Histone demethylase LSD1 is required for germinal center formation and BCL6-driven lymphomagenesis

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Germinal center (GC) B cells feature repression of many gene enhancers to establish their characteristic transcriptome. Here we show that conditional deletion of Lsd1 in GCs significantly impaired GC formation, associated with failure to repress immune synapse genes linked to GC exit, which are also direct targets of the transcriptional repressor BCL6. We found that BCL6 directly binds LSD1 and recruits it primarily to intergenic and intronic enhancers. Conditional deletion of Lsd1 suppressed GC hyperplasia caused by constitutive expression of BCL6 and significantly delayed BCL6-driven lymphomagenesis. Administration of catalytic inhibitors of LSD1 had little effect on GC formation or GC-derived lymphoma cells. Using a CRISPR-Cas9 domain screen, we found instead that the Lsd1 Tower domain was critical for dependence on LSD1 in GC-derived B cells. These results indicate an essential role for LSD1 in the humoral immune response, where it modulates enhancer function by forming repression complexes with BCL6.

Recent studies of gene enhancer chromatin during the GC reaction have unveiled a specific pattern of enhancer erasing and rewriting involving loss of H3K4me1/2 in about 2,800 sites, suggesting that histone demethylases probably contribute to the GC reaction. The first histone demethylase to be discovered, LSD1 (lysine-specific demethylase 1A, encoded by KDM1A in humans and Kdm1a in mice), specifically catalyzes demethylation of H3K4me1/2 (ref. 11). Lsd1 deletion results in developmental arrest and is lethal at early embryonic stages12–14. However, many of its cell-context-specific functions remain unknown. It was recently shown that inducible deletion of Lsd1 in early hematopoietic stem cells perturbs differentiation and terminal blood cell maturation, resulting in pancytopenia15. LSD1 overexpression has been observed in many tumor types, such as bladder, colorectal, breast and small cell lung cancer, and high LSD1 expression may function as biomarker for disease aggressiveness. In acute myeloid leukemia, LSD1 was shown to maintain leukemic stem cells, and LSD1 inhibition was shown to promote differentiation16–18. Here we explore the role of LSD1 in GC formation and the humoral immune response.

Results
LSD1 is required for the humoral immune response. To identify histone demethylases that might repress enhancers in GC B cells, we first mined RNA-sequencing (RNA-seq) data profiles to determine expression of the two known families of H3K4 demethylases,

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KDM1 and KDM5, in naïve B (NB) cells versus GC B cells in humans and mice. LSD1 was the most consistently upregulated from NB cells to GC B cells (Fig. 1a and Supplementary Fig. 1a). We confirmed this result using qPCR in purified human NB versus GC B cells, where we observed two-fold LSD1 induction, along with the expected upregulation of BCL6 and AICDA (Fig. 1b and Supplementary Fig. 1b), and LSD1 immunobLOTS showed a similar degree of upregulation (Fig. 1c). Immunohistochemistry (IHC) of tonsil sections showed higher LSD1 expression in GCs, especially in the proliferative dark zone (Supplementary Fig. 1c). LSD1 expression was maintained in post–GC B cells such as plasmaocytes and memory B cells (Supplementary Fig. 1d).

We next asked whether LSD1 is required for the development of GCs. We crossed conditional Lsd1fl/fl mice with the Cγ1-Cre strain, which expresses Cre recombinase in GC B cells. Next we immunized Lsd1fl/fl, Cγ1-Cre Lsd1fl/fl or Cγ1-Cre Lsd1fl/fl conditional mice with T cell–dependent antigen (sheep red blood cells (SRBCs)) to induce GC formation and analyzed the composition of their spleens 10 d later. Conditional homozygous and heterozygous Lsd1 deletion in GCs resulted in a marked and gene dose–dependent reduction in GCs, as shown by IHC using peanut agglutinin (PNA) (Fig. 1d,e), without any perturbation of B cell follicular structure, as shown by B220 IHC (Supplementary Fig. 1e). The numbers of splenic GCs formed were markedly reduced in Lsd1-deficient mice (Supplementary Fig. 1f). Concordant with the reduction in GC B cells, we observed a dose-dependent reduction in Ki67 (Fig. 1d,e) and bromodeoxyuridine-positive (BrDU+) cells after injection of BrDU 4 h prior to euthanasia at day 10 post antigen challenge (Fig. 1d,e).

Using flow cytometry staining to quantify relative abundance of GC B cells (Fas+GL7+ B220+ and FoxCD38B220+), we observed dose-dependent and significant loss of GC B cells in Lsd1-deficient mice (Fig. 1f,g). Lsd1 locus amplification in sorted GC B cells from Lsd1fl/fl or Cγ1-Cre Lsd1fl/fl mice confirmed loss of LSD1 in these residual cells (Supplementary Fig. 1g) and prompted us to monitor the GC reaction in Lsd1fl/fl versus Cγ1-Cre Lsd1fl/fl mice every 2 d, starting 4 d post antigen challenge. Although control Lsd1fl/fl animals showed robust GC formation at days 6, 8 and 10, Cγ1-Cre Lsd1fl/fl mice showed a severe loss in their ability to mount a full-blown GC response, and most of the incipient GC B cells were cleared within 10 d post antigen challenge (Supplementary Fig. 1h,i). Thus, LSD1 is required to support the expansion of newly forming GCs.

To assess the impact of Lsd1 loss of function on immunoglobulin affinity maturation, we challenged Lsd1fl/fl and Cγ1-Cre Lsd1fl/fl mice with NP12-cGGG, followed by a boost immunization at day 21. Measurement of high-affinity serum antibodies (binding NP-, BSA) or low-affinity serum antibodies (binding NP-, BSA) by ELISA revealed significantly lower titers of high-affinity IgG1 (P = 0.0007) in conditional Lsd1-deficient mice (Supplementary Fig. 1j). The ratio of high-affinity to low-affinity IgG1, IgG2b and IgG3 circulating antibody titers was also reduced (Supplementary Fig. 1k). Additionally, long-lived plasma cells secreting class-switched anti-NP immunoglobulins were markedly reduced in Cγ1-Cre Lsd1fl/fl mice versus Lsd1fl/fl mice at 40 d after their primary immunization, as shown using ELISPOT (Supplementary Fig. 1l).

These results raised the possibility that Lsd1 deletion earlier in B cell development might force premature differentiation into plasma cells or extracellular plasmablasts. To investigate this possibility, we crossed conditional Lsd1fl/fl mice with Cd19-Cre mice expressing Cre recombinase in all B lymphocytes. These animals also manifested significantly reduced GC formation (Supplementary Fig. 2a,b) and significant depletion of GC B cells (Supplementary Fig. 2c–e). However, we did not observe spontaneous differentiation of B cells into downstream lineages such as plasmaocytes (CD138B220+) or plasmablasts (CD138+, κ+, B220+) in the absence of Lsd1 (Supplementary Fig. 2f,g). Taken together, these results indicate that LSD1 plays an important role in GC formation and immunoglobulin affinity maturation.

**LSD1 is a key mediator of the GC transcriptional program.** To identify the LSD1-regulated transcriptome in GC B cells, we performed RNA-seq analysis of sorted GC B cells from three independent Lsd1fl/fl or Cγ1-Cre Lsd1fl/fl mice. We observed that 120 genes were significantly differentially expressed in Lsd1-deficient GC B cells (false discovery rate (FDR) < 0.05, fold change > 1.5), the majority of which were upregulated (n = 87, Fig. 2a), including canonical plasma and memory cell differentiation genes such as Irf4 and Prdm1, as well as Ccl80, which is important for B cell induction of follicular helper T cells; Cxcr3, which plays a critical role in plasma cell differentiation; and Stat4, which induces plasma cell differentiation in GC B cells downstream of IL-12 (ref. 13). Lsd1 loss of function also yielded upregulation of Gpr132, a gene that suppresses the proliferation of B cells14.

ATAC-seq in sorted viable GC B cells from Cγ1-Cre Lsd1fl/fl or Lsd1fl/fl mice revealed 733 loci that gained accessibility and 314 with reduced accessibility (FDR < 0.05, Fig. 2b,c). Loci that gained chromatin accessibility were mostly localized in intergenic and intronic regions (77%, Fig. 2d). Examples of genes linked to loci that gained accessibility included Prdm1 (FDR = 0.032), Ccl83, which encodes a marker induced in memory B cells (intronic, FDR = 0.00045) and Stat4 (intronic, FDR = 0.02) (Fig. 2e). Notably, gene transcripts linked to a gain in chromatin accessibility in Lsd1-deficient cells were significantly upregulated (Fig. 2f), whereas genes associated with loci with unaltered or reduced chromatin accessibility did not manifest significant differential expression. Along these lines, we observed significant enrichment for genes with increased chromatin accessibility (n = 610 genes) among those genes induced in Lsd1-deficient GC B cells (Fig. 2g).

Pathway analysis using curated gene sets linked to normal and malignant B cell functions revealed significant enrichment for genes linked to silenced or poised enhancers in the transition from NB cells to GC B cells (Fig. 2h), as well as genes involved in the terminal differentiation of GC B cells into plasma cells and memory B cells (Fig. 2h). Also enriched were direct target genes of CTCF and EP300 and, most notably, genes directly bound and repressed by BCL6. In an orthogonal analysis, we observed significant enrichment for genes repressed by BCL6 through promoter or enhancer binding among genes that are upregulated upon Lsd1 deletion in GC B cells14 (Fig. 2j). BCL6 contains two repressor domains, and Lsd1-deficient GC B cells showed upregulation of genes repressed by BCL6, specifically through its RD2 repression domain15. Taken together, both the phenotypic data and transcriptome data point to Lsd1 as being functionally linked to the actions of BCL6, a factor required for GC formation due in part to its blockade of plasma cell differentiation and supporting proliferation.

**BCL6 mediates Lsd1 recruitment to its direct target genes.** The data presented above suggest a functional connection between BCL6 and LSD1. To explore this possibility, we first tested whether BCL6 and LSD1 interact. We performed coimmunoprecipitation in diffuse large B cell lymphoma (DLBCL) cells (OCI-Ly1, SUDHL4 and HBL1) and showed that antibodies to LSD1 could precipitate BCL6 and, reciprocally, that antibodies to BCL6 enriched for LSD1 (Fig. 3a). We were also able to coimmunoprecipitate LSD1 and BCL6 in lysates from human tonsil–derived GC B cells (Fig. 3b). Additionally, we performed in vitro histone demethylation assays using LSD1 or BCL6 immunoprecipitates and bulk core histones that bear the various post-transcriptional modifications as substrates. As expected, precipitated LSD1 demethylated H3K4me1 compared to actin control, but BCL6 precipitates also demethylated H3K4me1 in an LSD1-dependent manner, because a specific LSD1 inhibitor blocked BCL6-mediated histone demethylation (Fig. 3c).
and Supplementary Fig. 3a). To determine whether the BCL6–LSD1 interaction is direct, we performed protein affinity chromatography with bacterially expressed GST-LSD1 and in vitro–synthesized BCL6. BCL6 was enriched by GST-LSD1 beads even with increasing concentrations of NaCl up to 350 mM, thus indicating direct and robust interaction between these proteins (Fig. 3d and Supplementary Fig. 3b).

To determine whether LSD1 is associated with BCL6 on chromatin, we characterized the genomic localization of LSD1 and its relationship to the BCL6 cistrome in GC-derived B cells...
was depleted, suggesting that BCL6 recruits LSD1 to these sites in Prdm1, Cd38, Lsd1, and -deficient cells compared to wild-type controls surrounding these regions.

Genes were ranked based on decreasing log2 fold gene expression changes comparing Lsd1-deficient cells to wild-type GC B cells, based on RefSeq. Values were calculated based on Benjamini-Hochberg procedure (BH).

Fig. 2 | LSD1 deletion alters the GC B cell transcription program. a, RNA-seq analysis of sorted GC B cells from biologically independent immunized conditional Lsd1fl/fl (n = 3) versus C1-Cre Lsd1fl/fl (n = 3) mice. Heat map represents row-based z scores of DESeq2-normalized read counts for differentially expressed genes (FDR < 0.05, fold change >1.5). b, Heat map of differentially accessible ATAC-seq peaks (FDR < 0.05) from sorted GC B cells from biologically independent immunized Lsd1fl/fl (n = 2) versus C1-Cre Lsd1fl/fl (n = 3) mice. Heat map represents row-based z scores of DESeq2-normalized Tn5 insertion counts for each differentially accessible ATAC-seq peak. c, Mean normalized Tn5 insertion signal and heat map of fragment density for loci that gained accessibility upon LSD1 deletion in GC B cells (RNA). Fisher’s exact test was used to calculate enrichment score (NES) and FDR values as implemented by GSEA; based on 10,000 sample permutations.

Using ChIP-seq, we identified 6,618 robust LSD1 peaks across replicate experiments (fold change = 5, P < 10^-10). Notably, a majority of LSD1 peaks (4,300/6,618, 67%) were lost when BCL6 was depleted, suggesting that BCL6 recruits LSD1 to these sites (Fig. 3e). 61% of these BCL6-dependent LSD1 peaks were localized to intronic and intergenic regions, whereas 35% localized to promoters (Fig. 3e). Further analysis revealed a significant reduction in BCL6 and LSD1 read counts at these sites after BCL6 knockdown via small interfering RNA (siRNA) (Fig. 3f,g). H3K4me1 ChIP-seq in BCL6-knockdown cells or controls showed that gain of this mark was more pronounced at intergenic and intronic BCL6-LSD1 peaks versus promoters (Fig. 3g), whereas neighboring regions (~50kb) were largely unaltered (Supplementary Fig. 3c). We confirmed that genomic loci occupied by BCL6 and LSD1 gained H3K4me1 upon

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BCL6 knockdown in an additional DLBCL cell line (SUDHL4, Supplementary Fig. 3d), and we showed that BCL6–LSD1 target sites also gain H3K4me2, another histone mark demethylated by LSD1 (Supplementary Fig. 3e).

We next tested whether genes directly bound by LSD1–BCL6 complexes were induced in Cty1-Cre Lsd1fl/fl GC B cells. Indeed, genes with intronic or intergenic LSD1–BCL6 complexes were significantly enriched among those upregulated in Cty1-Cre Lsd1fl/fl mice (Fig. 3h), as exemplified by Gfi1 and Gpr132 (Fig. 3i). BCL6–LSD1 targets were significantly enriched for genes linked to enhancers that are poised or lost from NB cells to GC B cells, genes with intronic and intergenic enhancers repressed by BCL6 in primary GC B cells and genes with enhancers activated by Kmt2d (Fig. 3j). Genes involved in plasma cell differentiation and GC exit such as B cell receptor signaling, signal transduction, MAPK signaling, cell cycle checkpoints and programmed cell death were also enriched (Fig. 3j). These results suggest that LSD1 is a BCL6 corepressor, directly recruited by BCL6 to repress enhancers for genes involved in GC exit, terminal differentiation and proliferation.

**LSD1 regulates GC B cell differentiation and BCL6 targets.** To more thoroughly characterize the regulated transcriptome of LSD1, we next performed RNA-seq on human GC-derived lymphoma cells transduced with two LSD1 short hairpin RNAs (shRNAs) (Fig. 4a) or a control shRNA. We found that most differentially regulated genes (fold change > 1.5, FDR < 0.05) were upregulated (n = 472, 69%) by both LSD1 hairpins, and 213 genes (31%) were significantly downregulated (Fig. 4b,c). Consistent with our murine GC B cell Cty1-Cre Lsd1fl/fl RNA-seq studies, significant enrichment for the plasma cell and memory B cell differentiation signature and BCL6 target genes was observed, including the RD2 domain-controlled transcriptome (Fig. 4d). We also observed enrichment for genes controlled by Kmt2d through enhancer and promoter H3K4 methylation and the histone acetyltransferase CREBBP, both of which counteract BCL6 as B cells exit the GC reaction by activating enhancers required for terminal differentiation into plasma cells (Fig. 4d).

We performed gene set enrichment analysis (GSEA) using the plasma/memory cell differentiation signature and the Cty1-Cre Lsd1fl/fl GC B cell–induced genes and observed enrichment among genes induced by LSD1 shRNA (Fig. 4e). Confirming the significance of its interaction with BCL6, LSD1 shRNA–induced genes were significantly enriched in BCL6-repressed genes, as were chromatin immunoprecipitation (ChIP)-seq–defined intergenic and intronic LSD1–BCL6 target enhancers (Fig. 4d,e). We validated the upregulation of the critical LSD1 target and GC exit gene Irf4 using qPCR (Fig. 4f). These data indicate similar functions for LSD1 in murine and human GCs and GC-derived lymphoma cells, linked to BCL6, cell proliferation and GC B cell differentiation pathways.

**Noncanonical LSD1 effect in GC B cells and lymphoma.** To understand the LSD1 mechanism of action in GC B cells, we next explored whether its function is mediated through its histone-demethylase activity. We first examined the effect on GC formation of a specific and irreversible LSD1 enzymatic inhibitor (GSK-LSD1; half-maximum effective concentration (EC50) < 10 nM in growth assays using acute myeloid leukemia cell lines) (22). To this end, we immunized mice with SRBCs and then initiated intraperitoneal injections of GSK-LSD1 inhibitor (0.5 mg/kg) at the highest dose recommended based on limiting thrombocytopenia or vehicle daily for 10 d (Fig. 5a). In contrast to the striking loss of GCs induced by Lsd1 conditional deletion, the effect of the LSD1 inhibitor on GC B cell populations by flow cytometry and PNA staining was modest, albeit still significant, although LSD1 treatment did not affect splenic GC counts (Fig. 5a,b). Unlike this moderate effect on GC B cell populations, LSD1 inhibition in vivo resulted in a significant reduction in Gr1hiCd11b– bone marrow–resident mature granulocytes, consistent with its known role in their differentiation (Supplementary Fig. 4a,b). LSD1 enzymatic inhibition of a panel of GC-derived lymphoma cell lines had very little effect, even in doses up to 10 μM, although it was highly effective at these doses against leukemia cells, which are known to be sensitive to LSD1 inhibitors (22) (Supplementary Fig. 4c). LSD1 inhibition induced H3K4me2 at LSD1 targets without inducing expression of these targets, in contrast to LSD1 knockdown, which does induce gene expression (Supplementary Fig. 4e). However, we confirmed that LSD1 is required to maintain the proliferation of GC-derived lymphoma cells by performing both viability and cell cycle analysis on lymphoma cell lines transduced with LSD1 shRNA (Supplementary Fig. 4f,g).

These data suggest that LSD1’s mechanism of action in GC B cells involves additional biochemical functions. To address this, we employed a CRISPR-Cas9–based structure–function approach to identify the LSD1 domain(s) that drive(s) the proliferation of GC-derived B cells. CRISPR-Cas9 genome editing frequently generates in-frame mutations instead of insertions or deletions, so that single guide RNAs (sgRNAs) targeting functional domains that sustain cell proliferation and survival are more deleterious to cells than are sgRNAs targeted to nonessential domains (23). We designed 30 green fluorescent protein (GFP)–expressing sgRNA vectors spanning the different LSD1 protein domains: the N-terminal SWIRM domain (amino acids (aa) 172–271), the C-terminal amino oxidase domain (aa 272–330), the RD2 domain–linked GC exit, terminal differentiation and proliferation.

**Fig 3 | LSD1 interacts with BCL6 in lymphoma cells.** a, Reciprocal chromatin immunoprecipitation of BCL6 and LSD1 (cropped image) from lymphoma cell extracts of OCI-Ly1, SUDHL4 and HBL1 cells. IgG antibody served as a negative control. Experiments were performed at least three times with OCI-Ly1, twice with SUDHL4 and once with HBL1 cells. b, Coimmunoprecipitation of BCL6 and LSD1 in tonsil GC B cell extracts (cropped image). IgG antibody served as a negative control. Experiment was performed twice with similar results. c, H3 and H3K4me1 immunoblot of histone-demethylation reactions (cropped image) with LSD1 and BCL6 immunoprecipitated (IP) from lymphoma B cell extracts and bulk core histone substitutes treated with 1 μg GS-K-LSD1 inhibitor or vehicle. Experiment was performed twice with similar results. d, GST pull-down assay using GST-LSD1 or GST alone as control. Purified proteins were incubated with radiolabeled BCL6 and washed with increasing NaCl concentrations. Radioactive signal indicating interaction was captured by fluorography (cropped image). Experiment was performed at least twice with similar results. e, Breakdown of LSD1 significantly depleted peaks after BCL6 knockdown or LSD1 peaks unaltered after BCL6 depletion. Pie chart indicates the genome-wide distribution of BCL6-dependent LSD1 peaks based on RefSeq. f, Fold enrichment of BCL6 and LSD1 ChIP-seq reads normalized to corresponding input in lymphoma cells treated with BCL6 siRNA or non-targeted control siRNA (P values were calculated by one-sided Kolmogorov-Smirnov test). Top panels show LSD1 peaks that occur in promoter regions (n = 2,557), and bottom panels show LSD1 peaks that are in intronic or intergenic regions (n = 3,503). g, Heat maps showing LSD1, BCL6 and H3K4me1 ChIP-seq peak density surrounding LSD1 peaks in promoters and intergenic/intronic regions shown in a with BCL6 siRNA or non-targeted control siRNA. h, GSEA based on genes ranked by decreasing log, fold gene expression changes of LSD1-deficient cells versus wild-type controls using gene sets linked to BCL6–LSD1 complexes through intronic or intergenic binding (NES and FDR as implemented by GSEA; based on 10,000 sample permutations). i, Examples of LSD1 and BCL6 ChIP-seq tracks surrounding the Gfi1 and Gpr132 promoter with or without siBCL6 treatment. Experiment was validated more than three times by qChIP. j, Pathway analysis of genes linked to BCL6-dependent LSD1 peaks. P values based on two-sided Fisher’s exact test for each gene set; FDR was calculated based on BH.
domain (AOD)—histone demethylase domain (amine oxidase, aa 271–852) and the central protruding ‘Tower’ domain26,27 (aa 417–522) (Fig. 5c). The LSD1 SWIRM domain, which is often found in chromatin-modifying and chromatin-remodeling complexes, forms interactions with the AOD but lacks DNA-binding properties26,27. The FAD-binding pocket and LSD1 catalytic center are located within the AOD, which accommodates the H3 tail as substrate for demethylation26–28. Finally, the Tower domain is a coiled-coil domain that mediates interactions with CoREST26,27. We initially generated two different OCI-Ly1 subclones that induce Cas9 expression upon doxycycline treatment. The effect of each sgRNA on cell survival was assessed by measurement of the proportion of GFP+ cells after doxycycline treatment independently for each of the two Cas9-expressing subclones (Fig. 5c and Supplementary Fig. 4h).

**Figure 5c**

- **a** Cell extract samples were subjected to IP with LSD1 or BCL6 antibodies. The IP and input samples were analyzed by western blotting with indicated antibodies.
- **b** Western blot analysis of LSD1 and BCL6 expression in OCI-Ly1 cells. The sample was subjected to IP with LSD1 or BCL6 antibodies.
- **c** Western blot analysis of LSD1 and BCL6 expression in OCI-Ly1 cells. The sample was subjected to IP with LSD1 or BCL6 antibodies.
- **d** GST beads were used for pull-down experiments. The IP was performed with LSD1 or BCL6 antibodies, and the IP and input samples were analyzed by western blotting with indicated antibodies.
- **e** Flow cytometry analysis of GFP+ cell proportion in OCI-Ly1 cells after doxycycline treatment.
- **f** Bar graph showing the fold enrichment of LSD1–LSD1 targets in intergenic/intronic regions.
- **g** Bar graph showing the fold enrichment of LSD1–BCL6 targets in intergenic/intronic regions.
- **h** Bar graph showing the fold enrichment of LSD1–BCL6 targets in intergenic/intronic regions.
- **i** Bar graph showing the fold enrichment of LSD1–BCL6 targets in intergenic/intronic regions.
- **j** Bar graph showing the fold enrichment of LSD1–BCL6 targets in intergenic/intronic regions.
For a positive control, we used sgRNAs targeting BCL6, which is essential for lymphoma growth,29,30. We confirmed CRISPR-Cas9-mediated genetic engineering of the LSD1 locus for our sgRNAs by T7-endonuclease digestion (Supplementary Fig. 4i). The most consistently deleterious sgRNAs across clones disrupted the LSD1 Tower domain, which mediates protein–protein interactions.26,27 (Fig. 5c and Supplementary Fig. 4h). Two sgRNAs targeting part of the AOD near its FAD-binding site also dropped out. Taken together, these results suggest that LSD1 GC B cell functions require both the Tower domain and the AOD, and that blocking the enzymatic activity of LSD1 alone is insufficient to fully elicit the LSD1 loss-of-function effect.

To validate this finding, we performed rescue experiments in two lymphoma cell lines (OCI-Ly1 and TMD8) expressing two different LSD1 shRNAs or control shRNA as well as FLAG-tagged LSD1 rescue constructs: wild-type LSD1 (LSD1$^{wt}$), AOD catalytically inactive LSD1 (LSD1$^{K661A}$),27, LSD1 lacking the Tower domain (LSD1ΔTower)27 and catalytically inactive LSD1 lacking the Tower domain (LSD1ΔK661AΔTower). Rescue protein transduction was validated via immunoblot (Fig. 5d). We found that similar to LSD1 wt, catalytically inactive mutant LSD1ΔK661A fully rescued the effect of LSD1 depletion on cell growth (Fig. 5e). However, LSD1 lacking the Tower domain (LSD1ΔTower) only partially rescued lymphoma cells, consistent with the CRISPR domain screen (Fig. 5c). Finally,
Fig. 5 | LSD1 lymphoma prosurvival effect is mediated through non-catalytic functions. a, Flow cytometry of GC B cell splenocytes, calculated as percent Fas+GL7+ cells among live B cells (B220+DAPI−) from biologically independent immunized mice (n=9 mice per group; mean±s.d.) treated daily with GSK-LSD1 (0.5 mg/kg) or PBS vehicle and euthanized at 10 d. P values were calculated using a two-sided unpaired t-test; *P<0.05). Data are representative of three independent experiments. b, Quantification of PNA-stained spleen sections from GSK-LSD1-treated mice versus vehicle-treated mice. Left, fraction of PNA stained area versus the total spleen section area. Middle, GC counts per spleen section. Right, representative IHC images of mouse spleen sections stained with PNA or Ki67 at two magnifications (vehicle versus GSK-LSD1 treated). Each point represents an individual mouse (data are pooled from two independent experiments; n=18 mice per group; mean±s.d.; P values were calculated using two-sided unpaired t-test; scale bars, 0.5 mm and 50 μm on the first and second columns from the left, respectively). c, Fold depletion of % GFP+ cells (mean±s.d.) bearing sgRNAs targeting different parts of the LSD1 open reading frame at day 40 post-doxycycline induction versus day 0. LSD1 protein structure with color-coded protein domains (N terminus, grey; SWIRM, blue; Tower, yellow; AOD, green) (PyMOL). Experiment was performed in n=4 technical replicates and was further replicated using an independent Cas9-expressing OCI-Ly1 clone (Supplementary Fig. 4h). d, Graphical representation of knockdown and rescue FLAG constructs and cropped immunoblot analysis of extracts of lymphoma cells transfected with FLAG-tagged LSD1 rescue constructs: wild-type LSD1 (LSD1wt), catalytically inactive LSD1 (LSD1K661A), LSD1 lacking the Tower domain (LSD1ΔTower) and catalytically inactive LSD1 lacking the Tower domain (LSD1ΔTower), all probed with anti-FLAG. Cells transfected with a Cas9-FLAG construct were used as controls. GAPDH served as protein loading control. Asterisk indicates each FLAG protein. Experiment representative of two blots. e, Viability cell counts (mean±s.d.) of two lymphoma cell lines (OCI-Ly1 and TMD8 cells in triplicate wells) transfected by control shRNA or two LSD1-targeted shRNAs and rescued with various LSD1 mutant constructs (d), normalized to cells rescued with wild-type LSD1.

The double mutant LSD1K661A,ΔTower was almost incapable of rescuing LSD1 loss of function (Fig. 5e). Therefore, LSD1’s actions in GC B cells involve both catalytic functions and structural functions mediated through the Tower domain.

The lymphomagenic effects of BCL6 require LSD1. GC B cells are the cells of origin of most non-Hodgkin lymphomas, including the DLBCLs, in which BCL6 is a central and critical oncoprotein. Given the functional cooperation of LSD1 and BCL6 in GC B cells,
Fig. 6 | LSD1 loss of function abolishes BCL6-driven GC hyperplasia and lymphomagenesis. a, Correlation of BCL6 and LSD1 mRNA levels in two independent DLBCL cohorts from ref. 11 (left) and ref. 7 (right). Heat maps were ranked by increasing BCL6 mRNA abundance. P value is based on Spearman correlation. b, IHC images of representative individual spleen sections from Lsd1−/− mice (n=3), IγBcl6 Cγ1-Cre Lsd1−/− mice (n=11) and IγBcl6 Cγ1-Cre Lsd1−/− mice (n=9) immunized with SRBCs and sacrificed 10 d post-immunization, stained with PNA, Ki67 and BrdU at two magnifications. Scale bars, 1mm and 100 μm, first and second columns from the left, respectively. c, Representative flow cytometry dotplots of mouse GC splenocytes. The gated area shows the percent of GC B cell populations (Fas−CD38−) among live B cells (B220+DAPI−). d, Flow cytometry analysis of mouse GC B cell splenocytes, identified as Fas−GL7− cells, from Lsd1−/− mice (n=15), IγBcl6 Cγ1-Cre Lsd1−/− mice (n=19), IγBcl6 Cγ1-Cre Lsd1−/− mice (n=18), IγBcl6 Cγ1-Cre Lsd1−/− mice (n=17) and IγFas+GL7+ (% B cells) at day 10 post immunization. Data are shown as percent of live B cells (B220+DAPI−) and were pooled from two independent experiments. e, Flow cytometry of mouse GC B cell splenocytes, identified as Fas−CD38− cells, from Lsd1−/− mice (n=8), IγBcl6 Cγ1-Cre Lsd1−/− mice (n=11), IγBcl6 Cγ1-Cre Lsd1−/− mice (n=9) and IγBcl6 Cγ1-Cre Lsd1−/− mice (n=3) at day 10 post immunization. Data are shown as percent of live B cells (B220+DAPI−). Flow cytometry of mouse GC B cells as percent of live B cells (B220+DAPI−), as Fas−CD38−B220+DAPI−. f, Quantification of PNA-stained area from Lsd1−/− mice (n=8), IγBcl6 Cγ1-Cre Lsd1−/− mice (n=11), IγBcl6 Cγ1-Cre Lsd1−/− mice (n=10), IγBcl6 Cγ1-Cre Lsd1−/− mice (n=9) and IγBcl6 Cγ1-Cre Lsd1−/− mice (n=3) mouse spleen sections, versus the total spleen section area. Each sample represents an individual mouse. P values indicate comparison to IγBcl6 Cγ1-Cre Lsd1−/− mice by two-sided unpaired t-test (**P < 0.01, ***P < 0.001, ****P < 0.0001); box plot edges show interquartile range; midlines indicate medians; and whiskers range minimum to maximum values. g, Overall survival of mice transplanted with bone marrow from Cγ1-Cre Lsd1−/− mice (n=20), Cγ1-Cre (n=18), IγBcl6 Cγ1-Cre Lsd1−/− (n=19) or IγBcl6 Cγ1-Cre Lsd1−/− (n=19) animals, assessed by measurement of time of death or euthanasia after bone marrow transplantation (BMT). P value calculated by log-rank test is indicated. h, Quantification of spleen/total body weight ratio (mean ± s.d.) of individual euthanized mice belonging to groups transplanted with bone marrow from Cγ1-Cre Lsd1−/− mice (n=5), Cγ1-Cre mice (n=5), IγBcl6 Cγ1-Cre Lsd1−/− mice (n=13), IγBcl6 Cγ1-Cre Lsd1−/− mice (n=5). P value indicates two-sided unpaired t-test.
we wondered whether LSD1 might also be important to the functions of BCL6 in lymphomagenesis. We first observed that LSD1 and BCL6 are concordantly expressed in primary human patients with DLBCL, based on gene expression profiling of two independent patient cohorts7,31 (Fig. 6a).

To determine whether LSD1 is required for BCL6 to induce lymphomagenesis, we crossed \textit{Cycl1-Cre Lsd1}^{fl/fl} mice with mice carrying the \textit{IgBcl6} allele that mimics BCL6 translocation and causes GC hyperplasia and DLBCL in vivo5. To examine whether \textit{Lsd1} deletion impairs BCL6-driven GC hyperplasia, we immunized \textit{IgBcl6} transgenic mice with conditional deletion of one \textit{Lsd1} allele (\textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/+} or both \textit{Lsd1} alleles (\textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/fl}) and compared them to littermate \textit{IgBcl6} animals (\textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/+}). In parallel, we immunized \textit{Lsd1}^{fl/fl} and GC-conditional \textit{Lsd1}-deficient mice (\textit{Cycl1-Cre Lsd1}^{fl/fl}) as controls. All animals manifested unperturbed splenic architecture and disposition of B cells 10 d after immunization (Supplementary Fig. 5a). As previously demonstrated, \textit{IgBcl6} mice (\textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/+}) manifested GC hyperplasia (Fig. 6b,1) as loss of one or both \textit{Lsd1} allele(s) (\textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/+} or \textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/fl}) yielded significantly reduced GC B cell populations, by flow cytometry and by PNA staining, compared to \textit{IgBcl6} Bcl6 γ mice (\textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/+} or \textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/fl}) (Fig. 6c–e). These enlarged \textit{IgBcl6} GCs contained increased numbers of Ki67+ cells and more in vivo BrdU labeling, consistent with BCL6-sustained proliferation of the GC compartment (Fig. 6b). On the contrary, conditional deletion of \textit{Lsd1} rescued the \textit{IgBcl6}-driven GC hyperproliferative phenotype in a dose-dependent manner (Fig. 6d,1), as loss of one or both \textit{Lsd1} allele(s) (\textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/+} or \textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/fl}) significantly reduced GC B cell populations, by flow cytometry and by PNA staining, compared to \textit{IgBcl6} Bcl6 γ mice (\textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/+} or \textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/fl}) (Fig. 6b–e). Additionally, \textit{IgBcl6} transgenic mice with conditional loss of one or two \textit{Lsd1} alleles formed fewer GCs per spleen section compared to \textit{IgBcl6} transgenic mice (Supplementary Fig. 5b). Unlike \textit{IgBcl6} transgenic mice, GC B cells from \textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/+} or \textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/fl} transgenic mice were not significantly enriched in replicating (BrdU+) cells (Supplementary Fig. 5c).

To determine whether loss of \textit{Lsd1} would also impair BCL6-driven lymphomagenesis, we transplanted bone marrow from \textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/+}, \textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/fl} or \textit{Cycl1-Cre Lsd1}^{fl/+} mice or from \textit{Lsd1}^{fl/+} control mice into lethally irradiated recipient mice. Animals were immunized with SBCs every 3 weeks to ensure continuous formation of GCs and were regularly monitored for survival and lymphomagenesis. \textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/+} mice manifested significantly delayed lethality compared to \textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/+} or \textit{Cycl1-Cre Lsd1}^{fl/+} controls (Fig. 6g). Lymphomagenesis in \textit{IgBcl6} mice is typically associated with massive splenomegaly. Accordingly, most \textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/+} mice featured significantly increased spleen weight-to-body weight ratios compared to \textit{Cycl1-Cre Lsd1}^{fl/+} or \textit{Cycl1-Cre} controls (Fig. 6h). Remarkably, spleens of \textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/+} mice were indistinguishable from those of controls and were significantly reduced compared to those of \textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/+} mice (Fig. 6h).

Pathological analysis of spleens, livers and other organs collected upon euthanasia revealed that most \textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/+} mice had developed lymphoma with complete effacement of lymphoid organs (Fig. 6g and Supplementary Fig. 5d). These contained large, atypical B220+ lymphoid cells with centroblast and immunoblast features. Moreover, \textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/+} mice featured invasive lymphoma infiltrates in their livers within cirrhotic structures and with sinusoid spreading, as well as in lungs and kidneys. Conversely, in \textit{IgBcl6 Cycl1-Cre Lsd1}^{fl/+} mice, lymphoid structures were mainly preserved with minimal effacement and no parenchymal infiltration, with only some large B220+ lymphoid elements seen within GCs and/or ectopically scattered in the lymphoid organs. Taken together, these data suggest that LSD1 is required for BCL6 to drive malignant transformation of GC B cells.

Discussion
Dampening expression of genes induced by T cell help is believed to be important for B cells to undergo immunoglobulin affinity maturation without premature interruption. Herein, we report that LSD1, a FAD-dependent demethylase, is essential for GC formation, and that LSD1 loss causes reactivation of GC-repressed enhancers, increased chromatin accessibility and gene upregulation, thus implicating that this chromatin modifier as a critical epigenetic regulatory factor in the GC reaction. LSD1 loss of function caused failure to repress genes induced by T cell help that encode molecules involved in GC exit and terminal differentiation, such as IRF4, XPB1 and PRDM1, which may explain why \textit{Lsd1}-deficient GCs are short-lived and fail to expand, preventing the formation of fully fledged GCs. In addition, LSD1 repressed several genes encoding molecules in B cell signaling interactions with T cells, including CD80 (the CLT4A receptor), CCXR3, Stat4, and the CD155 ligands TIGIT and CD226. Failure to proliferate could also be linked to upregulation of genes encoding molecules that inhibit cell-cycle progression. One of these genes, encoding the orphan G protein–coupled receptor Gpr132, was shown to arrest cell-cycle progression in B cells downstream of IKZF3.

LSD1 requires transcription factors for its recruitment to chromatin, and in GC B cells, BCL6 accounts for a large fraction of LSD1-binding sites in the genome. Recruitment of LSD1 is clearly important for BCL6 to repress its direct targets. However, we emphasize that LSD1’s histone-demethylase function alone is not sufficient for its actions in GC B cells, as we observed that the LSD1 Tower domain is required and essential for its actions in this cellular context. This finding suggests that recruitment of the CoREST complex (which also contains HDAC1/2) is critically required for LSD1 to mediate the repressive chromatin state and downstream biological effects in GC B cells. Notably, among all monoamine oxidases, including the AOD-containing homolog Lsd2 (Kdm1B), this coiled coil Tower domain is unique to LSD1, which may explain the apparent lack of compensatory effects of Kdm1B in \textit{Lsd1}-deficient GC B cells. These findings are in sharp contrast to LSD1’s mechanisms in myeloid leukemia, in which LSD1’s catalytic activity was shown to be crucial16,23,36. LSD1 inhibitors did cause a modest reduction in GC B cells, suggesting that loss of enzymatic activity might be slightly deleterious in primary GC B cells. However, this effect may also be due to perturbation of LSD1 function in other GC cell types, such as GC follicular helper T cells. Indeed, there is evidence that LSD1 plays an important role in T cell development. LSD1 is also known to act as a corepressor for PRDM1 (ref. 39), suggesting that LSD1 binds to and enables BCL6 to maintain the GC phenotype and may then switch to assist PRDM1 in inducing the plasma cell phenotype once BCL6 is downregulated.

Finally, consistent with our finding that LSD1 is an important BCL6 co-factor, our data indicate that LSD1 plays a critical role in lymphomagenesis, since it is required for this canonical lymphoma oncogene to induce malignant transformation. Although it is tempting to consider enzymatic inhibitors of LSD1 as a potential therapeutic strategy in lymphomas, these drugs are not sufficient to antagonize its actions. Instead, novel classes of inhibitors may need to be developed that could destabilize LSD1’s protein interactions, in addition to its enzymatic activity.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41590-018-0273-1.
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Author contributions

K.H. conceptualized the study, designed and performed the experiments, analyzed the data and wrote the manuscript. H.G., A.S.D. and C.M. performed bioinformatic analyses. R.L., M.C., C.D., H.S., M.N.C.V., T.B. and R.S. assisted in experiments. H.P.M. and R.G.K. provided GSK-LSD1 inhibitor and technical advice regarding drug treatments. A.M.M. edited the manuscript and provided technical advice with flow cytometry and IHC. G.I. performed pathological evaluation of transplanted mice. A.M.H. co-supervised the study. A.M.M. conceptualized and supervised the study and wrote the manuscript.

Competing interests

A.M.M. received research funding from GlaxoSmithKline. H.P.M. and R.G.K. are GlaxoSmithKline employees. R.S. is currently employed by Cancer Genetics Incorporated.

Additional information

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Methods

Mice. Conditional Lsd1-deficient mice (IoxP-flanked Lsd1 allele, Lsd1fl/fl) were purchased from the Jackson laboratory (023969). By crossing Lsd1fl/fl with the transgenic C1r-Cre strain (The Jackson Laboratory, 010611), we generated heterozygous C1r.Lsd1fl/+ mice, which were crossed to yield C1r-Cre Lsd1fl/+ mice. For controls, we used C1r-Cre-negative Lsd1fl/+ littermates. B cell conditional Lsd1 deletion was generated by crossing Lsd1fl/+ mice with CD19-Cre mice (Jackson Laboratory, 006785), where Cre is expressed from the pre-B cell stage. Mice were used for assessment of GC formation induced by immunization with SRBCs or affinity maturation by immunization with NP-CGG98. We also used IgB6/c mice (obtained from R. Dalla-Favera, Columbia University10) to generate IgB6/c C1r-Cre Lsd1fl/+ mice by crossing with C1r-Cre Lsd1fl/+ mice. These mice were used for GC formation assays or as bone marrow donors for transplantation into C57BL/6 recipients. Animal care and all experiments were performed in strict compliance with the institutional guidelines and protocols of Weill Cornell Medicine and Memorial Sloan-Kettering Cancer Center Institutional Animal Care and Use Committee, the Association for Assessment and Accreditation of Laboratory Animal Care International and in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals.

Germinal center formation experiments and flow cytometry. Age- and sex-matched C57BL/6 mice (8–12 weeks old) were immunized intraperitoneally with 0.5 ml of a 2% SRBC suspension in PBS (Cocalico Biologicals) and euthanized after 10 d. Single-cell suspensions from mouse spleens were separated by Ficoll gradient centrifugation and stained using fluorescent-labeled anti-mouse: PE-Cy7 anti-B220 (eBioscience, 25-0452, dilution 1:500), APC anti-CD3 (eBioscience, 7-0042, dilution 1:500), APC-Cy7 anti-CD19 (BioLegend, 101216, dilution 1:500), PE anti-GL7 (BD Biosciences, 553666, dilution 1:500), BV-421 anti-CD138 (BioLegend, 142507, 1:500), FITC anti-κ (BioLegend, 405095, 1:500) or APC-Cy7 anti-κ (BioLegend, 405093, 1:500), PE-Cy7 anti-CD11b (BioLegend, 101216, dilution 1:300) and APC-Cy7 anti-Gr1 (108424, dilution 1:300). DAPI was used for the exclusion of dead cells. When needed, cells were fixed and permeabilized using the BD Cytofix/Cytoperm Kit (554714, BD Biosciences). For cell cycle analysis, cells were stained in vitro by addition of BrDU (10 μM) for 20 min. 7AAD was used to determine DNA content in cell cycle analysis, along with V450 anti-BrDU staining (BD Pharmingen, V450, dilution: 1:50). For treatment with the LSD1 inhibitor GSK-LSD1, mice were injected intraperitoneally with drug or vehicle and then protein expression was induced with 1 mM IPTG for 3 h at 37 °C with shaking. Bacteria were pelleted, resuspended in lysis buffer (50 mM Tris HCl, pH 7.5, 130 mM NaCl, 1 mM EDTA, 1% Triton-X 100) supplemented with DNase I (1 μg/ml) and lysosyme (20μg/ml) and incubated for 30 min on ice and then spun for 30 min at 21,000g. Bacterial lysates were incubated with glutathione Sepharose beads (GE Healthcare) at 22 °C for 1 h with rocking, then washed 5x with PBS. Finally, GST or GST-LSD1 proteins were eluted with Glutathione Elution Buffer (50 mM Tris-HCl pH 8, 10 mM reduced glutathione). After gel-based quantification, 5μg of purified GST-tagged protein was bound to glutathione Sepharose beads in binding buffer for 1 h at 4°C (20 mM HEPES, pH 7.5, 150 mM KC1, 25 mM MgCl2, 10 mM DTT, 0.1 mM EDTA, 0.15% NP-50) and, after multiple washes, 20 μl of the radiolabeled in vitro transcription/translation reaction containing [35S]-labeled BCL6 protein was added to each sample and incubated 1 h at 37 °C with rocking. After washing the beads with increasing stringency salt concentrations, beads were boiled in 1x LDS sample buffer (Thermo Fisher NP0007) supplemented with 5% β-mercaptoethanol and supernatant was resolved by SDS–PAGE. Gels were then fixed (10% glacial acetic acid, 20% methanol (v/v) in water), incubated in liquid autoradiography enhancer for 1 h (EN3HANCE, Perkin Elmer), vacuum dried (70 °C, 3 h) and developed by autoradiography after exposing to film for 24 h at ~80 °C.

Immunoblotting. Lysates of DLBCL cells were prepared using 25 mM Tris, pH 7.4, 150 mM NaCl, 0.5% NP-40, 0.1% SDS lysis buffer with protease inhibitors (Roche). 5 μg of antibodies to BCL6 (Santa Cruz N3, sc-8588) or LSD1 (abcam 17721) or IgG control antibodies were added to precleared lysates and incubated overnight at 4°C with rocking. Protein A beads (Roche) were added for 2 h at 4°C with gentle agitation, washed four times (25 mM Tris, pH 7.4, 200 mM NaCl, 0.5% NP-40, 0.1% SDS) and then boiled in 1x LDS sample buffer (Thermo Fisher NP0007) supplemented with 5% β-mercaptoethanol, and supernatant was resolved by polyacrylamide gel electrophoresis (SDS–PAGE). For histone-demethylation assays, immunoprecipitated complexes were washed on-bead and incubated with 1 μg bulk histone (H4524, Sigma) in binding buffer (50 mM Tris HCl, 50 mM KCl, 5 mM MgCl2, 0.5% BSA, 5% glycerol) overnight. Reactions were denatured and resolved by 18% SDS–PAGE.

GST pull-down. Radiolabeled BCL6 was generated by TnT T7 Quick Transcription/Translation system (Promega) according to the manufacturer’s protocol supplemented with 0.02 μM EasyTag L-[^35S]-methionine (Perkin Elmer). GST and GST-LSD1 fusion pGEX-based vectors were transformed in BL21(DE3) competent Escherichia coli. Bacterial cultures were grown to OD600 0.5–0.6, and then protein expression was induced with 1 mM IPTG for 3 h at 37°C with shaking. Bacteria were pelleted, resuspended in lysis buffer (50 mM Tris-HCl, pH 7.5, 130 mM NaCl, 1 mM EDTA, 1% Triton-X 100) supplemented with DNase I (1 μg/ml) and lysosyme (20μg/ml) and incubated for 30 min on ice and then spun for 30 min at 21,000g. Bacterial lysates were incubated with glutathione Sepharose beads (GE Healthcare) at 22 °C for 1 h with rocking, then washed 5x with PBS. Finally, GST or GST-LSD1 proteins were eluted with Glutathione Elution Buffer (50 mM Tris-HCl pH 8, 10 mM reduced glutathione). After gel-based quantification, 5μg of purified GST-tagged protein was bound to glutathione Sepharose beads in binding buffer for 1 h at 4°C (20 mM HEPES, pH 7.5, 150 mM KC1, 25 mM MgCl2, 10 mM DTT, 0.1 mM EDTA, 0.15% NP-50) and, after multiple washes, 20 μl of the radiolabeled in vitro transcription/translation reaction containing [35S]-labeled BCL6 protein was added to each sample and incubated 1 h at 37 °C with rocking. After washing the beads with increasing stringency salt concentrations, beads were boiled in 1x LDS sample buffer (Thermo Fisher NP0007) supplemented with 5% β-mercaptoethanol and supernatant was resolved by SDS–PAGE. Gels were then fixed (10% glacial acetic acid, 20% methanol (v/v) in water), incubated in liquid autoradiography enhancer for 1 h (EN3HANCE, Perkin Elmer), vacuum dried (70 °C, 3 h) and developed by autoradiography after exposing to film for 24 h at ~80 °C.

Immunoblotting. Lysates of DLBCL cells line and isolated cell populations were prepared using 25 mM Tris, pH 7.4, 150 mM NaCl, 0.5% NP-40, 0.1% SDS and complete protease inhibitor cocktail (Roche) lysis buffer. Protein lysates were resolved by SDS–PAGE, transferred to PVDF membrane and probed with primary antibodies to the following: LSD1 (Santa Cruz, B22E5, sc-33875), FLAG (F1804, M2, Sigma), GAPDH (Santa Cruz, FL-3535, sc-25778), actin (sc-81760). Membranes were then incubated with corresponding HRP-conjugated secondary antibodies, and signal was detected using enhanced chemiluminescence (ChemilumDoc Touch, Bio-Rad Laboratories). Densitometry values were obtained using ImageJ 1.44o software (NIH).

ELISA. Mice were immunized by intraperitoneal injection of NP-CGG98 (200 μg) in alum and were boost-immunized with the same dose 21 d after the primary immunization. Serum samples were collected at 26 d post primary NP-CGG immunization, and the abundance of NP hapten-specific immunoglobulin titers was assessed by ELISA. Serum was tested for binding of NP-specific antibodies to low-haptenated BSA (NP−-BSA)– versus high-haptenated BSA (NP+−BSA)– coated plates using HRP-conjugated antibodies (SouthernBiotect, dilution 1:500). Optical density (OD) at 450 nm was measured in a plate reader (BioTek), and the absorbance ratio was calculated by dividing the mean OD of NP−-BSA-coated wells by the mean OD of NP+−BSA-coated wells. Titters were calculated by logarithmic interpolation of the dilutions with readings immediately above and immediately below a chosen ODcut threshold equal to two-fold of background ODcut, as previously described16.
ELISpot. Mice were euthanized 40 d after NP-CGG immunization, and splenocytes were collected and analyzed for secretion of NP-specific IgG1 antibodies. ELISpot plates (Millipore, MAHA4510) were activated by incubation with 35% ethanol, coated with NP-BSA and NP2-BSA (Biosearch Technologies) and used for overnight culture of splenocytes seeded at 3×10^5 cells without disturbing overnight. Secreted antibodies binding to the coated reagent were visualized by HRP-conjugated anti-IgG1 and DAB substrate. The plates were scanned, and the number of spots were analyzed by ZellNet Consulting.

Plasmids, shRNAs and virus production. shRNAs targeting LSD1-1 and LSD1-2 were delivered via lentiviral infection. Viral particles were produced using calcium phosphate transfection of 293T cells with pLKO.1 vector along with pSPax2 and pMD2.G at a 4:1 ratio. Infected cells were selected via puromycin treatment (1 μg/mL). Negative antisense sequences of shRNA were as follows: hsiLSD1-1, 5‘-CCACGAGTCAAACCTTTATTT-3‘; hsiLSD1-2, 5‘-CCACGAGTCAAACCTTTATTT-3‘. LSD1 wild-type and rescue mutant expression constructs were cloned into lentiviral Cas9-Blast (Addgene 52962), replacing Cas9 using Gibson Assembly Master Mix (NEB, E2611). Tower-deleted constructs contained a pentaglycin loop that links the two parts of the AOD, in place of the LSD1 ‘Tower domain’.  

CRISPR-Cas9 domain screen. Two lymphoma cell lines were subcloned after the genome with LSD1-AOD regions were removed using SAMtools and Picard, respectively. Sequences mapped uniquely to reference genome hg38, and non-unique mapping reads and PCR duplicates were generated using the Illumina ChIP-seq Library preparation Kit, starting on 1 ml of ATAC-seq resuspension buffer (RSB: 10 mM Tris-HCl, pH 7.4, 10 mM NaCl, and 3 mM MgCl2 in water) and were centrifuged at 5000 × g for 5 min. Cell pellets were resuspended in 50 μl of ATAC-seq RSB containing 0.1% NP40, 0.1% Tween-20 and 0.01% digitonin and were incubated on ice for 3 min. Following lysis, 1 ml of ATAC-seq RSB containing 0.1% Tween-20 was added, and nuclei were centrifuged at 9000 × g (4°C, 10 min). Pellet nuclei were resuspended in 50 μl of transposition mix (25 μl 2 × TD buffer, 2.5 μl Tagment DNA enzyme, 16.5 μl PBS, 0.5 μl 1% digitonin, 0.5 μl 10% Tween-20, and 5 μl water) and were incubated overnight at 37°C. 50,000 purified GC B cells were resuspended in 1 ml of ATAC-seq resuspension buffer (RSB: 10 mM Tris-HCl, pH 7.4, 10 mM NaCl, and 3 mM MgCl2 in water) and were centrifuged at 5000 × g for 5 min. All libraries were sequenced on a HiSeq 2000 (Illumina).

ATAC-seq pair-end sequencing data tracks were trimmed to remove adapter sequences using NGmer with options ‘-a’, and trimmed reads were aligned to mm10 reference genome using Bowtie2 with options ‘-X 2000 –local’. Alignments were filtered using SAMTools v1.8 to retain only uniquely mapping read pairs and to discard mitochondrial mapping reads. Read duplicates were removed using the MarkDuplicates program in Picard Tools 2.18.11 (http://broadinstitute.github.io/picard). Peak calling was performed by pooling all the samples to control for recovery using the MACS2 peakcalling command with parameters ‘–shift -75 –extsize 150 –pval 0.01 –keep-dup all –call-summits’. A matrix of non-nucleosomal Tin5 insertion counts was generated for the common set of peaks. The count matrix was used to identify differentially accessible peaks with DESeq2. Reads within the final set of peaks were taken as library size for normalization. Differential peaks at FDR <0.05 were retained for analysis. The peaks were annotated to RefSeq mouse genes and located using upstream promoter and regulatory regions (basal region plus upstream extension to the nearest gene up to 1 Mb). Genes near differentially opening peaks were used in pathway enrichment analysis by GSEA. Mean-normalized Tin5 insertions at differential peaks were computed for pooled replicates in both Ct1-Cre LSD1 and LSD1 mice and smoothed over a 120-bp window. Average signal values were plotted using deepTools.  

Pathway analysis. Pathway analysis was performed as previously described. Gene sets for canonical pathway and Gene Ontology (GO) terms were downloaded from the Molecular Signatures Database (MSigDB) using C5 collection. The BC cell- and lymphoid-specific signatures were curated by studies published from the Stanford laboratory29 or Melnick laboratory30-31. This database contains approximately 250 gene sets/signatures associated with normal lymphoid biology and lymphoid neoplasms. Fisher’s exact test was used to calculate enrichment P values for each of those gene sets. The Benjamini–Hochberg method32 was used for FDR control.

Statistics and reproducibility. Two-sided unpaired t-test was used where indicated. All proportional numerical values provided in the text are written as the mean ± standard deviation. All statistical analyses were done in Prism 7.0 (Graphpad). Experiments were performed at least two times, with similar results obtained each time. For animal studies, the number of animals was chosen to ensure 90% power with 5% error based on observed standard deviation from previous studies. Additional information can be found in the Life Sciences Reporting Summary.

Data availability. Sequence data that support the findings of this study have been deposited in GEO under SuperSeries GSE118783 with the accession codes GSE106417 (RNA-seq for GC B cell), GSE106416 (RNA-seq for lymphoma) and GSE107920 (ChIP-seq). Other data that support these findings are available from the corresponding author upon reasonable request.

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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
- Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- Clearly defined error bars
- State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

BD FACS Canto II, Panoramic Viewer Software

Data analysis

FlowJo v10, ImageJ 1.44, Graphpad Prism v7.0, DESeq2, STAR, BWA, GSEA, MACS2, deepTools 3.0.2, Picard Tools 2.18.11, SAMtools v1.8

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Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
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Data availability

Sequence data that support the findings of this study have been deposited in GEO under SuperSeries GSE118783 with the accession codes GSE106417 (RNA-seq for
GC B cell), GSE106416 (RNA-seq for lymphoma) and GSE107920 (ChIP-seq). Other data that support these findings are available from the corresponding author upon reasonable request.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

For animal studies, the number of animals was chosen to ensure 90% power with 5% error based on observed SD from previous studies. No statistical analyses were performed to predetermine sample sizes for in vitro experiments, but our sample sizes are similar to those generally employed in the field.

Data exclusions

No data were excluded from any analyses with the exception of one LSD1fl/fl and one Cγ1-Cre; Lsd1fl/fl ATAC-seq sample that were excluded due to very low sequencing coverage. In addition, in our survival analysis we censored 3 mice that died less that one month after bone marrow transplantation lacking distinct pathological features due to failed BM engraftment, one animal that was euthanized due to tail necrosis and one euthanized due to dermatitis based on Research Animal Resource Center recommendations.

Replication

All experiments were performed independently more than once using biologically independent replicates. All results reported were successfully replicated.

Randomization

Randomization of experimental groups were not applicable in most experiments in this study. For treatment with LSD1 inhibitor GSK-LSD1 (Figure 5a-b), C57Bl/6 mice were randomized before treating with active compound or PBS control.

Blinding

Blinded review and lymphoma diagnosis was performed for reporting pathology results in Figure 6.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a Involved in the study

- Unique biological materials
- Antibodies
- Eukaryotic cell lines
- Palaeontology
- Animals and other organisms
- Human research participants

Methods

n/a Involved in the study

- ChIP-seq
- Flow cytometry
- MRI-based neuroimaging

Antibodies

Antibodies used

Flow cytometry antibodies: PE-Cy7 anti-B220 (eBioscience, 25-0452, dilution 1:500), APC anti-CD38 (eBioscience, 17-0381, dilution 1:500), APC anti-B220 (BD Biosciences, 553092, dilution 1:500), PE anti-FAS (BD Biosciences, 554258, dilution 1:500), FITC anti-GL7 (BD Biosciences, 55366, dilution 1:500), BV421 anti-CD138 (BioLegend, 124507, 1:500), FITC anti-IgK (BioLegend, 409509, lot#B195860, 1:500) or APC-Cy7 anti-IgK (BioLegend, 409503, lot#B232635, 1:500), PE-Cy7 anti-CD11b (BioLegend, 101216, dilution 1:500), FITC anti-IgK (BioLegend, 409509, lot#B268267), APC-Cy7 anti-Gr1 (108424, dilution 1:500), and V450 anti-BrdU (BD Pharmingen, V450, dilution: 1:50).

ChIP-seq antibodies: LSD1 (abcam 17721, lot#GR3193508-2), BCL6 (Santa Cruz N3, sc-858, lot#I2607) and H3K4me1 (abcam, ab8895, lot#GR193882-2).

Western Blot: LSD1(Santa Cruz, 1B2E5, sc-53875, dilution 1:500), GAPDH (Santa Cruz, 3850, dilution 1:500), Actin (Santa Cruz, sc-81760, dilution 1:500), and H3K4me1(abcam, ab8895, dilution 1:5000, lot#GR193882-2).

Validation

All antibodies are validated upon arrival to the lab, no matter what is claimed on the data sheets. In this study, antibodies were...
Eukaryotic cell lines

Policy information about cell lines

| Cell line source(s) | OCI-LY1: Ontario Cancer Institute (OCI)  
|                     | TMD8 and HBL-1:  
|                     | Kindly provided by Dr. Jose Angel Martinez-Climent, Centre for Applied Medical Research (CIMA), Pamplona, Spain  
|                     | SUDHL4: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) |

Authentication

GC derived B-lymphoma cell lines were obtained from the cell lines repositories, American Type Culture Collection (ATCC) and the Ontario Cancer Institute (OCI). Cell lines are kept in our institutional bio-repository and we conduct semi-annual genomic identification by matching short tandem repeat DNA profiles with those deposited in databases from repositories and monthly testing for mycoplasma and other potential contaminants. Serum is also purchased in bulk and is validated on a number of cell lines before use. All CRISPR-generated and newly derived cell lines were characterized and validated by surveyor assay.

Mycoplasma contamination

Biannual testing for mycoplasma was performed. All cell lines tested negative for mycoplasma contamination.

Commonly misidentified lines

(See ICLAC register)

No cell line used is part of ICLAC.

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

Laboratory animals

The Research Animal Resource Center of Weill Cornell Medicine approved all mouse procedures. Conditional Lsd1 knockout mice (loxP-flanked Lsd1 allele, Lsd1fl/fl) were purchased from the Jackson laboratory (023969). By crossing Lsd1fl/fl with the transgenic Cy1-Cre strain (The Jackson Laboratory, 010611) we generated heterozygous Cy1-Cre Lsd1fl/+ mice, which were crossed to yield Cy1-Cre Lsd1fl/fl mice. As control group, we used Cy1-Cre negative Lsd1fl/fl littermates. Age- and sex-matched C57Bl6 mice were immunized intraperitoneally at 8 to 12 weeks of age were used for assessment of the germinal center formation, which were induced by intra-peritoneal injection of 2% SRBC solution or affinity maturation by intra-peritoneal injection of NP-CGG28-30 in alum. We also used Iγ-Bcl6 mice (obtained from Dr. Ricardo Dalla-Favera, Columbia University) to generate Iγ-Bcl6 Cy1-Cre Lsd1fl/fl by crossing with Cy1-Cre Lsd1fl/fl. These mice were used for germinal center formation assays induced by SRBC IP injections or harvested bone marrow to perform transplantation to C57Bl6 recipients. Finally, B cell conditional LSD1 model was generated by crossing Lsd1fl/fl mice with CD19-Cre mice (Jackson Laboratory, 00678) where Cre is expressed from the pre-B cell stage.

For treatment with LSD1 inhibitor GSK-LSD1, mice were injected intraperitoneally with drug or vehicle drug or PBS vehicle starting the following day after induction of GC by SRBC and administered daily at a concentration of 50 mg/kg per day for 9 consecutive days after which the mice were sacrificed (day 10). All studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed the Institutional Animal Care and Use Committee either at GSK or by the ethical review process at the Research Animal Resource Center of Weill Cornell Medicine. For murine bone marrow transplantation assays, bone marrow cells from 6-8 week old male donors were harvested. One million bone marrow cells of each genotype (Iγ-Bcl6 Cy1-Cre Lsd1fl/+), Iγ-Bcl6 Cy1-Cre Lsd1fl/fl and Cy1-Cre Lsd1fl/fl mice or Lsd1fl/fl) were injected into the tail veins of lethally irradiated female C57Bl6 mice. All mice were followed until any one of several criteria for euthanizing were met, including severe lethargy, more than 10% body weight loss, and palpable splenomegaly that extended across the midline, in accordance with our Weill Cornell Medicine and Memorial Sloan-Kettering Cancer Center Institutional Animal Care and Use Committee–approved animal protocols. Animal care was in strict compliance with institutional guidelines established by Weill Cornell Medicine, the Memorial Sloan-Kettering Cancer Center, the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences 1996) (Silverman et al., 2006), and the Association for Assessment and Accreditation of Laboratory Animal Care International.

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve field collected samples.

ChIP-seq

Data deposition

- Confirm that both raw and final processed data have been deposited in a public database such as GEO.
- Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

Sequence data that support the findings of this study have been deposited in GEO under SuperSeries GSE118783 with accession code GSE107920.
Methodology

Replicates

For ChIP-seq experiments with cells treated with siRNAs, OCI-Ly1 or SUDHL4 cells were transfected using Nucleofector 96-well Shuttle system (Lonza) with 1μM siRNA against BCL6 (HSS100968) or non-targeted siRNA (46-2001) (Stealth RNAi, Invitrogen) for 24h. Briefly cells were fixed, lysed, and sonicated to generate fragments less than 400 bp. Sonicated lysates were incubated with antibodies against LSD1 (abcam 17721), BCL6 (Santa Cruz N3, sc-858), H3K4me1 (abcam, ab8895) or IgG control (abcam, ab171870) and after increasing stringency washes immunocomplexes were recovered and DNA was isolated. ChIP enrichment was confirmed by qPCR. H3K4me1 ChIPs in SUDHL4 also contained Drosophila spike-in chromatin control (750ng per ChIP, Active Motif, 53083) added during precipitation along with Drosophila antibody (2ug, Active Motif, 61686) per manufacturer’s recommendation for proper signal normalization. ChIP-seq libraries were prepared using the Illumina ChIP-seq Library preparation Kit following the manufacturer’s instructions with minor modifications. Briefly 10ng of purified ChIP DNA (quantified using Qubit 2.0 fluorometer, Invitrogen) was end repaired by conversion of overhangs to phosphorylated blunt ends. A’ bases were added to the 3’ ends of the DNA fragments and Illumina adapters (1:30 dilution) were ligated to the ends of ChIP fragments. After adaptor ligation DNA was separated by electrophoresis and size selected by isolating a gel band of 250 ± 25bp. Size selected fragments were PCR amplified for 15cycles using Illumina genomic DNA primers 1.1 and 1.2 with the following program (30 s at 98°C, 15cycles of 10 at 98°C, 30 s at 65°C, 30 s at 72oC and 5min extension at 75oC). Q-PCR was repeated to confirm retention of relative enrichment. For ChIP-seq, raw images generated went through primary image analysis and basecalling (RTA v1.6) that was followed by Illumina Genome Analyzer Off-Line Basecaller (QLB v1.6) analysis. Raw ChIP-seq reads were trimmed and filtered for quality using Trim Galore and FASTQC. Reads were aligned using BWA-mem against human reference genome hg38, and non-uniquely mapping reads and PCR duplicates were removed using samtools and Picard, respectively only sequences mapped uniquely to the genome with not more than 2 mismatches were used for downstream analysis. Several reads mapping to the same exact location (clonal reads) were considered amplification artifacts and were excluded from the analysis. The determine the quality of individual ChIP-seq experiments we used ENCODE consortium quality metrics (Landt, Marinov et al. 2012). Strand cross-correlation analysis was performed to asses signal-to-noise ratios, and samples were retained for analysis that passed a normalized strand coefficient (NSC) threshold >1.05 and a relative strand correlation (RSC) threshold >0.8. Read density tracks were visualized using the UCSC browser. Peak calling was performed using MACS2[41]. Each ChIP-seq dataset was normalized to its corresponding input. H3K4me1 signals from SUDHL4 cells were normalized based on the scaling factor calculated from the ratio of human and drosophila mapped reads as previously described. Peaks were annotated based on the RefSeq database (hg38). Peaks localized +/-kb of the TSS were defined as promoter peaks, peaks localized +/-kb of the TES were defined as 30 end peaks, and peaks > 2kb from genes were defined intergenic.

Sequencing depth

The sequencing depth of our ChIP-seq results is roughly between 26-58 million reads.
OCI-Ly1 LSD1 baseline total: 32,609,339 uniquely mapped: 26,324,718 (single end)
OCI-Ly1 LSD1 NT total: 54,122,363 uniquely mapped: 40,127,234 (single end)
OCI-Ly1 LSD1 si total: 51,843,752 uniquely mapped: 36,676,237 (single end)
OCI-Ly1 BCL6 NT total: 43,932,942 uniquely mapped: 32,334,558 (single end)
OCI-Ly1 BCL6 si total: 56,680,286 uniquely mapped: 42,924,695 (single end)
OCI-Ly1 H3K4me1 NT total: 41,902,231 uniquely mapped: 34,859,650 (single end)
OCI-Ly1 H3K4me1 si total: 47,129,185 uniquely mapped: 38,791,112 (single end)
**OCI-Ly1 Input_NT total:** 42,943,750 uniquely mapped: 32,968,653 (single end)

**OCI-Ly1 Input_si total:** 42,214,823 uniquely mapped: 32,889,628 (single end)

**SUDHL4 LSD1 total:** 20,709,196 uniquely mapped: 19,598,046 (single end)

**SUDHL4 BCL6 total:** 61,010,362 uniquely mapped: 55,939,001 (single end)

**SUDHL4 H3K4me1 NT total:** 68,690,586 uniquely mapped: 61,844,189 (single end)

**SUDHL4 H3K4me1 si total:** 50,686,624 uniquely mapped: 45,978,833 (single end)

**SUDHL4 Input_NT total:** 46,291,950 uniquely mapped: 43,428,408 (single end)

**SUDHL4 Input_si total:** 53,543,411 uniquely mapped: 50,756,036 (single end)

**Antibodies**
- LSD1 (abcam 17721)
- BCL6 (Santa Cruz N3, sc-858)
- H3K4me1 (abcam, ab8895)

**Peak calling parameters**
Peak calling was performed using MACS version 2.1.1.20160309 with the following parameters:
- OCI-Ly1_LSD1 baseline > FC=5, p val < 10^-10 25,922 peaks
- OCI-Ly1_LSD1 NT > FC=5, p val < 10^-10, 10,536 peaks
- OCI-Ly1_LSD1 si > FC=5, p val < 10^-10 4,878 peaks
- OCI-Ly1_BCL6 NT > FC=8, p val < 10^-10, 13,458 peaks
- OCI-Ly1_BCL6 si > FC=8, p val < 10^-10 3,745 peaks
- SUDHL4_BCL6 > FC=8, p val < 10^-10 13,056 peaks
- SUDHL4_LSD1 > FC=5, p val < 10^-10, 13,119 peaks

**Data quality**
Raw ChIP-seq reads were trimmed and filtered for quality using Trim Galore and FASTQC. Reads were aligned using BWA against human reference genome hg38, and non-uniquely mapping reads and PCR duplicates were removed using samtools and Picard, respectively. An input control was used as internal control for peak calling using MACS2. Peaks were identified using MACS2. The determine the quality of individual ChIP-seq experiments we used ENCODE consortium quality metrics (Landt, Marinov et al. 2012). Strand cross-correlation analysis was performed to asses signal-to-noise ratios, and samples were retained for analysis that passed a normalized strand coefficient (NSC) threshold >1.05 and a relative strand correlation (RSC) threshold >0.8.

**Software**
For ChIP-seq, raw images generated went through primary image analysis and basecalling (RTA v1.6) that was followed by Illumina Genome Analyzer Off-Line Basecaller (OLB v1.6) analysis, where reads were aligned to the human genome (UCSC hg38) using BWA 0.6.2. Read density tracks were visualized using the UCSC browser. Peak calling was performed using MACS version 2.1.1.20160309. Each ChIP-seq dataset was normalized to its corresponding input. Peaks were annotated based on the RefSeq database (hg38). Peaks localized +/-2kb of the TSS were defined as promoter peaks, peaks localized +/-2kb of the TES were defined as 30 end peaks, and peaks > 2kb away from genes were defined intergenic.

**Flow Cytometry**

**Plots**
- Confirm that:
  - The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
  - The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
  - All plots are contour plots with outliers or pseudocolor plots.
  - A numerical value for number of cells or percentage (with statistics) is provided.

**Methodology**

**Sample preparation**
Single-cell suspensions from mouse spleens were ficoll separated and stained using fluorescent-labeled anti-mouse antibodies, incubated on ice in the dark for 30min, then washed 2x with PBS with 0.5% BSA and 5mM EDTA and resuspended in 200ul washing buffer for acquisition.

**Instrument**
FLOW Canto II

**Software**
FlowJo v10.2

**Cell population abundance**
Sorted human and murine GC B cells were confirmed to be >90% following each sorting.

**Gating strategy**
For selection of single cells, cells were first gated based on SSC-A/FSC-A, then FSC-H/FSC-W followed by SSC-H/SSC-W gating. For Sorting: B cells were gated on APC-B220 positive population on a SSC-A/APC-A dotplot. GC B cells were gated on GL7+FAS+ populations on a BV421-A/PE-Cy7 boxplot (Log axes). For regular flow: live B cells were selected as B220APC+DAPI- cells on APC-A/DAP1-A dotplot followed by gating GC B cells either as GL7-FITC+/FAS-PE+ or CD38-APC negative /FAS-PE positive on dotplot with logarithmic axes. All gating strategies are shown in Figure S6.

- Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.