation with anti-IgM. The level of PIK3IP1 in stimulated cells relative to unstimulated cells is shown for each condition. Data represent mean +/− SEM, n = 3–8. * $p < 0.05$, ** $p < 0.01$ by one way ANOVA. (C) Purified splenic B cells from wild type or Foxo3−/− mice were harvested immediately (open bars) or stimulated with media alone (gray bars) or anti-IgM F(ab)'$_2$ fragments (black bars) for 17 hrs. The level of PIK3IP1 relative to GAPDH is shown. Data represent mean +/− SD, n = 3. *$p < 0.05$, ns = not significant by unpaired Student's t-test. (D) Purified splenic B cells were treated with DMSO vehicle (DMSO) or LY294002 for 17 hours. The level of PIK3IP1 in LY294002 treated cells relative to DMSO treated cells is shown. n = 3. ***$p < 0.001$ by unpaired Student's t-test. E) PIK3IP1 levels were measured by real time PCR in purified B cells from CD19-cre (open bar) or CD19-cre.PIK3IP1f/f (black bar) mice. The level of PIK3IP1 relative to GAPDH is shown. Data represent mean +/− SEM, n = 5–6. **$p<0.01$ by unpaired Student's t-test.

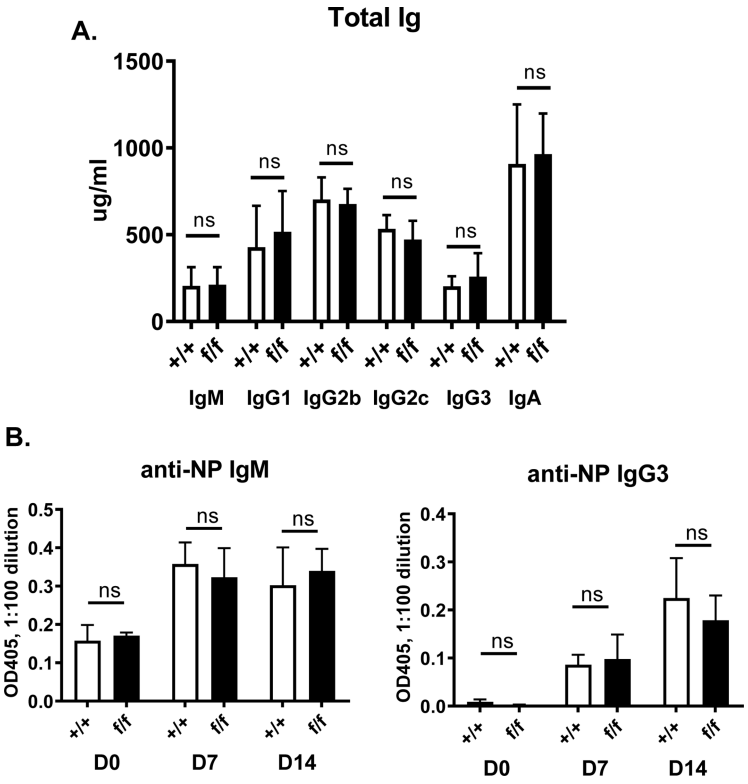

Figure 2: Normal basal Ig levels and T-independent responses in CD19-cre.PIK3IP1f/f mice.

A) Total Ig levels of the indicated isotypes are shown for CD19-cre (open bars) and CD19-cre.PIK3IP1f/f (black bars) mice. Data represent mean +/− SD, n = 6. There was no significant difference between CD19-cre and CD19-cre.PIK3IP1f/f mice. B) CD19-cre (open bars) and CD19-cre.PIK3IP1f/f (black bars) mice were immunized with NP-Ficoll and bled at day 0 (prior to immunization), 7, and 14. Anti-NP IgM and IgG3 was measured by ELISA using a 1:100 dilution of serum. Data represent mean +/− SEM, n = 3. There was no significant difference between CD19-cre and CD19-cre.PIK3IP1f/f mice.

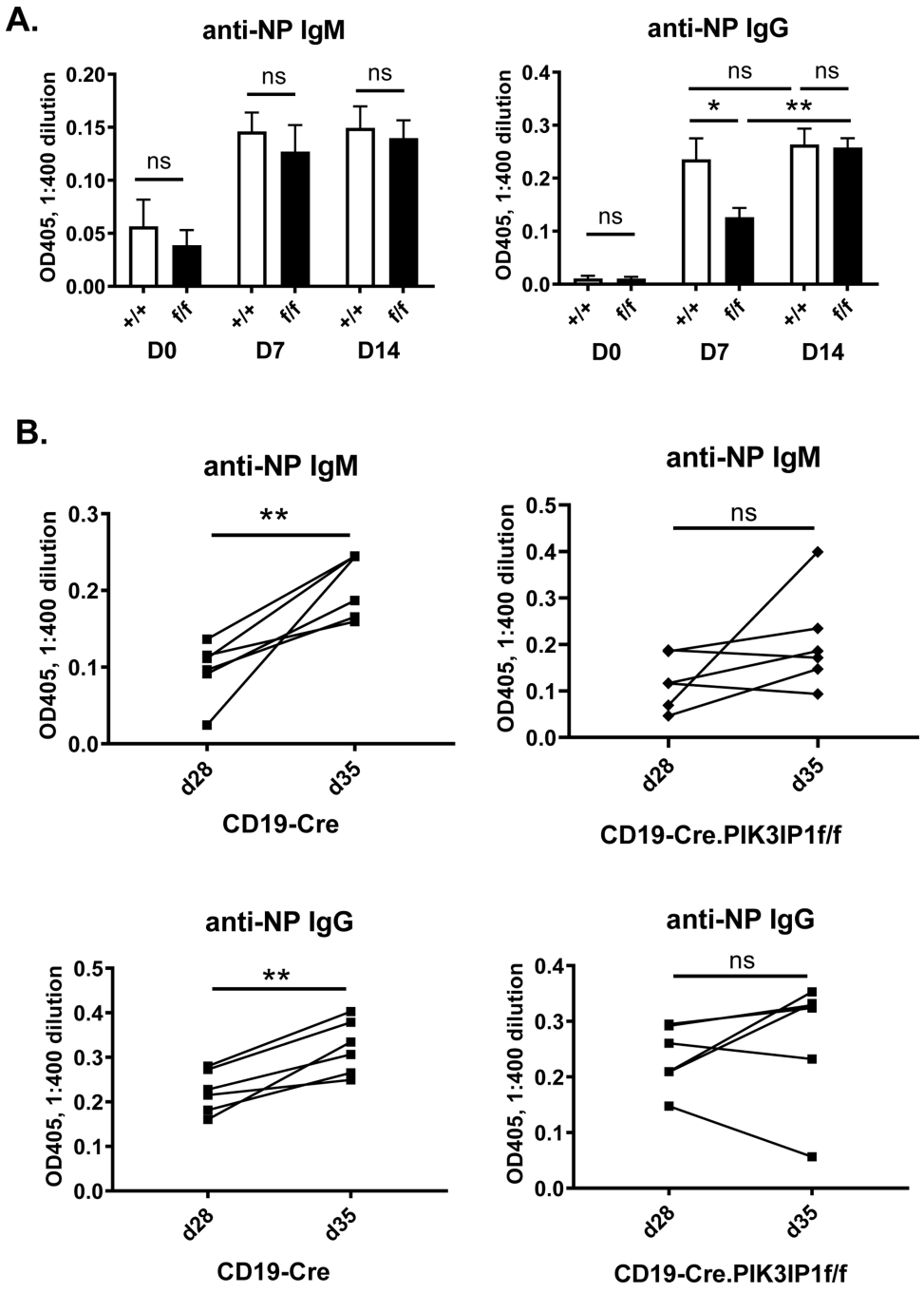

Figure 3: Delayed class switching and impaired memory responses to T-dependent antigens in CD19-cre.PIK3IP1f/f mice.

A) CD19-cre (open bars) and CD19-cre.PIK3IP1f/f (black bars) mice were immunized with NP-KLH in alum and bled at day 0 (prior to immunization), 7, and 14. Anti-NP IgM and IgG was measured by ELISA using a 1:400 dilution of serum. Data represent mean +/− SEM, n = 5–6. * $p < 0.05$, ** $p < 0.01$, ns = not significant by one way ANOVA. B) The mice in (A) were bled at day 28 post immunization, boosted with NP-KLH in PBS, and bled 7 days later. Anti-NP IgM and IgG was measured by ELISA using the indicated dilutions of

serum. Each line connects a pre and post boost sample from an individual mouse. ** $p < 0.01$, by paired Student's t-test. ns = not significant.

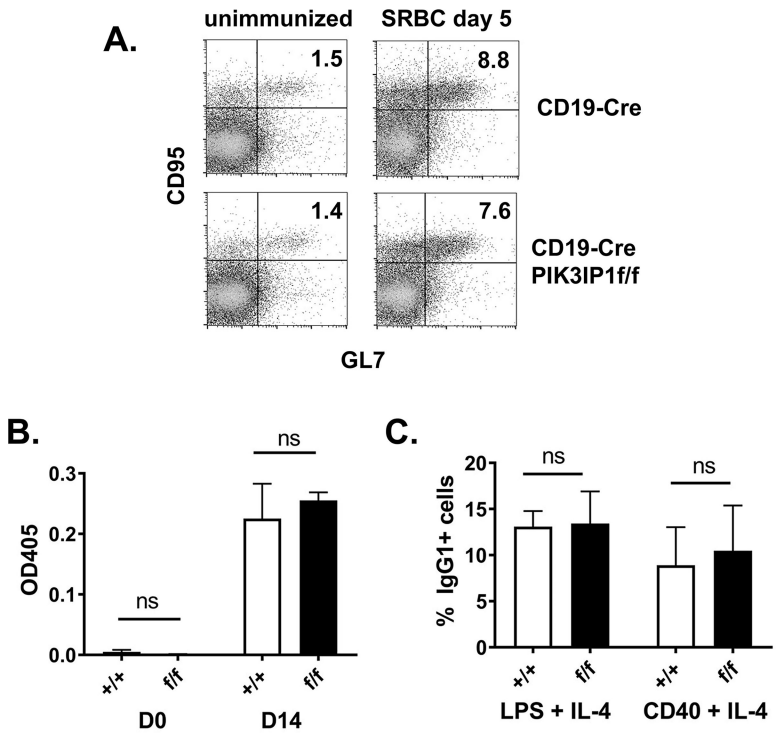

Figure 4: Normal germinal center responses and in vitro class switching in CD19-cre.PIK3IP1f/f mice.

A) CD19-cre and CD19-cre.PIK3IP1f/f mice were immunized with SRBCs or a PBS control and splenocytes analyzed by flow cytometry 5 days later. Germinal center B cells are defined as B220+CD95+GL7+. Representative flow cytometry plots are gated on B220+ cells. B) A 1:400 dilution of sera from CD19-cre (open bars) and CD19-cre.PIK3IP1f/f (black bars) mice immunized with NP-KLH in alum was subjected to ELISA analysis using plates coated with NP-BSA_8 to measure high affinity antibodies. Data represent mean +/− SEM, n = 6. There was no significant difference between CD19-cre and CD19-cre.PIK3IP1f/f mice. C) Purified splenic B cells were stimulated for 72 hours in LPS + IL-4 or anti-CD40 + IL-4. The frequency of IgG1+ cells was measured by flow cytometry. Data represent mean +/− SD, n = 6 for LPS and 3 for anti-CD40. There was no significant difference between CD19-cre (open bars) and CD19-cre.PIK3IP1f/f (black bars) mice.

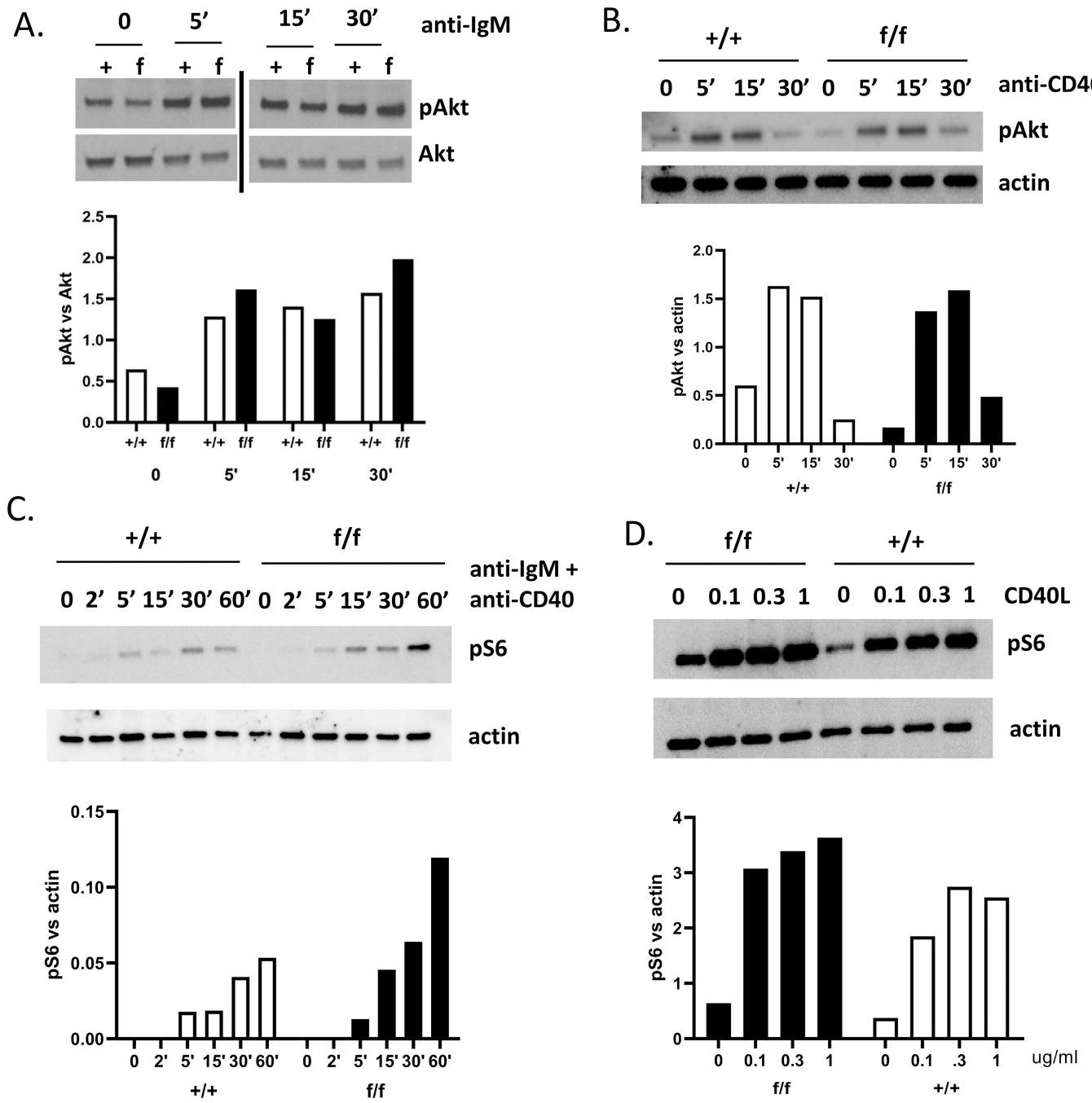

Figure 5: Increased pS6 phosphorylation in PIK3IP1 deficient B cells stimulated with anti-IgM + anti-CD40 or CD40L.

A, B) Purified splenic B cells from CD19-cre (+/+) and CD19-cre.PIK3IP1f/f (f/f) mice were stimulated with (A) 10 ug/ml anti-IgM or (B) 10 ug/ml anti-CD40 for the indicated times. Whole cell lysates were subjected to Western blot for anti-pAkt S473 and total Akt or β-actin loading controls. Results are representative of 3 independent experiments. C, D) Purified splenic B cells from CD19-cre and CD19-cre.PIK3IP1f/f mice were stimulated with (C) 10 ug/ml anti-IgM plus 10 ug/ml anti-CD40 for the indicated times or (D) the indicated dose of CD40L for 15 min. Whole cell lysates were subjected to Western blot for anti-pS6

and β-actin loading control. Results are representative of 3 independent experiments. Quantitation of each blot is shown in the graph below (open bars = CD19-cre, black bars = CD19-cre.PIK3IP1f/f).

Table I:

Normal B cell development in CD19-cre.PIK3IP1f/f mice

Population	CD19-cre	CD19-cre.PIK3IP1f/f
Bone marrow (x 10^6) (n = 4)		
Pro and Pre B ($B220^+IgM^-$)	2.9 +/− 1.2	3.3 +/− 1.4
Immature B ($B220^+IgM^+CD93^+$)	1.2 +/− 0.84	1.1 +/− 0.43
Mature B ($B220^{hi}IgM^+CD93^-$)	1.8 +/− 0.91	1.8 +/− 1.0
Spleen (x 10^7) (n = 9)		
Total splenocytes	11.1 +/− 2.7	9.2 +/− 2.1
Newly formed ($B220^+CD23^-CD21^-$)	0.4 +/− 0.21	0.33 +/− 0.13
Follicular ($B220^+CD23^+CD21^+$)	3.73 +/− 0.92	3.07 +/− 0.88
Marginal zone ($B220^+CD23^{lo/-}CD21^{hi}$)	0.16 +/− 0.072	0.17 +/− 0.94
Peritoneal cavity (% of lymphocytes) (n = 3)		
B-2 (B220+CD5−)	25.4 +/− 8.5	27.3 +/− 8.4
B-1 (B220+CD5+)	10 +/− 2.3	9.14 +/− 1.8

Total numbers (bone marrow, spleen) or frequency (peritoneal cavity) of cells in each of the indicated populations are indicated.