PHGDH is required for germinal center formation and is a therapeutic target in MYC-driven lymphoma

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The synthesis of serine from glucose is a key metabolic pathway supporting cellular proliferation in healthy and malignant cells. Despite this, the role that this aspect of metabolism plays in germinal center biology and pathology is not known. Here, we performed a comprehensive characterization of the role of the serine synthesis pathway in germinal center B cells and lymphomas derived from these cells. We demonstrate that upregulation of a functional serine synthesis pathway is a metabolic hallmark of B cell activation and the germinal center reaction. Inhibition of phosphoglycerate dehydrogenase (PHGDH), the first and rate-limiting enzyme in this pathway, led to defective germinal formation and impaired high-affinity antibody production. In addition, overexpression of enzymes involved in serine synthesis was a characteristic of germinal center B cell–derived lymphomas, with high levels of expression being predictive of reduced overall survival in diffuse large B cell lymphoma. Inhibition of PHGDH induced apoptosis in lymphoma cells, reducing disease progression. These findings establish PHGDH as a critical player in humoral immunity and a clinically relevant target in lymphoma.

Introduction

B cells play a critical role in the humoral immune response that eliminates threats to the host by secreting highly specific antibodies. Activated B cells (ABCs) can either differentiate into extrafollicular plasmablasts essential for early protective immune responses or enter a germinal center (GC). In the GC, B cells undergo affinity maturation and eventually differentiate into plasma cells, which secrete high-affinity antibody critical to eliminate the infectious agent, or memory B cells that confer long-lasting protection from secondary infection (1–4). In the GC reaction, B cells are activated by antigen engagement via their B cell receptor (BCR) and subsequent CD4+ T cell help, leading to MYC induction (5). The cell-cycle regulator MYC is essential for the formation and maintenance of GCs (6). MYC is commonly dysregulated in many high-grade B cell malignancies, including GC-derived lymphomas such as Burkitt lymphoma (BL) and diffuse large B cell lymphoma (DLBCL; ref. 7). MYC is a master regulator of metabolism, regulating the activity of many metabolic pathways including glycolysis and glutaminolysis. B cell proliferation, either in the context of a GC reaction or in B cell lymphomas, requires significant alterations in cellular metabolism to sustain the demands of dividing cells (8–10). B cells upregulate glycolysis following BCR engagement, a metabolic switch that is also characteristic of many cancers, including high-grade lymphomas (11–17). However, little is known about which metabolic pathways are involved in the utilization of glucose to support proliferating B cells.

One pathway that has emerged as a key metabolic node in cellular proliferation is the serine synthesis pathway (SSP). This uses a downstream product of glycolysis, 3-phosphoglycerate, to produce serine by the action of phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase 1 (PSAT1), and phosphoserine phosphatase (PSPH) (18). Notably, all 3 of these enzymes are known MYC targets (19). Serine is necessary for glycinic, phospholipid production, and it acts as a 1-carbon donor to the folate cycle, with serine-derived 1-carbon units being used for the synthesis of purine nucleotides to support cell growth (18, 20–22). Overexpression of SSP enzymes and increased serine biosynthesis from glucose is a feature of many types of cancer (23). While some cancers acquire amplification or overexpression of PHGDH, the first and rate-limiting step in this pathway, other types of cancers activate oncogenes such as MYC, MDM2, KRAS, and NRF2, leading to increased SSP enzyme expression (19, 23–26). Upregulation of the SSP allows cells to increase de novo synthesis of serine from glucose when extracellular serine availability is limiting in the tumor microenvironment (27–33). In support of this, a recent study has shown that inhibition of PHGDH...
is able to attenuate the growth of brain metastasis in vivo (34–36). Despite this, little is known about the role of the SSP in B cell lymphoma, with no reports on its role in normal GC biology. Here, we performed a comprehensive characterization of the role of SSP in the GC reaction and lymphomagenesis. We reveal that upregulation of the SSP is a metabolic hallmark of B cell activation and lymphoma, with PHGDH being a critical player in humoral immunity and a clinically relevant target in lymphoma.

**Results**

**Resting human naive B cells lack expression of SSP enzymes that are induced upon activation.** To understand the role of the SSP during B cell responses in vivo, we analyzed the expression of SSP genes in B cell subsets isolated from reactive human tonsils of cancer-free individuals by single-cell RNA-Seq (37). We observed elevated expression of PHGDH, PSAT1, and PSPH in cycling GC B cells compared with naive and nonproliferating B cells, suggesting an important role of the SSP in B cell proliferation (Figure 1A). We then examined the expression of SSP enzyme proteins and mRNA in naive B cells isolated from the peripheral blood of healthy individuals (Supplemental Figure 1; supplemental material available online with this article; https://doi.org/10.1172/JCI153436DS1). While resting naive B cells expressed very low to negligible amounts of PHGDH and PSAT protein, these enzymes were robustly induced 24–48 hours after stimulation by anti-IgM/G, CD40L, and IL-4 — signals that mimic those delivered in vivo to induce GC responses and B cell proliferation (Figure 1, B–E). In contrast, PSPH was constitutively expressed, becoming further elevated after stimulation (Figure 1, B–E). Treatment of naive B cells by these stimuli alone or in combination revealed that upregulation of PHGDH and PSAT was predominantly driven by BCR stimulation, which was synergistic with costimulation by CD40L and/or IL-4 (Supplemental Figure 2, A and B). Treatment of naive B cells by CpG to activate B cells via TLRs also resulted in induction of PHGDH and PSAT1 expression, although not to the degree of that seen after stimulation by the combination of anti-IgM/G, CD40L, and IL-4 (Supplemental Figure 2C). The temporal dynamics of PHGDH and PSAT1 were noted to be different, with PSAT1 expression being induced more rapidly than PHGDH, a pattern also observed in their mRNA transcripts (Figure 1, E and F). Importantly, IHC analysis of reactive human tonsils showed striking expression of PHGDH and PSAT1 within GCs, but not in mantle zone (MZ) areas (Figure 1, G and H, and Supplemental Figure 2D), confirming the upregulation of these enzymes as a hallmark of human GC B cells in vivo. We next assessed the dynamics of serine metabolism in ABCs. We cultured isolated human B cells with U-[13C]-glucose and examined the steady-state incorporation of [13C]-glucose–derived carbon into serine and glycine using liquid chromatography–mass spectrometry (LC-MS). While resting B cells fail to incorporate U-[13C]-glucose into serine, approximately 50% of the intracellular serine pool was labeled from glucose in stimulated B cells, with 40% of serine carbon being fully labeled (Figure 1I). When serine is directly derived from fully labeled glucose, it can be expected that all 3 of serine’s carbons will carry the 13C label (m+3). However, partially labeled serine isotopologues (m+1 and m+2) were also detected, likely due the interconversion of [13C]-labeled and unlabeled serine and glycine (Figure 1I), indicating the bidirectional nature of this pathway. Taken together, these data indicate that resting human naive B cells lack expression of SSP enzymes, which are induced upon activation to provide a functional ability to synthesize serine and glycine from glucose.

**Characterization of the SSP in mice after activation in vivo.** We characterized the expression of SSP enzymes in healthy resting murine B cells isolated from different tissue compartments (Supplemental Figure 3A). Consistent with the human data, there was low expression of PHGDH and PSAT1 protein in the peripheral blood, spleen, and lymph nodes of WT mice (Figure 2A). IHC analysis of murine spleen and lymph nodes also confirmed low expression of SSP enzymes in resting B cells (Figure 2B). The expression of PHGDH and PSAT1 was assessed in murine spleen and lymph nodes by flow cytometry and IHC 8 days after immunization with sheep RBCs, a T cell–dependent antigen that elicits robust GC responses. PHGDH and PSAT1 were specifically detected within peanut agglutinin– (PNA+) GCs in the spleen (Figure 2, B and C, and Supplemental Figure 3B) and lymph nodes (Supplemental Figure 3C). Expression of SSP-involved enzymes was also increased in splenic B cells following in vitro stimulation for 24–48 hours (Figure 2, D–F, and Supplemental Figure 3D). Comparable with human B cells, activation of murine B cells resulted in a clear increase in their ability to synthesize serine and glycine from U-[13C]-glucose (Figure 2G).

**Genetic loss and pharmacological inhibition of PHGDH impairs GC responses.** To interrogate the role of the SSP in B cell differentiation and GC responses, we targeted PHGDH, the first enzyme in the SSP, genetically and pharmacologically. We generated a conditional KO murine model in which Phgdh was specifically deleted in B cells by crossing mice carrying floxed Phgdh alleles with mice expressing the Cre recombinase under control of the Cd19 promoter (Phgdh<sup>fl/fl;Cd19-Cre</sup>). As expected, B220<sup>+</sup> B cells isolated from these mice did not show an increase in PHGDH expression following in vitro stimulation with anti-IgM/G antibody, CD40L, and IL-4, but they retained the ability to upregulate PSAT1 (Supplemental Figure 4A). We then analyzed animals before and 8 days after immunization with sheep RBCs to assess the impact of deletion of Phgdh on GC responses (Figure 3A). Numbers of B220<sup>+</sup>CD38<sup>+</sup>Fas<sup>+</sup> GC B cells were reduced in Phgdh<sup>fl/fl;Cd19-Cre</sup> mice following immunization, reflecting a reduction in both light zone (LZ) and dark zone (DZ) B cells (Figure 3, B and C). These observations were also confirmed by IHC analyses showing a significant reduction in average PNA+ GC area and proportion of splenic sections occupied by PNA+ GCs in Phgdh<sup>fl/fl;Cd19-Cre</sup> mice (Figure 3D and Supplemental Figure 4B). Further confirmation of the B cell–specific nature of the Phgdh KO was provided by a complete absence of PHGDH expression in GCs, in contrast to PSAT1, while PHGDH was expressed in the T cell–rich periaortical lymphoid sheaths (Supplemental Figure 4C). Thus, conditional KO of Phgdh in B cells results in an impaired GC response. Notably, we did not observe any significant difference in numbers of Pro-B cells, Pre-B cells, immature B cells, and mature B cells in BM when comparing Phgdh<sup>fl/fl;Cd19-Cre</sup> and Phgdh<sup>fl/fl;Cd19-Cre</sup> mice (Supplemental Figure 5, A and B). In addition, there was no significant difference in numbers of transitional, marginal zone, and follicular B cells in the spleen when comparing Phgdh<sup>fl/fl;Cd19-Cre</sup> and


**Figure 1. Upregulation of the SSP is a metabolic hallmark of GC B cells.**

(A) Uniform Manifold Approximation and Projection (UMAP) of tonsillar B cell single-cell RNA clusters (including naive, activated, pre-GC, total GC, plasmablasts, memory [MBC], and cycling B cells) (left). Expression of SSP–network genes in B cell subsets (right). (B) Analysis of PHGDH, PSAT1, and PSHF protein levels in human naive B cells isolated from blood bank volunteers by immunoblotting (n = 6). MDA-MB-231 and MDA-MB-468 cell lines were used as control for low and high SSP–enzyme expression, respectively. (C) Quantification of specific transcript levels relative to β-actin mRNA levels. (D) Representative immunoblot of PHGDH, PSAT1, and PSHF in resting and activated human naive B cells. Human B cells were left unstimulated (−) or stimulated (+) with anti-IgM/G antibody, CD40 ligand (CD40L), and IL-4 for 3, 24, and 48 hours. (E) Quantification of protein levels shown in D normalized to HSC70. (F) Relative mRNA expression of SSP enzyme genes in resting and activated human B cells determined by qPCR. Isolated human B cells were left unstimulated (−) or stimulated with (+) with anti-IgM/G antibody, CD40L, and IL-4 for 3, 24, and 48 hours before mRNA extraction. Transcript levels were determined relative to β-actin mRNA levels (n = 4). (G and H) Representative IHC staining for PHGDH and PSAT1 in germinal center (GC) and mantle zone (MZ) areas in sequential sections of human reactive tonsils (×5 and ×20 magnification) and quantification (n = 10). (I) Mass isotopologue distribution of [U-13C]-glucose–derived serine and glycine from human resting and activated B cells. B cells were unstimulated stimulated with anti-IgM/G antibody, CD40L, and IL-4 for 48 hours. Cells were cultured for 2 hours in serine/glycine deplete media containing [U-13C]-glucose. Data are shown as the mean ± SEM. *P < 0.05, **P < 0.01, and ***P < 0.0001, by Mann-Whitney U test (E, F, and H).

Phgdh−/−;Cd19-Cre mice (Supplemental Figure 5, C–F). We would hypothesize that these observations can be explained by lower deletion efficiency of Cd19-Cre in BM B cells (38), combined with the relative impact of Phgdh deletion on B cells proliferating in serine-deplete microenvironments.

We next assessed whether inhibition of GC responses could be replicated by pharmacological inhibition of PHGDH using a specific inhibitor, PH-755 (35, 36). Mice were injected with sheep RBCs 24 hours before starting treatment with PH-755 or vehicle control. GC responses were assessed by flow cytometry and IHC as before (Figure 3E). Treatment with PH-755 resulted in significantly reduced numbers of GC B cells comparable with that seen with the conditional KO (Figure 3, F and G). IHC analysis also showed a reduction in PNA+ GC area, with an overall reduction in the proportion of splenic sections occupied by PNA+ GC B cells but with preservation of PHGDH expression (Figure 3H and Supplemental Figure 6, A and B). We then proceeded to assess the impact on NP-specific plasma cell responses after 4-hydroxy-3-nitrophenyl acetyl–chicken γ-globulin (NP-CGG) immunization. PH-755 treatment resulted in a significant reduction of numbers of NP-specific GC B cells and total NP-specific and NP-specific IgG1 plasma cells that correlated with reduced titres of anti–NP IgG1 antibodies in the sera of these mice (Figure 3, I and J), with no effect on NP-specific IgM responses (Supplemental Figure 6, C–E). The lack of impact of PHGDH inhibition on NP-specific IgM responses could, therefore, reflect increased extracellular serine availability outside of GCs, leading to a relative sparing of extrafollicular responses. Taken together, these data show that PHGDH inhibition impairs GC formation with a resultant reduction in high-affinity antibody production.

PHGDH inhibition impairs B cell proliferation and de novo serine and glycine synthesis. Based on previous observations showing the crucial role of the SSP in cell growth and proliferation, we proceeded to assess the impact of PHGDH inhibition on the behavior of primary stimulated murine B cells. Since GC B cells cannot survive ex vivo due to the rapid inception of a proapoptotic program (39), we performed these experiments on B220+ cells isolated from spleens. Stimulated primary B cells from either Phgdhfl/fl;Cd19-Cre mice or Phgdh−/−;Cd19-Cre mice were tested for their ability to synthesize serine and glycine from glucose by incubating them with U-[13C]-glucose. Comparable experiments were performed with stimulated B cells taken from WT mice treated with either PH-755 or control. As expected, both genetic KO and pharmacological inhibition of PHGDH almost completely abolished the ability of the cells to incorporate labeled glucose into serine at these time points (Figure 4A). We then assessed the impact of PHGDH inhibition on the proliferative capacity of B cells from Phgdhfl/fl;Cd19-Cre mice in vitro following stimulation, compared with Phgdh−/−;Cd19-Cre control B cells. B cell proliferation measured by dye-dilution assay (Figure 4B) revealed only a slight reduction in proliferation of Phgdhfl/fl;Cd19-Cre B cells as compared with Phgdh−/−;Cd19-Cre cells when stimulated in serine-glycine replete medium (Figure 4C). However, in media lacking serine and glycine, the proliferation of Phgdhfl/fl;Cd19-Cre B cells was completely abrogated, whereas PHGDH induction following stimulation sustained the proliferation of Phgdh−/−;Cd19-Cre B cells (Figure 4, B and C). Notably, the proliferative capacity of these PHGDH-deficient B cells could be partially rescued by the addition of glycine with formate as a 1-carbon donor (32). In addition, providing Phgdh−/−; Cd19-Cre B cells with formate and glycine allowed them to proliferate optimally in the absence of serine (Figure 4, B and C). We then proceeded to investigate whether the changes in proliferation were accompanied by altered cell cycling and/or apoptosis. Notably, the reduction in proliferation was mirrored by a decrease in the fraction of cells in S phase, particularly when genetic KO of Phgdh was accompanied by the removal of extracellular serine and glycine (Figure 4D). This decrease in cells in S phase was accompanied by an accumulation of cells in G0/G1 phase (Figure 4D). Supplementing serine-starved Phgdhfl/fl;Cd19-Cre B cells with glycine and formate partially rescued the fraction of cells in S phase, as reflected by cell proliferation (Figure 4D). We then proceeded to repeat these experiments with WT B cells cultured in various combinations of serine, glycine, and formate, with PH-755 or vehicle control. Treatment with PH-755 largely recapitulated the pattern observed with the genetic ablation of Phgdh, with the exception that PH-755 treatment was able to partially inhibit B cell proliferation even in the presence of extracellular serine and glycine (Figure 4, E–G). Notably, we did not observe increased Caspase-3 activation as a marker of apoptosis in the Phgdh-KO or drug-treated B cells under any of the conditions (Figure 4H). In summary, these results show that inhibition of PHGDH effectively blocks de novo synthesis of serine and glycine from glucose, which in turn has a cytostatic effect on primary murine B cells in the absence of extracellular serine and glycine.

Activation of the SSP pathway in human MYC-driven GC lymphomas. One of the key emerging concepts in the cancer metabolism field over the last few years is that the metabolic phenotype of cancer cells reflects their cell-of-origin in combination with other factors such as oncogenic drivers and the tumor metabolic micro-
environment (40). In light of this, we analyzed the role of the SSP in GC lymphomas. These were of particular interest due to the role of MYC in their pathogenesis and as a regulator of the SSP. The expression of PHGDH and PSAT1 was assessed by IHC in a series of diagnostic biopsies from patients with BL, DLBCL, and chronic lymphocytic leukemia (CLL). Notably, very high expression of these 2 proteins was observed in BL, consistent with a recent report (41), with intermediate to high expression in DLBCL and relatively low expression in CLL (Figure 5, A and B). Although biopsies from CLL patients showed the lowest expression, PHGDH and PSAT1 staining was significantly increased within proliferation centers, which are microanatomical sites in lymphoid tissues where CLL cells proliferate and where MYC is expressed (Figure 5, C and D; refs. 42, 43). Given the heterogeneity of expression in DLBCL

Figure 2. Characterization of the SSP in WT mice after activation in vivo. (A) Analysis of PHGDH, PSAT1, and PSPH protein levels in resting B cells isolated from mouse spleen (SPL), peripheral blood (PB), and lymph nodes (LN). NIH3T3 murine cells were used as control for high expression of SSP-related enzymes. (B) Representative IHC staining for PNA as GC marker, PHGDH, and PSAT1 on consecutive spleen sections derived from mouse spleens 8 days after sheep RBC immunization (×5 magnification). (C) Expression of PHGDH and PSAT1 in GC B cells and non-GC B cells harvested from mouse spleen 8 days after immunization with sheep RBC. (D) Representative immunobLOTS of PHGDH, PSAT1, and PSPH proteins levels in murine resting and activated B cells. (E) Mouse B cells were isolated from spleen and left unstimulated (−) or stimulated (+) with anti-IgM/G antibody, CD40L, and IL-4 for 24 hours before protein extraction and quantification of protein levels normalized to HSC70. Individual samples (dots) and means (bars) values are plotted (n = 4). (F) Relative mRNA expression of SSP enzyme genes in resting and activated mouse B cells as determined by qPCR. Isolated mouse B cells were left unstimulated or stimulated with anti-IgM/G antibody, CD40L, and IL-4 for 24 and 48 hours before mRNA extraction. Specific transcript levels were determined relative to β-actin mRNA levels (n = 4). (G) Mass isotopologue distribution of U-[13C6]-glucose–derived serine and glycine from murine resting and activated B cells. Cells were left unstimulated or stimulated with anti-IgM/G antibody, CD40L, and IL-4 for 48 hours. Cells were then cultured for 2 hours in serine/glycine-deplete media containing U-[13C]-glucose. [13C]-isotopologue distribution in serine and glycine was determined by LC-MS. Data are shown as the mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, by Mann-Whitney U test (C, E) or by 1-way ANOVA (F).
Figure 3. Genetic loss and pharmacological inhibition of PHGDH impairs GC responses. (A) Mice were injected with sheep RBCs and spleens examined by IHC and flow cytometry 8 days after immunization. (B) Representative flow cytometric analysis of splenic B cells from Phgdh<sup>−/−</sup>;Cd19-Cre (WT) or Phgdh<sup>fl/fl</sup>;Cd19-Cre (F/F) before and after immunization to identify GC B cells (CD19<sup>+</sup>B220<sup>+</sup>CD38<sup>lo</sup>CD95<sup>hi</sup>), as well as DZ (CD86<sup>lo</sup>CXCR4<sup>hi</sup>) and LZ (CD86<sup>hi</sup>CXCR4<sup>lo</sup>) B cells, within GC splenic population. (C) Flow cytometric analysis of absolute numbers of B cell subsets within CD19<sup>+</sup>B220<sup>+</sup>CD38<sup>lo</sup>CD95<sup>hi</sup> splenic population from Phgdh<sup>fl/fl</sup>;Cd19-Cre (n = 6) and Phgdh<sup>−/−</sup>;Cd19-Cre (n = 4) after immunization. (D) Average GC area (left) and proportion (%) of GC area per spleen area (right) from Phgdh<sup>−/−</sup>;Cd19-Cre (F/F) and Phgdh<sup>−/−</sup>;Cd19-Cre (WT) mice after immunization. (E) WT mice were immunized with sheep RBCs before PH-755 treatment (300 mg/kg PH-755 orally twice daily for 7 days). Spleens were analyzed 8 days after immunization. (F) Representative flow cytometric analysis of splenic B cells from mice immunized with sheep RBCs and treated with vehicle/PH-755 to identify GC, DZ, and LZ B cells within GC splenic population. (G) Flow cytometric analysis of absolute number of GC, DZ, and LZ B cells within B220<sup>+</sup> splenocytes collected from mice 8 days after sheep RBCs immunization; mice were treated with vehicle (n = 5) or PH-755 (n = 5). (H) Average GCs area (left) and proportion (%) of GC area per spleen area (right) from vehicle- and PH-755–treated mice 8 days after sheep RBC immunization. (I) Summary of NP<sub>2</sub>-specific plasma cells (PCs; left) and NP<sub>2</sub>-specific IgG1 PCs (right; total number per popliteal lymph nodes). (J) Serum antibody titers for NP<sub>2</sub>-specific IgG1 8 days after NP-CGG immunization. Data are shown as the mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001, by Mann-Whitney U test (C, G, I, and J) or unpaired t test (D and H).
Figure 4. PHGDH inhibition impairs B cell proliferation and de novo serine and glycine synthesis. (A) Mass isotopologue distribution of U-[13C6]–glucose-derived serine and glycine in B220+ B cells isolated from Phgdh+/+;Cd19-Cre (WT) or Phgdhfl/fl;Cd19-Cre (F/F) or WT C57BL/6J mice. Isolated cells were stimulated with anti-IgM/G antibody, CD40L, and IL-4 for 48 hours and were then cultured for 2 hours in serine/glycine-deplete media with U-[13C]-glucose (and treated with/without PH-755 for WT B cells). (B and C) Representative proliferation profiles and quantification of B220+ B cells from either Phgdh+/+;Cd19-Cre (WT) or Phgdhfl/fl;Cd19-Cre (F/F) mice. Cells were labeled with the Cell Proliferation Dye eFluor 670 and then cultured for 3 days with anti-IgM/G antibody, CD40L, and IL-4 in complete media, serine/glycine-free medium, or serine/glycine-free medium containing glycine and formate (n = 4 per genotype). (D) Cell-cycle analysis of B220+ cells isolated from Phgdh+/+;Cd19-Cre (WT) or Phgdhfl/fl;Cd19-Cre (F/F) mice. Cells were cultured for 48 hours with anti-IgM/G antibody, CD40L, and IL-4 in complete media, serine/glycine-free medium, or serine/glycine-free medium containing glycine and formate; they then underwent BrdU labeling and 7-AAD staining to assess cell cycle. (E and F) Representative proliferation profiles and quantification of B220+ B cells from WT mice. Cells were labeled with the Cell Proliferation Dye eFluor 670 and were then cultured for 3 days with anti-IgM/G antibody, CD40L, and IL-4 in complete media, serine/glycine-free medium, or serine/glycine-free medium containing glycine and formate in combination with DMSO or 10 μM PH-755 (n = 5 per group). (G) Cell-cycle analysis of B220+ B from WT mice. Cells were cultured for 48 hours with anti-IgM/G, CD40L, and IL-4 in complete medium, serine/glycine-free medium, or serine/glycine-free medium containing glycine and formate in combination with DMSO or 10 μM PH-755 before cell-cycle analysis. (H) Activate Caspase-3 on B220+ B cells from either Phgdh+/+;Cd19-Cre (WT) or Phgdhfl/fl;Cd19-Cre (F/F) mice. Cells were cultured for 48 hours with anti-IgM/G antibody, CD40L, and IL-4 in complete media, serine/glycine-free medium, or serine/glycine-free medium containing glycine and formate in combination with DMSO or 10 μM PH-755 before cell-cycle analysis. (I) and on B cells isolated from WT mice (right panel; n = 3 per group) and treated as described in A, and on B cells isolated from WT mice (right panel; n = 3 per group) and treated as described in G. Data are shown as the mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001, by 1-way ANOVA (H and I).
patients, we next interrogated a published data set (GSE10846; https://www.ncbi.nlm.nih.gov/) to investigate the relationship between SSP gene expression and patient survival (44). Importantly, high expression of PSAT1 was significantly associated with poorer overall survival in DLBCL (Figure 5E), with a trend toward patients with high expression of PHGDH also having reduced overall survival. The expression of PHGDH and PSAT1 in ABC-like or GC B cell–like (GCB-like) DLBCL was also assessed due to the prognostic importance of these profiles (45). There was no difference between the expression of PHGDH and PSAT1 between these 2 subsets, although there was a weak positive correlation between MYC expression and the expression of SSP enzymes (Supplemental Figure 7, A and B). The lack of difference in PHGDH and PSAT1 expression when comparing ABC- and GCB-like DLBCL likely reflects our observations regarding the strong induction of these 2 enzymes upon activation of human B cells both in vitro and in GCs in vivo (Figure 1, D–H). Overall, the upregulation of the SSP is a feature of GC malignancies and can predict impaired overall survival in DLBCL.

**PHGDH inhibition impairs proliferation and promotes apoptosis in BL cells.** We hypothesized that the SSP may represent a therapeutic target in human lymphoma. The expression of SSP

**Figure 5. Human GC lymphomas are characterized by activation of the SSP pathway.** (A and B) Representative immunohistochemical staining (×20 magnification) and quantification for PHGDH and PSAT1 abundance in sections of human diagnostic biopsies from patients with Burkitt lymphoma (BL), Diffuse Large B cell lymphoma (DLBCL), and Chronic lymphocytic leukemia (CLL). The statistical difference was analyzed using the ordinary 1-way ANOVA. (C and D) IHC analysis (×40 magnification) for PHGDH and PSAT1 in proliferation centers (PC) and resting zone (RZ) areas in sections from biopsies collected from patients with chronic lymphocytic leukemia (CLL), and quantification. Individual samples (dots) and means (bars) values are plotted. The statistical difference was analyzed using the Mann-Whitney U test. (E) Kaplan-Meier survival analysis of patients with DLBCL from a published data set (GSE10846) (44). Patients whose PHGDH/PSAT1 mRNA levels were within the top quartile were grouped as PHGDH/PSAT1 high; those with PHGDH/PSAT1 mRNA levels within the bottom quartile were grouped as PHGDH/PSAT1 low. Data are shown as the mean ± SEM. ****P < 0.0001, by 1-way ANOVA (B) or by Mann-Whitney U test (D). Survival analysis were conducted with log-rank (Mantel-Cox) test (E).
A Human B cell–derived lymphoma cell lines

| BL   | DLBCL | MCL   |
|------|-------|-------|
| Daudi| Ramos | Raji  |
| BJAB | Daudi | Raji  |
| Narm  | SK-H4 | SJ-L6 |
| KARPAS 422 | Daudi | Raji |
| OCI-Ly3 | OCI-Ly7 | Jeko-1 |
| Hela  | Ramos | Raji  |

PHGDH
PSAT1
PSPH
HSC70

B Ramos

Ramos

PHGDH
PSAT1
PSPH
HSC70

C

D Ramos

Ramos

PHGDH
PSAT1
PSPH
HSC70

Daudi

Daudi

PHGDH
PSAT1
PSPH
HSC70

C

D

D

D
enzymes was assessed in a panel of human lymphoma cell lines (Figure 6A and Supplemental Figure 8A). In contrast to other BL cell lines, Daudi cells had no expression of PHGDH but did express PSAT1 and PSPH. Consequently, Daudi cells were unable to enter S phase when cultured without extracellular serine and glycine, with cycling being partially rescued by glycine and formate for 24 and 24 hours in medium lacking serine and glycine in presence of U-[13C]-glucose (10 mM) and treated with DMSO or 10 μM PH-755. Serine, glycine, ATP, and GTP levels were measured by LC-MS. The percentage distribution of each isotopologue for their respective metabolite pool is shown. Data are presented as mean ± SEM of 6 repeats and are representative of 3 independent experiments.

We assessed the ability of these cell lines to synthesize serine, glycine, and the purine nucleotides adenosine triphosphate (ATP) and guanine triphosphate (GTP) from glucose. The cell lines were cultured with U-[13C]-glucose for 2–24 hours in the absence of serine and glycine, with labeling being assessed by LC-MS. Ramos and Raji cells grown in the absence of serine and glycine diverted glucose into de novo serine and glycine synthesis, which was prevented when treated with PH-755 (Figure 6D and Supplemental Figure 8B). Furthermore, labeling of higher isotopologues (≥m+6) of ATP and GTP demonstrated that these nucleotides were being synthesized via the SSP, and this could again be inhibited by PH-755 (Figure 6D and Supplemental Figure 8B). Unlike Ramos and Raji cells, this labeling was virtually absent in Daudi cells, consistent with their lower ability to proliferate in the absence of exogenous serine (Figure 6D). In conclusion, these results show that inhibition of PHGDH in the absence of extracellular serine and glycine is able to inhibit purine synthesis and block entry into the S phase of the cell cycle and promote apoptosis of human lymphoma cell lines.

**Discussion**

Our results establish the importance of the SSP in supporting the proliferation of GC B cells. We show that the SSP is upregulated during B cell activation and is a metabolic hallmark of the GC reaction. Inhibition of PHGDH, either genetically or pharmaco-
logically, leads to a defect in GC formation and a reduction in high-affinity antibody production. In addition, overexpression of SSP enzymes is a characteristic of GC-derived lymphomas, with high levels of expression predicting a poorer prognosis in DLBCL. In contrast to healthy ABCs, where PHGDH blockade has a cytostatic effect, inhibition of this enzyme in lymphoma cells induces apoptosis, likely reflecting the impact of MYC overexpression. Importantly, inhibition of PHGDH, either specifically within lymphoma cells using an inducible KO transplantation model or globally using a pharmacological agent, reduces dis-
ease progression, highlighting this enzyme as a potentially novel therapeutic target in lymphoma.

These data highlight the interaction between the availability of extracellular serine and the SSP. Collectively, these results suggest that the concentration of serine is rate limiting in GCs, resulting in a requirement for proliferating B cells to synthesize serine from glucose. A recent study has shown that serine is an essential metabolite for effector T cell proliferation (50). Notably, these authors observed that, while effector T cells also upregulate the SSP after activation, they derive the majority of their serine from extracellular sources, with dietary serine availability dictating T cell responses in vivo. Our data suggest an important difference between T cell and B cell biology, due to the anatomical localization of humoral immune responses to the GC. This raises the intriguing possibility that PHGDH inhibition could selectively target the humoral response with relative preservation of T cell responses, with important implications for the treatment of autoimmune disease and lymphoma.

The role that the SSP plays in cancer is complex and an area of active investigation. Much of the focus has been on the role of PHGDH and its potential as a target for therapy. Notably, while PHGDH does seem to be important for the development of some cancers, including metastatic breast cancer, observations in other cancer models have suggested that this enzyme is dispensable for tumor development (23, 27, 51). A previous report suggested that MYC-driven lymphomagenesis can occur in the absence of PHGDH. However, those observations were based on the use of λ- MYC; Phgdh−/+ mice crossed with Phgdh−/+ mice, in a system that generated lymphoma-prone mice with varying expression of PHGDH (52). It is quite possible that even low levels of PHGDH permit MYC-driven lymphomagenesis, which would explain the differences we observed with our systems. Indeed, MYC-driven lymphoma may be particularly susceptible to serine deprivation, as previous work has demonstrated that the progression of lymphoma in Eμ-Myc mice can be slowed by reducing the availability of extracellular serine and glycine by dietary restriction (25). This interaction between extracellular serine and PHGDH inhibition may open up new therapeutic opportunities, either in terms of combining pharmacological inhibition of PHGDH with a serine/glycine-free diet or in treating lymphoma in serine/glycine-depleted environments such as the CNS (36). Tumor cells may become increasingly dependent on de novo serine synthesis as the disease progresses, as the increasing mass of cancer cells consumes microenvironmental serine.

Our observations support the hypothesis that it is serine and glycine themselves that are important for lymphoma cell proliferation and survival, as these cells retain the ability to cycle and have low rates of apoptosis when PHGDH is inhibited in serine/glycine-replete conditions. Serine appears to be the more important, given our observations that culture with glycine and formate, glycine-replete conditions. Serine appears to be the more important, given our observations that culture with glycine and formate, glycine-replete conditions. Serine appears to be the more important, given our observations that culture with glycine and formate, glycine-replete conditions. Serine appears to be the more important, given our observations that culture with glycine and formate, glycine-replete conditions. Serine appears to be the more important, given our observations that culture with glycine and formate, glycine-replete conditions. 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euthanized according to license guidelines, and lymph nodes and spleens were collected for further analysis.

For PHGDH excision experiments, 2 × 10^6 Eμ-MyC/+;Rosa26-CreERT2/+-Phgdh^-/- or Eμ-MyC/+;Rosa26-CreERT2/+-Phgdh^-/- cells were injected via tail vein into 7- to 12-week-old C57BL/6J mice. Three days after lymphoma injection, mice were randomized to receive vehicle (sunflower oil; MilliporeSigma, S5007) or 3 mg at day 1 and 2 days after lymphoma injection, mice were randomized to receive 20 µg/mL goat F(ab')2 anti-mouse IgM + IgG (Stratech Scientific, 115-006-068), 250 ng/mL mouse recombinant MEGACD40L (Enzo, ALX-522-120), and 20 ng/mL recombinant mouse IL-4 (R&D system, 404-ML). Cells were collected at specific time points for analysis. For all serine- and glycine-deprivation experiments, cells were cultured in standard media deprived of L-serine and L-glycine.

**Cell culture**

**Cell lines.** All the human cell lines used in this study were obtained from Cell Services at the Francis Crick Institute. All cell lines underwent routine quality control, which included mycoplasma detection, short tandem repeats (STR) profiling, and species identification for validation. Cells were cultured at 37°C in a humidified atmosphere of 5% CO2. Daudi, Ramos, and Namalwa cells were cultured in RPMI 1640 medium (Thermo Fisher Scientific, 21875091) supplemented with 5% FCS; JEKO-1, Raji, DOHH2, and DG-75 cells were cultured in RPMI 1640 medium supplemented with 10% FCS; BJAB and OCI-LY3 cells were cultured in RPMI 1640 medium supplemented with 20% FCS; KARPAS 422 and SUDHL4 cells were cultured in ATCC RPMI 1640 medium (Thermo Fisher Scientific, A1049101) supplemented with 20% and 10% FCS, respectively; GRANTA-519 cells were cultured in DMEM (Thermo Fisher Scientific, 14966052) supplemented with 10% FCS and 2 mM L-glutamine; and OCI-LY17 cells were cultured in IMDM (Thermo Fisher Scientific, 21980032) supplemented with 20% FCS.

**Primary cells.** Buffy cones were obtained from the UK National Blood Service for investigation of healthy B cells. Human naive B cells were isolated using the MACSxpress Whole Blood Naïve B Cell Isolation Kits (Miltenyi Biotec, 130-098-186) according to the manufacturer’s instructions. Residual erythrocytes were lysed using 1× Red Blood Cell Lysis Buffer (Invitrogen, 00-433). Cells were then washed in full medium consisting of RPMI 1640 (Thermo Fisher Scientific, 21875091), 10% FCS (Thermo Fisher Scientific, 26400044), 2.5% 1M HEPES buffer (MilliporeSigma, H0887), 1% GlutaMAX (Thermo Fisher Scientific, 35050038), 1% penicillin-streptomycin (Thermo Fisher Scientific, 15140122), and 0.1% 0.05M β-mercaptoethanol (MilliporeSigma, M3148). Murine resting B cells were stimulated with 20 µg/mL goat F(ab')2 anti–mouse IgM + IgG (Stratech Scientific, 115-006-068), 250 ng/mL mouse recombinant MEGACD40L (Enzo, ALX-522-120), and 20 ng/mL recombinant mouse IL-4 (R&D system, 404-ML). Cells were collected at specific time points for analysis.

**Immunoblot analysis**

Cells were lysed on ice for 30 minutes using lysis buffer (1% [vol/vol] Nonidet P-40, 20 mM Tris-HCl [pH 8.0], 150 mM NaCl, and 5 mM EDTA with protease inhibitors [Roche Diagnostics, 11873580011]) and phosphatase inhibitors (sodium fluoride and sodium orthovanadate;MilliporeSigma, S7920 and S6508). Samples were centrifuged (21,000g, 15 minutes, 4°C), and the protein content of the supernatant was measured using the Protein Assay Dye Reagent (Bio-Rad, 500-0006). Immunoblotting was performed using from 10 µg to 50 µg of protein lysate. Antibodies were used for detecting the following proteins: PHGDH (Cell Signaling Technology; human specific, 66550; mouse-specific, 13428), PSAT1 (Thermo Fisher Scientific, PA5-22124), PSPH (Thermo Fisher Scientific, PA5-22003), anti-HSC70 (Santa Cruz Biotechnology, sc-7298). All secondary HRP-conjugated antibodies were from Cell Signaling Technology. Membranes were exposed using Amersham Hyperfilm ECL (GE Healthcare) and developed using a Carex 60 developer (Agfa). Films were scanned and quantified using ImageJ 1.50c (NIH). All values were normalized to the HSC70 loading control, and relative fold-change was calculated with the isotype control antibody treated cells taken as 100% of expression.

**qPCR**

Total RNA was isolated from cells using the RNeasy Mini-Kit (Qiagen, 74104) according to the manufacturer’s instructions. RNA was reverse transcribed using oligo(dT) primers (Promega, C1101) and M-MLV (Promega, M1701) in the presence of RNase inhibitor (Promega, N2511). Quantitative PCR (qPCR) was performed on a QuantStudio 12 Flex Real-Time (Applied Biosystems). A standard curve was generated for each human gene from untreated HeLa cells and for each mouse gene from untreated NIH3T3 cells. The average complementary DNA concentration was determined using the standard curve method and made relative to β-actin. Primers used for qPCR were all purchased from Applied Biosystems and are as follows: TaqMan probe for human PHGDH, Hs01106329_m1; TaqMan probe for human PSAT1, Hs00795278_mH; TaqMan probe for human PSPH, Hs00190154_m1; TaqMan probe for β-actin, Hs01060665_g1; TaqMan probe for human PHGDH, Mm01623589_g1; TaqMan probe for human PSAT1, Mm02793542_g1; TaqMan probe for human PSPH, Mm01197775_m1; and TaqMan probe for β-actin, Mm02619580_g1.

**IHC**

All tissues were fixed in 10% neutral buffered formalin and were embedded in paraffin. Tissue microarrays (TMAs) of triplicate 1 mm diameter cores were prepared from paraffin blocks using a manual
tissue arrayer (Beecher Scientific) as previously described (54). Cut sections or TMAs were fully drained and placed in a 60°C oven for a minimum of 2 hours. Slides were then deparaffinized in xylene and rehydrated using a series of absolute ethanol solutions and distilled water. Heat-induced epitope retrieval (HIER) was performed for 10 minutes in a pressure cooker using boiling citric acid-based antigen unmasking solution (Vector, H3300). After retrieval, slides were placed into a wash buffer containing 1× TBS-tween (Agilent Dako, S3306) before IHC staining. Specific primary antibodies were diluted in Agilent antibody diluent (catalog S080983-2) and incubated for 40 minutes at RT. The dilutions and manufacturer details of all the antibodies used are listed in Table 1. The slides were washed and then incubated with biotinylated secondary antibodies (Vector; anti–rat BA-9401, anti–rabbit BA-1000) for 30 minutes at RT. The slides were washed again before staining with the VECTASTAIN Elite ABC peroxidase kit (Vector, PK-6100), in combination with a DAB substrate (eBioscience, 14-2504), anti–CD23-PE-Cy7 (BioLegend, 740414), anti–IgM-APC (eBioscence, 17-5790-82), and anti–NP-PE (generated in-house). To identify B cell populations in BM, cells were incubated with the following combination of monoclonal antibodies: anti–IgD-FITC (eBioscence, 11-5993-82), anti–CD138-PE-Cy7 (BioLegend, 142504), anti–CD23-PE-Cy7 (BioLegend, 103247), anti–IgM-APC (eBioscence, 17-5790-82), and anti–B220-BV710 (BioLegend, 103247). To identify splenic B cell populations, cells were incubated with directly conjugated monoclonal antibodies: anti–CD93-FITC (BioLegend, 136508), anti–CD138-PE (BioLegend, 142504), anti–CD23-PE-Cy7 (BioLegend, 740414), and anti–IgM-APC (eBioscence, 17-5790-82), and anti–B220-BV510 (BioLegend, 103247).

To assess PHGDH and PSAT1 levels in vivo, splenocytes were first surface stained for GC markers and then washed twice in staining buffer and treated with fixation/permeabilization solution Cytosix/Cytoperm (BD Bioscience, 554722) for 30 minutes on ice in the dark. Cells were washed twice in 1× Perm/Wash buffer (BD Bioscience, 554723) and then incubated with either anti-PHGDH (1/250) (Cell Signaling Technology, 13428) or anti-PSAT1 (1/400) (Thermo Fisher Scientific, PA5-22124) in staining buffer for 1 hour on ice in the dark and washed twice as previously. To identify GC B cells populations, cells were stained with the secondary antibody anti-rabbit Alexa Fluor 647 (1/500) (Cell Signaling Technology, 44144) for 30 minutes at RT. Cells were then washed, resuspended in staining buffer, and kept at 4°C until analysis. For intracellular active Caspase-3 staining, cells were stained with fixable viability dye eFluor780 staining (eBioscience, 65-0865-14) for 30 minutes on ice protected from light. Cells were then washed and resuspended in staining buffer and kept at 4°C until analysis. To identify NP-specific plasma cells following NP-CGG immunization, splenocytes were incubated with directly conjugated monoclonal antibodies (anti–CD19-BUV395 [BD Horizon, 563557], anti–B220-BV510 [BioLegend, 103247], anti–FAS-PerC/P-Cy5.5 [eBioscence, 45-5892-82], anti–CD38-PE-Cy7 [BioLegend, 103247], anti–IgM-APC [eBioscence, 17-5790-82], anti–IgG1-BV421 [BD Horizon, 562580], anti–CD138-BV786 [BD Bioscience, 740880], and anti–NP-PE [generated in-house]). To identify B cell populations in BM, cells were incubated with the following combination of monoclonal antibodies: anti–IgD-FITC (eBioscence, 11-5993-82), anti–CD138-PE (BioLegend, 142504), anti–CD23-PE-Cy7 (BioLegend, 103247), and anti–IgM-APC (eBioscence, 17-5790-82), and anti–B220-BV510 (BioLegend, 103247).

Flow cytometry

For surface staining, single-cell suspension was washed twice in staining buffer (PBS containing 2% FCS and 2 mM EDTA). To detect sheep RBC–induced GC, DZ, and LZ B cells, splenocytes were incubated with directly conjugated monoclonal antibodies (anti–CD19-PE [BD Bioscience, 707361], anti–B220-BV510 [BioLegend, 103247], anti–FAS-BV421 [BD Bioscience, 562633], anti–CD38-PE-Cy7 [BioLegend, 102718], anti–CXCR4-Alexa488 [eBioscence, 53-9991-80], anti–CD86-APC [BioLegend, 105012]) and Fixable viability dye eFluor780 staining (eBioscence, 65-0865-14) for 30 minutes on ice protected from light. Cells were then washed and resuspended in staining buffer and kept at 4°C until analysis. To identify NP-specific plasma cells following NP-CGG immunization, splenocytes were incubated with directly conjugated monoclonal antibodies (anti–CD19-BUV395 [BD Horizon, 563557], anti–B220-BV510 [BioLegend, 103247], anti–FAS-PerC/P-Cy5.5 [eBioscence, 45-5892-82], anti–CD38-PE-Cy7 [BioLegend, 103247], anti–IgM-APC [eBioscence, 17-5790-82], anti–IgG1-BV421 [BD Horizon, 562580], anti–CD138-BV786 [BD Bioscience, 740880], and anti–NP-PE [generated in-house]). To identify B cell populations in BM, cells were incubated with the following combination of monoclonal antibodies: anti–IgD-FITC (eBioscence, 11-5993-82), anti–CD138-PE (BioLegend, 142504), anti–CD23-PE-Cy7 (BioLegend, 103247), and anti–IgM-APC (eBioscence, 17-5790-82), and anti–B220-BV510 (BioLegend, 103247).

To assess PHGDH and PSAT1 levels in vivo, splenocytes were first surface stained for GC markers and then washed twice in staining buffer and treated with fixation/permeabilization solution Cytosix/Cytoperm (BD Bioscience, 554722) for 30 minutes on ice in the dark. Cells were washed twice in 1× Perm/Wash buffer (BD Bioscience, 554723) and then incubated with either anti-PHGDH (1/250) (Cell Signaling Technology, 13428) or anti-PSAT1 (1/400) (Thermo Fisher Scientific, PA5-22124) in staining buffer for 1 hour on ice in the dark and washed twice as previously. To identify GC B cells populations, cells were incubated with directly conjugated monoclonal antibodies: anti–CD93-FITC (BioLegend, 136508), anti–CD138-PE (BioLegend, 142504), anti–CD23-PE-Cy7 (BioLegend, 740414), and anti–IgM-APC (eBioscence, 17-5790-82), and anti–B220-BV510 (BioLegend, 103247).

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Table 1. Details of antibodies used for IHC

| Target | Manufacturer | Catalog | Dilution | HIER | Protocol | Detection |
|--------|--------------|---------|----------|------|----------|-----------|
| Anti-mouse antibodies | | | | | |
| PNA | Vector | B-1075 | 1 in 500 | HIER | Standard | Lectin |
| PHGDH | Thermo Fisher Scientific | PA5-24633 | 1 in 400 | HIER | Standard | Anti-rabbit |
| PSAT1 | Thermo Fisher Scientific | PA5-22224 | 1 in 200 | HIER | Standard | Anti-rabbit |
| B220 | BD Biosciences | 550286 | 1 in 50 | None | Standard | Anti-rat |
| MYC | Abcam | ab32072 | 1 in 500 | HIER | Standard | Anti-rabbit |
| Ki67 | Dako | M7249 | 1 in 100 | HIER | Standard | Anti-rat |
| Anti-human antibodies | | | | | |
| PHGDH | MilliporeSigma Prestige | HPA021241 | 1 in 1000 | HIER | Standard | Anti-rabbit |
| PSAT1 | Thermo Fisher Scientific | PA5-22224 | 1 in 100 | HIER | Standard | Anti-rabbit |
| CD20 | Dako | M0755 | 1 in 2000 | HIER | Standard | Anti-mouse |
| IgD | Novocastra | NCL-L-IgD | 1 in 1500 | HIER | Standard | Anti-mouse |
respectively; and observe full scan range at 70–1,050 m/z acetonitrile. MS was performed with positive/negative polarity switch-solution of ammonium hydroxide at 35% in water, and solvent B was ammonium carbonate solution in water supplemented by 4 mL/L of aning the column at 80% of B during 4 minutes. Solvent A was 20 mM minutes, holding at 5% of B during 3 minutes, and finally reequilibrat-total run time of 25 minutes. The elution gradient was programmed using a gradient program at a constant flow rate of 300 μm particle size, polymeric, 150 × 4.6 mm) chromatographic separation was performed on a SeQuant Zic pHILIC coupled with a Vanquish UHPLC system (Thermo Fisher Scientific). TheTriplicates of identically seeded and treated cells were analyzed. as an internal extraction standard) were added to each dried sample,pretreated with 10 M PH-755 (Raze Therapeutics) diluted in DMSOtransferring to dry ice. For PHGDH inhibition experiments, cells were were then washed with PBS before being metabolically quenched by transferring to dry ice. For PHGDH inhibition experiments, cells were pretreated with 10 μM PH-755 (Raze Therapeutics) diluted in DMSO or DMSO alone for 1 hour before labeling with 13C-U-glucose. Metabolites were extracted by resuspending the cell pellet in ice-cold HPLC-grade methanol, acetonitrile, and H₂O at a volume ratio 50:30:20 for 1 hour at 4°C with 3 sonication steps (8 minutes each step) within the hour. After sonication, samples were centrifuged for 10 minutes at 21,000g (4°C), and the supernatant was collected. The extraction solvent was dried in a glass insert placed in a LC-MS vial (Agilent, 5182-0716) using a SpeedVac (Christ RVC 2-33 CDplus). Then, 20 μL of metabolite extraction buffer containing 5 μM 13C,3N-Valine (used as an internal extraction standard) were added to each dried sample, and metabolite samples were stored at ~80°C for subsequent analyses. Triplicates of identically seeded and treated cells were analyzed. Metabolite analysis was performed by LC-MS using a Q-Exactive Plus (Orbitrap) mass spectrometer (Thermo Fisher Scientific) coupled with a V Vanquish UHPLC system (Thermo Fisher Scientific). The chromatographic separation was performed on a SeQuant Zic pHILIC (Merck Millipore) column (5 μm particle size, polymeric, 150 × 4.6 mm) using a gradient program at a constant flow rate of 300 μL/min over a total run time of 25 minutes. The elution gradient was programmed as a decreasing percentage of solvent B from 80% to 5% during 17 minutes, holding at 5% of B during 3 minutes, and finally reequilibrat-ing the column at 80% of B during 4 minutes. Solvent A was 20 mM ammonium carbonate solution in water supplemented by 4 mL/L of a solution of ammonium hydroxide at 35% in water, and solvent B was acetonitrile. MS was performed with positive/negative polarity switch-ing using a Q-Exactive Orbitrap (Thermo Fisher Scientific) with a HESI II probe. MS parameters were as follows: spray voltage 3.5 and 3.2 kV for positive and negative modes, respectively; probe temperature 320°C; observe sheath and auxiliary gases at 30 and 5 arbitrary units, respectively; and observe full scan range at 70-1,050 m/z, with set-tings of AGC target and resolution as balanced and high (3 × 10⁴ and 70,000), respectively. Data were recorded using Xcalibur 4.2.47 soft-ware (Thermo Fisher Scientific). Mass calibration was performed for both ESI polarities before analysis using the standard Thermo Fisher Scientific Calmix solution. To enhance calibration stability, lock-mass correction was also applied to each analytical run using ubiquitous low-mass contaminants. Parallel reaction monitoring (PRM) acquisi-tion parameters were as follows: resolution was 17,500, and collision energies were set individually in high-energy collisional dissociation (HCD) mode. Metabolites were identified and quantified by accurate mass and retention time and by comparison with the retention times, mass spectra, and responses of known amounts of authentic standards using TraceFinder 4.1 EFS software (Thermo Fisher Scientific). Label incorporation and abundance was estimated using TraceFinder 4.1 EFS software. The level of labeling of individual metabolites was esti-mated as the percentage of the metabolite pool containing 1 or more 13C atoms after correction for natural abundance isotopes. Abundance was given relative to the internal standard.

Metabolite extraction and LC-MS
Cells were seeded at 5 × 10⁶ per well of serine/glycine-free complete media for 1 hour. After 1 hour, cells were transferred to serine/gly-cine-free media containing 13C-U-glucose (2 g/L) (Cambridge Iso-tope Laboratories, CLM-1396-PK) for an additional 2 hours. Cells were then washed with PBS before being metabolically quenched by transferring to dry ice. For PHGDH inhibition experiments, cells were pretreated with 10 μM PH-755 (Raze Therapeutics) diluted in DMSO or DMSO alone for 1 hour before labeling with 13C-U-glucose. Metabolites were extracted by resuspending the cell pellet in ice-cold HPLC-grade methanol, acetonitrile, and H₂O at a volume ratio 50:30:20 for 1 hour at 4°C with 3 sonication steps (8 minutes each step) within the hour. After sonication, samples were centrifuged for 10 minutes at 21,000g (4°C), and the supernatant was collected. The extraction solvent was dried in a glass insert placed in a LC-MS vial (Agilent, 5182-0716) using a SpeedVac (Christ RVC 2-33 CDplus). Then, 20 μL of metabolite extraction buffer containing 5 μM 13C,3N-Valine (used as an internal extraction standard) were added to each dried sample, and metabolite samples were stored at ~80°C for subsequent analyses. Triplicates of identically seeded and treated cells were analyzed. Metabolite analysis was performed by LC-MS using a Q-Exactive Plus (Orbitrap) mass spectrometer (Thermo Fisher Scientific) coupled with a V Vanquish UHPLC system (Thermo Fisher Scientific). The chromatographic separation was performed on a SeQuant Zic pHILIC (Merck Millipore) column (5 μm particle size, polymeric, 150 × 4.6 mm) using a gradient program at a constant flow rate of 300 μL/min over a total run time of 25 minutes. The elution gradient was programmed as a decreasing percentage of solvent B from 80% to 5% during 17 minutes, holding at 5% of B during 3 minutes, and finally reequilibrat-ing the column at 80% of B during 4 minutes. Solvent A was 20 mM ammonium carbonate solution in water supplemented by 4 mL/L of a solution of ammonium hydroxide at 35% in water, and solvent B was acetonitrile. MS was performed with positive/negative polarity switch-ing using a Q-Exactive Orbitrap (Thermo Fisher Scientific) with a HESI II probe. MS parameters were as follows: spray voltage 3.5 and 3.2 kV for positive and negative modes, respectively; probe temperature 320°C; observe sheath and auxiliary gases at 30 and 5 arbitrary units, respectively; and observe full scan range at 70-1,050 m/z, with set-tings of AGC target and resolution as balanced and high (3 × 10⁴ and 70,000), respectively. Data were recorded using Xcalibur 4.2.47 soft-ware (Thermo Fisher Scientific). Mass calibration was performed for both ESI polarities before analysis using the standard Thermo Fisher Scientific Calmix solution. To enhance calibration stability, lock-mass correction was also applied to each analytical run using ubiquitous low-mass contaminants. Parallel reaction monitoring (PRM) acquisi-tion parameters were as follows: resolution was 17,500, and collision energies were set individually in high-energy collisional dissociation (HCD) mode. Metabolites were identified and quantified by accurate mass and retention time and by comparison with the retention times, mass spectra, and responses of known amounts of authentic standards using TraceFinder 4.1 EFS software (Thermo Fisher Scientific). Label incorporation and abundance was estimated using TraceFinder 4.1 EFS software. The level of labeling of individual metabolites was esti-mated as the percentage of the metabolite pool containing 1 or more 13C atoms after correction for natural abundance isotopes. Abundance was given relative to the internal standard.

Single-cell RNA-Seq analysis of human tonsillar B cell subsets
B cell populations from previously published tonsillar immune single-cell RNA-Seq (37) were identified by using unbiased clustering of gene expression and analysis of their antibody repertoires (i.e., isotype fre-quences, somatic hypermutation levels, and clonal expansion) using Seurat (v3) (56). Gene expression markers used to define the groups presented here include the following: naïve (TXNIP, FCER2, FCMR), activated (EGRI, CD69, JUN), pre-GC (MIR155HG, BHLHE40, PSME2, CCND2), memory (TNFRSF13B, CD44, VIM, FCRL4), GC (BCL6, CD38, EZR, SERPINA9, LMO2), cycling GC (AURKB, MKI67, UBE2C), and plasmablast (XBPI, PRDM1, MZB1). Gene expression counts were imputed for visualization using MAGIC (57).

Statistics
Statistical significance was assessed Prism 9.1.1 software (GraphPad Software). Groups were compared using a 2-tailed unpaired t test, Mann-Whitney U test, or 1-way ANOVA with Tukey’s post hoc test, depending on the number of groups and distribution. For survival comparison, a log-rank test was used. Data are shown as the mean ± SEM. A P value of less than or equal to 0.05 was considered to indicate statistical significance. When analyzing variables with more than 2 categories, P values were adjusted for multiple comparisons.

Study approval
All animal studies were conducted in compliance with UK Home Office approved licences (Animals [Scientific Procedures] Act 1986 and the EU Directive 2010). Animal experiments were subject to ethical review by the Francis Crick Animal Welfare and Ethical Review Body and carried out under UK Home Office project licence P319AE968. Lymphoma samples were obtained from Barts Cancer Institute tissue bank (London, United Kingdom). Ethical approval was confirmed by the East London & The City Health Authority Local Research Ethics Committee (no. 10/H0704/65), and written informed consent was also obtained in accordance with the Declaration of Helsinki.

Author contributions
AD designed and performed the experiments, analyzed and interpreted data, and wrote the manuscript; MT and ECC designed experiments and analyzed and interpreted data; NL and JIM designed and performed the metabolic experiments, as well as analyzed and interpreted the LC-MS data; RLS, KK, and AJC designed, performed, and analyzed the IHC experiments; HWK and LKJ analyzed and interpreted the single-cell RNA-Seq data.
sets; PC analyzed and interpreted the statistical data; ASG and LZ designed the flow cytometry and ELISA experiments, and they analyzed and interpreted data; JGG and DPC designed experiments and edited the manuscript; KHV designed experiments, analyzed and interpreted the data, edited the manuscript, and supervised the study; and JCR designed and performed the experiments, analyzed and interpreted the data, wrote and edited the manuscript, and supervised the study. All authors approved the final submission.

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