The RNA-binding protein PTBP1 is necessary for B cell selection in germinal centers

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Antibody affinity maturation occurs in germinal centers (GCs), where B cells cycle between the light zone (LZ) and the dark zone. In the LZ, GC B cells bearing immunoglobulins with the highest affinity for antigen receive positive selection signals from helper T cells, which promotes their rapid proliferation. Here we found that the RNA-binding protein PTBP1 was needed for the progression of GC B cells through late S phase of the cell cycle and for affinity maturation. PTBP1 was required for proper expression of the c-MYC-dependent gene program induced in GC B cells receiving T cell help and directly regulated the alternative splicing and abundance of transcripts that are increased during positive selection to promote proliferation.

Germinal centers (GCs) are specialized areas of secondary lymphoid tissues in which B cells undergo antibody affinity maturation. The GC can be divided into a dark zone (DZ), characterized by extensive proliferation and somatic hypermutation, and a light zone (LZ), where B cells are less proliferative and make contacts with follicular dendritic cells and T cells. There, B cells are positively selected to survive and undergo further rounds of proliferation in the DZ. Help provided by T cells promotes faster progression through the cell cycle, a greater number of cell divisions and increased frequency of somatic hypermutation, which results in increased antibody affinity maturation. Positive selection in the LZ results in the transient expression of the transcription factor c-MYC necessary for the progression of selected cells through the cell cycle. c-MYC also induces the transcription of genes encoding products important for anabolic metabolism. Moreover, activity of the metabolic checkpoint kinase complex mTORC1 is increased during positive selection and promotes the anabolic gene-expression program. In addition to c-MYC, the transcription factors AP4, FOXO1 and BATF are important during the LZ–DZ transition of GC B cells. Such findings highlight the existence of B cells in relays of transcription factors that are responsive to T cell help and promote B cell proliferation and mutation.

Alternative splicing (AS), alternative polyadenylation (APA), mRNA decay and translation have the potential to regulate cell fate. However, relatively little is known about the relevant changes in and regulation of AS during immune responses, and the molecular regulation of APA is only beginning to be appreciated. The impact of RNA-binding proteins (RBPs) that have the potential to integrate multiple aspects of gene expression in B cells undergoing selection in the GC is only emerging. One class of RBPs with pleiotropic functions is the polypyrimidine tract–binding proteins (PTBPs) that have the potential to regulate post-transcriptional processing and the expression of genes encoded in GC B cells. These proteins include PTBP1, which is both a repressor of AS and an activator of AS, and has been shown to be co-expressed with PTBP1 in B lymphocytes. PTBP1 is both a repressor of AS and an activator of AS, and has been linked to APA, mRNA decay and translational regulation. In CD4+ T cells, PTBP1 has been shown to enhance the stability of mRNA encoding the B cell–stimulatory molecule CD40L, and in human B cells, it has been shown to be responsive to the receptor TLR9. Although PTBP1 has the potential to regulate post-transcriptional gene-expression programs in lymphocytes, its physiological functions in the development and activation of lymphocytes are unknown.

Here we found that PTBP1 acted in concert with c-MYC to ensure the selection of B cell clones with the highest affinity for antigen by promoting the proliferation of GC B cells. PTBP1 guaranteed proper post-transcriptional processing and the expression of genes induced as part of the c-MYC-dependent gene-expression program.

Results

PTBP1 expression is increased in positively selected GC B cells. In contrast to the expression of mRNA encoding other members of the PTBP family, the expression of Ptbp1 mRNA was ~1.5-fold higher in GC B cells than in naive B cells (Supplementary Fig. 1a,b). Ptbp2 transcripts were rare and showed evidence of skipping of exon 10 (Supplementary Fig. 1b), which generates mRNAs degraded by nonsense-mediated RNA decay (NMD). Ptbp1 mRNA increased 1.4-fold after c-MYC expression in LZ B cells, but Ptbp3 mRNA did not (Fig. 1a), and Ptbp1 mRNA was also increased in GC B cells that had received the greatest levels of T cell help, relative to its abundance in GC B cells that had received less help from T cells (Supplementary Fig. 1c,d). A panel of monoclonal antibodies validated as recognizing PTBP1, PTBP2 or PTBP3 that were specific for each PTBP and able to detect the proteins by flow cytometry (Supplementary Fig. 2a,b) detected PTBP1 and PTBP3, but not PTBP2, in B cells (Fig. 1b and Supplementary Figs. 1f and 2a,b,2c). PTBP1 protein was ~1.6-fold more abundant in GC B cells than in non-GC B cells (Fig. 1b) and was ~1.4-fold more abundant in GC
We next studied PTBP1 is dispensable for B cell development. With, c-MYC in GC B cells responding to help from T cells. in B cells29. Thus, PTBP1 might act downstream of, or in parallel and that c-MYC binds to the promoter of the gene encoding PTBP1 shown that PTBP1 expression correlates positively with c-MYC28 B cells negative for GFP–c-MYC (Fig. 1c). Published studies have fluorescent protein (GFP)-tagged c-MYC (GFP–c-MYC) than in GC Myc B cells positive for a reporter transgene (MycGFP) encoding green fluorescent protein (GFP)–tagged c-MYC (GFP–c-MYC) than in GC B cells negative for GFP–c-MYC (Fig. 1c). Published studies have shown that PTBP1 expression correlates positively with c-MYC28 and that c-MYC binds to the promoter of the gene encoding PTBP1 in B cells29. Thus, PTBP1 might act downstream of, or in parallel with, c-MYC in GC B cells responding to help from T cells.

PTBP1 is dispensable for B cell development. We next studied mice with conditional knockout (cKO) of Pax5-flanked Ptbp1 alleles (Ptbp1fl/fl), from the pro-B cell stage onward, via Cre recombinase expressed from the locus encoding the immunoglobulin α-chain CD79A (Cd79aCre) and confirmed the absence of PTBP1 protein at the appropriate stage (Supplementary Fig. 2b–d). B cell development was normal in the absence of PTBP1 (Supplementary Fig. 2c,e,f). Moreover, in lethally irradiated CD45.1+ B6.SJL mice reconstituted with a 1:1 mixture of bone marrow cells from B6.SJL mice and Cd79aCre+Ptbp1fl/fl mice, the number of follicular B cells that arose from the Cd79aCre+Ptbp1fl/fl (cKO) bone marrow was not lower than the number of such cells that arose from B6.SJL bone marrow (data not shown). In cells that had deleted Ptbp1, expression of PTBP2 was evident from the pro-B cell stage onward (Supplementary Fig. 2d). The loss of PTBP1 and expression of PTBP2 was confirmed by immunoblot analysis (Supplementary Fig. 2a). As expected15, Ptbp1-deficient B cells also contained a greater amount of the long isoform of PTBP3 than did Ptbp1-sufficient B cells (Supplementary Fig. 2a). This indicated that although PTBP1 was not necessary for B cell development, its deletion was consequential at the molecular level.

PTBP1 in B cells is necessary for the GC B cell response. We immunized Cd79aCre+Ptbp1fl/fl and Cd79aCre+Ptbp1fl/fl mice with 4-hydroxy-3-nitrophenyl-acetyl conjugated to keyhole limpet

B cells positive for a reporter transgene (MycGFP) encoding green fluorescent protein (GFP)–tagged c-MYC (GFP–c-MYC) than in GC B cells negative for GFP–c-MYC (Fig. 1c). Published studies have shown that PTBP1 expression correlates positively with c-MYC28 and that c-MYC binds to the promoter of the gene encoding PTBP1 in B cells29. Thus, PTBP1 might act downstream of, or in parallel with, c-MYC in GC B cells responding to help from T cells.

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Fig. 2 | PTBP1 is necessary for GC B cell responses. a, Gating strategy for GC B cells (left) and DZ and LZ GC B cells (right) from the spleen of Cd79a−/−Ptbp1fl/fl and Cd79a−/−Ptbp1Cre/+ mice (left margin) 7 d after immunization with NP-KLH, pre-gated on B220−CD19+ cells at left. Numbers in outlined areas indicate percent cells in each gate. b, Frequency (top row) and number (bottom row) of GC B cells and DZ and LZ GC B cells in mice as in a (key), identified as in a, and ratio of DZ GC B cells to LZ GC B cells (DZ/LZ) in such mice (bottom right). Each symbol represents an individual mouse in one area. Numbers in the bracket indicate mean ± s.d. (c) or mean ± s.e. (d) from the data in c, NS, not significant (P > 0.05); *P ≤ 0.05 and **P ≤ 0.001 (two-tailed paired Student’s t-test). c, ELISA of the endpoint titers of anti-NP20 IgG1 (high- and low-affinity IgG1; left) or anti-NP2 IgG1 (high-affinity IgG1 only; right) in the serum of Cd79a−/−Ptbp1fl/fl and Cd79a−/−Ptbp1Cre/+ mice (key) at various times (horizontal axis) after immunization with NP-KLH. d, Ratio of anti-NP1 IgG1 to anti-NP2 IgG1 (NP1/NP2), calculated from the data in c, NS, not significant (P > 0.05); *P ≤ 0.05 and ***P ≤ 0.0001. Cd79a−/−Ptbp1fl/fl versus Cd79a−/−Ptbp1Cre/+ (two-way analysis of variance (ANOVA) plus Sidak’s multiple-comparisons test). Data are from one experiment representative of four independent experiments (a,b) or one experiment representative of two independent experiments with similar results, with three Cd79a−/−Ptbp1fl/fl mice and six Cd79a−/−Ptbp1Cre/+ mice (c,d; mean ± s.d. (c) or mean ± s.e. (d)).

PTBP1 is necessary for antibody affinity maturation. Cd79a−/−Ptbp1fl/fl (cKO) mice produced lower amounts of high-affinity antibodies than did Cd79a−/−Ptbp1Cre/+ (control) mice (Fig. 2c). In Cd79a−/−Ptbp1Cre/+ (control) mice, the ratio of high-affinity antibodies to high- and low-affinity antibodies increased over time, but this ratio remained low in Cd79a−/−Ptbp1fl/fl (cKO) mice (Fig. 2d). Antibodies from mice lacking Ptbp2 in B cells (Cd79a−/−Ptbp1Cre/+ mice) showed no defect in affinity maturation relative to that of antibodies from Cd79a−/−Ptbp1Cre/+ mice (Supplementary Fig. 3f,g). Cd79a−/−Ptbp1Cre/+ (cKO) GC B cells had switched to immunoglobulin G1 (IgG1) in vivo at a greater frequency than had Cd79a−/−Ptbp1Cre/+ (control) GC B cells (Supplementary Fig. 3h), which indicated the presence of functional activation-induced cytidine deaminase (AID) in Ptbp1-deficient GC B cells. Additionally, mutations were found at a similar frequency in the intronic region of the gene encoding heavy-chain joining region 4 in GC B cells sorted from Cd79a−/−Ptbp1Cre/+ mice and those from Cd79a−/−Ptbp1fl/fl mice (Supplementary Fig. 3i,j). Thus, PTBP1 was necessary in B cells for optimal antibody affinity maturation, but this probably did not stem from diminished function of AID.

PTBP2 partially compensates for the loss of PTBP1 in GC B cells. The expression of PTBP2 in Ptbp1-deficient GC B cells might compensate for the absence of PTBP1 in GC cells[1,2]. To address this in the absence of confounding effects due to the deletion of genes during the bone marrow stages of B cell development, we introduced a transgene encoding Cre expressed under the promoter of the gene encoding AID (AicdaTg-Cre) to generate mice with single conditional knockout of Ptbp1 alone (AicdaTg-CrePtbp1Cre/+ (cKO)) or double conditional knockout of both Ptbp1 and Ptbp2 (AicdaTg-CrePtbp1Cre/+Ptbp2Cre/+ (dcKO)). After immunization with sheep red...
PTBP1 regulates mRNA abundance and AS in GC B cells. We performed mRNA sequencing (mRNAseq) on LZ and DZ B cells 21 d after immunization with NP-KLH (data not shown). Thus, PTBP2, but not PTBP3, partially compensated for the loss of PTBP1. 

Fig. 3k). The residual *Aicda*-*Cre*-*Ptbp1*^fl/+*-*Ptbp2*^fl/+* (dcKO) GC B cells had the phenotype typical of LZ B cells (Fig. 3a,b), and they expressed PTBP3, had lower expression of PTBP1 than that of *Ptbp1*-sufficient GC B cells (from *Ptbp1*^fl/+*-*Ptbp2*^fl/+* mice) but expressed no PTBP2 (Fig. 3c,d). No NP-specific IgG1-antibody-secreting cells were detected in *Aicda*-*Cre*-*Ptbp1*^fl/+*-*Ptbp2*^fl/+* (dcKO) mice 21 d after immunization with NP-KLH (data not shown). Thus, PTBP2, but not PTBP3, partially compensated for the loss of PTBP1.

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from \textit{Cd79a}^{+/+}\textit{Ptbp}^{l\text{WT}} (control) mice and \textit{Cd79a}^{Cneo}\textit{Ptbp}^{l\text{WT}} (PTBP1-cKO) mice. In addition, we generated a transcriptome-wide inventory of PTBP1-binding sites in mitogen-activated B cells using individual-nucleotide-resolution cross-linking and immunoprecipitation (iCLIP) (Supplementary Table 1). By combining mRNAseq with iCLIP of PTBP1, we were able to distinguish the direct effects of PTBP1 on the transcriptome from its indirect effects and, through the consideration of positional information, we were able to deduce the likely mechanism of action of PTBP1. Changes in mRNA abundance at the whole-gene level in control LZ B cells relative to those in control DZ B cells were consistent with published changes\textsuperscript{19} and were conserved in \textit{Ptbp1}-deficient GC B cells (Fig. 4a, Supplementary Fig. 4a,b and Supplementary Table 2).

We found 998 genes with increased mRNA abundance and 980 genes with decreased mRNA abundance in PTBP1-cKO LZ GC B cells relative to their abundance in control LZ GC B cells, and 409 genes with increased mRNA abundance and 270 genes with decreased mRNA abundance in PTBP1-cKO DZ GC B cells relative to their abundance in control DZ GC B cells (Fig. 4b and Supplementary Table 2). The changes in genes that were expressed differentially in LZ B cells and DZ B cells due to deletion of \textit{Ptbp1} showed a strong positive correlation (Supplementary Fig. 4c), which indicated that the absence of PTBP1 affected the same genes in LZ B cells that it affected in DZ B cells. PTBP1 can increase mRNA stability when bound to 3′ untranslated regions (UTRs)\textsuperscript{19,23}. Among genes with either increased mRNA abundance or decreased mRNA abundance, the proportion of genes bound by PTBP1 in
Fig. 5 | The positive-selection gene-expression program is reduced after deletion of Ptbp1. a, Genes with different mRNA abundance (DA) in LZ GC B cells due to deletion of Ptbp1, assessed for the following gene sets: targets of c-MYC (GSEA HALLMARK_MYC_TARGETS_V1) (left), genes with higher expression in GC B cells receiving high levels of T cell help than in those not receiving high levels of T cell help (middle), and genes with higher expression in c-MYC+AP4+ LZ GC B cells (right). Each symbol represents an individual mouse; bar tops indicate the mean. *P < 0.05; †P < 0.05 (unpaired two-tailed Student’s t-test). b, Differential AS events (FDR < 0.05) with an inclusion-level difference greater than 10% due to Ptbp1 deletion in LZ GC B cells, assessed for genes in sets as in a (above plots); right margin, gene symbol, followed by the type of AS event (as in Fig. 4c) and the rMATS identification number (Supplementary Table 3; genes bound by PTBP1 near the AS event, Supplementary Table 5). Data are from four independent experiments with four mRNAseq libraries per condition (a,b,e) or are representative of one experiment (c,d).
**Fig. 6 | Late S-phase progression is impaired in the absence of PTBP1.**

**a.** Gating strategy for GC B cells (CD19+CD38+CD95+) at various stages of the cell cycle among splenocytes from Cd79a−/+Ptbp1fl/fl and Cd79a−/+Ptbp1flox/flox mice (above plots) 7 d after immunization with NP-KLH, assessed by flow cytometry on the basis of staining of BrdU and with the membrane-impermeable DNA-intercalating dye 7-AAD. Numbers adjacent to outlined areas indicate percent cells in each phase. **b.** Frequency of cells in each phase of the cell cycle as in a. NS, not significant (P > 0.05); *P < 0.05 and ***P < 0.001 (two-tailed unpaired Student’s t-test). **c.** Gating strategy for the flow-cytometry analysis of dead cells (with the viability dye eFluor780) among LZ (CD86+CXCR4+) or DZ (CD86+CXCR4+) GC B cells (CD95+CD38+CD19+B220) from Cd79a−/+Ptbp1fl/fl and Cd79a−/+Ptbp1flox/flox mice (above plots). Numbers adjacent to outlined areas indicate percent eFluor780− (live) cells (left) or eFluor780+ (dead) cells (right). **d.** Frequency of dead (eFluor780+) cells in mice as in c (key). NS, not significant (P > 0.05); *P < 0.05 (two-tailed unpaired t-test). Each symbol (a-d) represents an individual mouse; bar tops indicate the mean. Data are representative of two mice (a, c) or are from one experiment representative of three independent experiments with similar results (b, d).

PTBP1 is necessary for the c-MYC-dependent program induced after positive selection. To determine if PTBP1 regulated changes in annotated gene-ontology (GO) pathways, we assessed genes with changes at the level of mRNA abundance or AS due to Ptbp1 deletion separately. Several GO terms relevant to the biology of GC B cells showed enrichment for genes with a difference in mRNA abundance due to deletion of Ptbp1 (Supplementary Fig. 5 and Supplementary Table 4). No pathways showed enrichment (false discovery rate, FDR, < 0.1) when AS changes were analyzed (Supplementary Table 4). Instead, individual genes of different pathways were regulated by PTBP1 at the level of AS.

Pathways involved in nucleotide biosynthesis and cell proliferation (Supplementary Fig. 5a–c) showed enrichment for genes with differential mRNA abundance in LZ B cells and DZ B cells due to the lack of PTBP1. In LZ B cells, most of the differentially expressed genes within these pathways had decreased mRNA abundance (Supplementary Fig. 5b). LZ B cells showed enrichment for The Ras protein signaling pathway was also enrichment in LZ B cells with genes that had different mRNA abundance, but most of these genes had increased mRNA abundance due to the lack of PTBP1 (Supplementary Fig. 5b). Pathways linked to B cell differentiation, lymphocyte migration, cholesterol metabolism and the regulation of apoptosis in leukocytes (Supplementary Fig. 5a,c) showed enrichment for genes with different mRNA abundance in DZ B cells due to deletion of Ptbp1. Among the 243 genes with different mRNA abundance that belonged to the enriched pathways, we found that 64 were bound by PTBP1 in their 3′ UTR and only 10 had AS in the absence of PTBP1 (Supplementary Fig. 5b,c). Thus, PTBP1 directly regulated some of these genes but also had for control LZ GC B cells versus control DZ GC B cells (Fig. 4c, 582 events). The 52% overlap of AS events that were different in control LZ B cells versus control DZ B cells and those affected by Ptbp1 deletion (Supplementary Fig. 4g) indicated that PTBP1 affected a substantial proportion of the AS events that differed in LZ B cells versus DZ B cells.

379 AS events were more skipped and 477 AS events were more included (in 670 genes) in PTBP1-cKO LZ B cells than in control LZ B cells (Fig. 4d and Supplementary Table 3). 416 AS events were more skipped and 544 AS events were more included (in 747 genes) in PTBP1-cKO DZ B cells than in control DZ B cells (Fig. 4d and Supplementary Table 3). The proportion of AS events directly regulated by PTBP1, inferred from the binding of PTBP1 in the vicinity of the event, varied depending on the type of AS event and ranged from ~50% in mutually exclusive exons to ~7% in alternative 3′ UTR and only 6% in the LZ and in 8% the DZ (Fig. 4e). Similarly, there was a small overlap between genes with different mRNA abundance and those with different AS due to deletion of Ptbp1 was 6% in the LZ and in 8% the DZ (Fig. 4e). Similarly, there was a small overlap between genes with changes in abundance and AS in the comparison of Cd79a−/+Ptbp1flox/flox (control) LZ B cells versus control Cd79a−/+Ptbp1fl/fl (control) DZ B cells (Fig. 4e). Together our data identified PTBP1 as a post-transcriptional regulator in GC B cells, in which it controlled mRNA abundance and five different types of AS events.
an indirect effect on the expression of genes in these pathways. We investigated further how the lack of PTBP1 affected the expression of genes that are part of the c-MYC hallmark and also of genes induced in response to T cell help. In LZ B cells lacking PTBP1, there was a globally lower mRNA abundance of the genes induced after positive selection relative to the mRNA abundance of such genes in control LZ B cells (Fig. 5b and Supplementary Table 3). We reconstructed the resultant mice with SRBCs and found that the proportion of GFP–c-MYC+ cells or GFP–c-MYC– cells or GFP–c-MYC− cells was similar in each group of mice (Fig. 5c,d). Furthermore, the abundance of Tcap4 mRNA, which encodes AP4 (Fig. 5b), and the phosphorylation of ribosomal protein S6 at Ser240 and Ser244 in SRBCs (Supplementary Fig. 6b in CD79aCre+/Ptpbp1fl/+ (cKO) LZ GC B cells was similar to that in CD79aCre+/Ptpbp1fl/+ (control) LZ GC B cells. Additionally, Cxcr4 and Cxcr5 (which encode chemokine receptors), Bcl6, Bach2, Foxo1 and Batf (which encode transcription factors), Aicda (which encodes AID) and Il21r (which

Fig. 7 | PTBP1 regulates the abundance of Tyms mRNA through control of AS. a, Expression of Tyms (FPKM) in GC B cells and naive B cells (left), in GC B cells receiving high levels of T cell help (Anti-DEC-Ctrl) or not (Anti-DEC-OVA) (middle), or in LZ and DZ B cells expressing c-MYC and AP4 or not (right). b, mRNAseq analysis of Tyms in CD79aCre+/Ptpbp1fl/+ (Ctrl) and CD79aCre+/Ptpbp1fl/+ (P1-cKO) LZ GC B cells (top two rows), presented as 'sashimi plots' showing read coverage and reads that map to exon–exon junctions (arrows; numbers indicate reads that map to that junction), and iCLIP analysis of PTBP1 (below); scale bar (bottom), number of independent X-link sites (PTBP1-binding sites) identified. c, Ratio of NMD reads (mapping to exons and retained introns that would generate an NMD transcript; blue bins in b) to FL reads (mapping to the first three exons needed for the full-length Tyms transcript; blue bins in b) (NMD/FL) for mRNAseq libraries from GC B cells receiving high levels of T cell help or not (left) and in LZ and DZ GC B cells expressing c-MYC and AP4 or not (right) (as in a). d, Expression of Tyms mRNA in cells as in c, presented as DESeq2-normalized read counts. NS, not significant (adjusted P value > 0.1 (DESeq2)); ***P < 0.001 (DESeq2; adjusted P value). e, Flow-cytometry analysis of TYSMS in non-GC B cells (CD19+CD95+CD5+CD23–) and in LZ cells (CD86+CXCR5+) or DZ cells (CD86+CXCR4+) among GC B cells (CD19+CD95+CD38–) from the spleen of mice b d after immunization with SRBCs. *P < 0.05 and ***P < 0.001 (Student’s two-tailed t-test). Each symbol (a–f) represents an individual mRNAseq library (a–e) or mouse (f); bar tops indicate the mean. Data are representative of four independent experiments with two (b) or four (d,e) mRNAseq libraries per condition (b,d,e) or are from one experiment (f).
PTBP1 regulates AS of Pkm. a, Abundance of Pkm1 and Pkm2 transcripts (FPKM) in GC B cells and naïve B cells (left), in GC B cells receiving high levels of T cell help or not (middle) or in LZ and DZ GC B cells expressing c-MYC and AP4 or not (right) (as in Fig. 7a), calculated with the Cuffnorm program of Cufflinks software. b, rMATS inclusion values for exon 9 of Pkm (Pkm1 E9) in LZ and DZ cells (below plot) from Cd79a+/+/Ptbp1fl/fl and Cd79a+/+/Ptbp1Cre/Cre mice (key). c, mRNAseq analysis of Pkm exons 8-10 in Cd79a+/+/Ptbp1fl/fl and Cd79a+/+/Ptbp1Cre/Cre LZ GC B cells (top two rows), and iCLIP analysis of PTBP1 (presented as in Fig. 7b). d, Immunoblot analysis of PKM1 and PKM2 in lysates of B cells freshly isolated from the spleen of Cd79a+/+/Ptbp1fl/fl and Cd79a+/+/Ptbp1Cre/Cre mice (above plots; one mouse per lane) and analyzed without further treatment (Ex vivo; left) or stimulated in vitro for 48 h with lipopolysaccharide (LPS; right), presented along with Ponceau S staining of the blots (cropped images; full images, Supplementary Fig. 8). Left margin, size (in kilodatons). e, Pkm1 and Pkm2 transcripts (FPKM) in our mRNAseq libraries from Cd79a+/+/Ptbp1fl/fl and Cd79a+/+/Ptbp1Cre/Cre (key) LZ and DZ GC B cells (below plots), calculated with Cuffnorm. f, CellTrace Violet profiles of splenic B cells stimulated in vitro for 62 h with antibody to (Anti-) CD40 plus IL-4 and IL-5 or with antibody to IgM plus IL-4 (above plots) in the presence of DASA-58 or vehicle (DMSO, in the same amount as that used for 50 μM DASA-58). g, Quantification of the proliferation profiles in f (left and middle) and absolute number of cells recovered from the cultures (right). NS, not significant (P > 0.05); **P < 0.05, ***P < 0.01 and ****P < 0.001 (unpaired Student’s two-tailed t-test). Each symbol (a,b,e,g) represents an individual mRNAseq library (a.b.e.g) or mouse (g); bar tops indicate the mean. Data are from four independent experiments with four (b,e) or two (c) mRNAseq libraries per condition (b,c.e) or are from one experiment with several mice (d,f,g).

PTBP1 encodes a cytokine receptor that had equivalent expression and AS patterns in Ptbp1-deficient and Cd79a+/+/Ptbp1fl/fl (control) GC B cells (Supplementary Fig. 6c–e and Supplementary Table 3). Thus GC B cells do not require PTBP1 for the sensing of T cell help or the induced expression of c-MYC.

28% of the mRNAs that were part of the c-MYC-dependent gene-expression program induced after positive selection and that were decreased in LZ B cells due to Ptbp1 deletion were bound by PTBP1 in their 3′ UTR (Supplementary Fig. 6a and Supplementary Table 5). This indicated a direct role for PTBP1 in stabilizing a fraction, but not all, of c-MYC-dependent transcripts. Moreover, 22 genes that were induced after positive selection showed differential AS (inclusion-level difference > 10%) in Ptbp1-deficient LZ B cells relative to their AS in Cd79a+/+/Ptbp1fl/fl (control) LZ B cells (Fig. 5c and Supplementary Fig. 6a), and six of these were bound by PTBP1 near the AS event (Supplementary Table 5). Pkm (which encodes pyruvate kinase), Abcb1b (which encodes an ATP-binding-cassette transporter), Tspan33 (which encodes a tetraspanin protein), Phb2 (which encodes the repressor Bcap37) and Dkc1 (which encodes the dyskerin pseudouridine synthase DKC1) were the only genes with
both decreased mRNA abundance and differential AS (inclusion-level difference > 10%) due to the lack of PTBP1. Thus, PTBP1 regulated expression of the c-MYC-dependent gene-expression program both directly and indirectly in positively selected GC B cells by regulating mRNA abundance and AS.

PTBP1 regulates the proliferation of GC B cells. We investigated whether PTBP1 was required for GC B cell proliferation by measuring DNA content and incorporation of the thymidine analog bromodeoxyuridine (BrdU) into GC B cells in vivo. The proportion of Cd79a<sup>Cre</sup>/Ptbp1<sup>fl/fl</sup> (cKO) GC B cells in late S phase (BrdU<sup>-</sup> with high DNA content) was lower than that of Cd79a<sup>Cre</sup>/Ptbp1<sup>+/+</sup> (control) GC B cells in late S phase, in mice immunized with NP-KLH (Fig. 6a,b). In contrast, the proportion of cells that were in early S phase (BrdU<sup>-</sup> with low DNA content) was similar among Cd79a<sup>Cre</sup>/Ptbp1<sup>fl/fl</sup> (cKO) GC B cells and Cd79a<sup>Cre</sup>/Ptbp1<sup>+/+</sup> (control) GC B cells. Additionally, Cd79a<sup>Cre</sup>/Ptbp1<sup>fl/fl</sup> (cKO) GC B cell populations had a greater proportion of cells in G2 and M phases than did Cd79a<sup>Cre</sup>/Ptbp1<sup>+/+</sup> (control) GC B cell populations (Fig. 6a,b). The proportion of cells in late S phase was lower among both LZ B cells (in five of five experiments) and DZ B cells (in four of five experiments) from Cd79a<sup>Cre</sup>/Ptbp1<sup>fl/fl</sup> (cKO) mice than among those from Cd79a<sup>Cre</sup>/Ptbp1<sup>+/+</sup> (control) mice (Supplementary Fig. 7a). We also observed a lower proportion of cells in late S phase among GC B cells from Aicda<sup>Tg-Cre</sup>/Ptbp1<sup>fl/fl</sup> (cKO) mice than among those from Aicda<sup>Tg-Cre</sup>/Ptbp1<sup>+/+</sup> or Ptbp1<sup>i<sup>Cre</sup></sup>/Ptbp1<sup>+/+</sup> mice, after immunization with SRBCs (Supplementary Fig. 7b). The proportion of GC B cells in late S phase was also lower among CD45.2<sup>Ptbp1<sup>−</sup></sup>-deficient cells than among CD45.2<sup>Ptbp1<sup>+</sup></sup>-sufficient cells from B6.SJL competitive chimera reconstituted with a 1:1 mixture of bone marrow cells from CD45.1<sup>B6.SJL</sup> mice and CD45.2<sup>Cd79a<sup>Cre</sup>/Ptbp1<sup>fl/fl</sup></sup> (cKO) mice or from CD45.1<sup>B6.SJL</sup> mice and CD45.2<sup>Cd79a<sup>Cre</sup>/Ptbp1<sup>+/+</sup></sup> (control) mice and then immunized with NP-KLH (Supplementary Fig. 7c), which showed that this was a cell-autonomous defect. In contrast to results obtained for GC B cells, the proportion of cells at different stages of the cell cycle was normal among the highly proliferative early-pre B cells of Cd79a<sup>Cre</sup>/Ptbp1<sup>+/+</sup> (cKO) mice (Supplementary Fig. 7d). Thus, PTBP1 was not universally required in cells that were undergoing cell division but was necessary for the progression of GC B cells through late S phase.

Closer analysis of the expression of genes encoding molecules important for progression through the cell cycle revealed that the expression of Ccn2, Ccn3 and Ccn2 was unaffected in Cd79a<sup>Cre</sup>/Ptbp1<sup>fl/fl</sup> (cKO) GC B cells relative to such expression in Cd79a<sup>Cre</sup>/Ptbp1<sup>+/+</sup> (control) GC B cells (Supplementary Fig. 7e), which suggested that the enrichment for proliferation and nucleotide-biosynthetic GO pathways did not arise from a failure to express early cell-cycle-progression factors. Impaired nucleotide synthesis could cause replication stress as cells progress through S phase and could cause cell death<sup>31</sup>. Flow cytometry using a fixable viability dye to detect non-viable cells showed a higher proportion of dead cells among Cd79a<sup>Cre</sup>/Ptbp1<sup>fl/fl</sup> DZ GC B cells than among Cd79a<sup>Cre</sup>/Ptbp1<sup>+/+</sup> DZ GC B cells (Fig. 6c,d). In contrast, the proportion of dead cells among LZ B cells were similar for Ptbp1<sup>−</sup>-deficient cells and Ptbp1<sup>+</sup>-sufficient cells (Fig. 6c,d). Thus, PTBP1 was necessary for progression through the S and G2-M phases of the cell cycle and promoted the survival of DZ cells.

PTBP1 controls AS of c-Myc-dependent genes that encode molecules important for proliferation. Given the impaired proliferation of Ptbp1<sup>−</sup>-deficient GC B cells, we looked for evidence of PTBP1-dependent AS isoforms among genes that were part of the c-MYC-dependent gene-expression program and encoded molecules important for proliferation. mRNA from the gene encoding thymidylate synthase (Tym<sup>s</sup>, a target of c-MYC, Fig. 5a), which is necessary for de novo nucleotide synthesis, was 30-fold greater in abundance in GC B cells than in naive B cells and increased after positive selection in GC B cells (Fig. 7a). Tym<sup>s</sup> mRNA was spliced differently in the absence of PTBP1 (Fig. 7b). Deletion of Ptbp1 resulted in a complex AS pattern, with increased inclusion of exons and retained introns (yellow bins, Fig. 7b) that generated transcript isoforms predicted to be degraded by NMD. We quantified the ratio of mRNAseq reads that mapped to segments generating transcripts predicted to be degraded by NMD (NMD reads); yellow bins, Fig. 7b) to mRNAseq reads that mapped to the first three exons of Tym<sup>s</sup>, which encode the full-length (FL) protein (FL reads); blue bins, Fig. 7b). The ratio of NMD reads to FL reads was reduced after positive selection in GC B cells (Fig. 7c), which indicated an increase in protein-coding Tym<sup>s</sup> mRNA in positively selected GC B cells. The ratio of NMD reads to FL reads was higher in Ptbp1<sup>−</sup>-deficient LZ and DZ B cells than in Ptbp1<sup>+</sup>-sufficient LZ and DZ B cells (Fig. 7d). The abundance of Tym<sup>s</sup> mRNA (at the whole-gene level) was reduced due to deletion of Ptbp1 in LZ B cells (Fig. 7e). TMY<sup>s</sup> protein was decreased in abundance due to deletion Ptbp1 in LZ and DZ B cells (Fig. 7f). Consistent with a direct role for PTBP1 in regulating those Tym<sup>s</sup> AS events, icLIP analysis showed Ptbp1<sup>−</sup> bound to the AS region of Tym<sup>s</sup> (Fig. 7b). These data indicated that PTBP1 ensured increased expression of TYM<sup>s</sup> during positive selection by regulating AS of Tym.<sup>s</sup>

The pyruvate kinase PKM catalyzes the conversion of phosphoenolpyruvate to pyruvate in glycolysis and is also encoded by a target of c-MYC<sup>26</sup> that was spliced differentially due to deletion of Ptbp1 (Fig. 5e). Pkm encodes two protein isoforms generated from mutually exclusive inclusion of exon 9 (PKM1) or exon 10 (PKM2). Whereas PKM1 exists only as a highly active tetrameric form, PKM2 interchanges among less-active monomeric and dimeric forms as well as a fully active tetramer in response to nutrient availability and energy demands<sup>26</sup>. B cells expressed Pkm<sup>2</sup> almost exclusively, and Pkm<sup>2</sup> was induced after positive selection in GC B cells, but Pkm<sup>1</sup> was not (Fig. 8a). In the absence of PTBP1, the inclusion level of exon 9 increased from 0.05 to 0.2 (Fig. 8b,c). Immunoblot analysis of proteins from naive and mitogen-activated B cells in vitro revealed that PKM1 was hardly detected in Cd79a<sup>Cre</sup>/Ptbp1<sup>fl/fl</sup> B cells but was readily detected in Cd79a<sup>Cre</sup>/Ptbp1<sup>+/+</sup> B cells (Fig. 8d). This AS event has been shown to be regulated by PTBP1 in human cell lines to favor the production of PKM2<sup>26,31</sup>. Our icLIP data revealed binding of PTBP1 close to the intronic 3’ splice site of Pkm exon 9 in B cells (Fig. 8e). Thus, PTBP1 promoted skipping of this exon in favor of the inclusion of exon 10 and suppression of Pkm<sup>1</sup> mRNA in GC B cells (Fig. 8e).

Expression of PKM1 has been shown to impair the proliferation of malignant cells and non-transformed fibroblasts, and the small molecule DASA-58 activates the tetramerization of PKM2 and inhibits the proliferation of transformed cells<sup>26</sup>. DASA-58 reduced the proliferation of mouse B cells in response to in vitro stimulation with antibody to the costimulatory receptor CD40 plus the cytokines IL-4 and IL-5 or stimulation with antibody to IgM plus IL-4 (Fig. 8f,g). These findings were consistent with the hypothesis that increased activity of pyruvate kinase, as would be expected after PKM1 expression, is detrimental for B cell proliferation.

**Discussion**

PTBP1 is either induced by and acts downstream of c-MYC or forms part of a previously unrecognized pathway that acts in parallel with c-MYC to drive GC B cell proliferation. We favor the former hypothesis, as there is evidence that PTBP1 is encoded by a c-MYC-responsive gene in other cell systems<sup>38,39</sup>. A proliferation defect of Ptbp1<sup>−</sup>-deficient embryonic stem cells and human CD4<sup>+</sup> T cells with reduced levels of PTBP1 has been observed<sup>38,39</sup>, which indicates that PTBP1 is necessary for cell proliferation in other systems, although the basis for the reported effects is not clear. However, the requirement for PTBP1 in proliferation is not universal, as the
proliferation of Ptbp1-deficient early pre-B cells was normal. This difference could reflect compensatory mechanisms or the distinct environment of the GC versus that of the bone marrow.

PTBP1 controlled gene expression by regulating multiple processes in the biogenesis and fate of mRNA. The mRNA abundance or AS of 213 genes that were part of the c-MYC-dependent gene-expression program induced after positive selection was PTBP1 dependent, but there were additional direct and indirect targets of PTBP1 that were not part of the MYC-dependent program. Among those, we observed changes in the AS of Sema4d, Pdlim7, Pbrm1, Acly and Ick3β. Changes in semaphorin 4d (CD100; encoded by Sema4d) could affect the migration of GC B cells through the LZ and DZ and interactions with T cells. Changes in enigma (encoded by Pdlim7) could alter expression of the tumor suppressor p53. Altered splicing of the gene encoding ATP citrate lyase (Acly) could have an effect on lipid biosynthesis. Polybromo-1 (BAF180; encoded by Pbrm1) is part of the SWI–SNF-B (PBAF) chromatin-remodeling complex and is a cofactor of c-MYC that might help propagate the c-MYC-induced gene-expression program, and alterations in Aiolos (encoded by Ick3β) might influence plasma cell formation. Despite the potential for molecules encoded by these genes to have roles in GC B cell biology, most of the alternative isoforms found have not been studied before, to our knowledge. Elucidation of the functions of molecules encoded by these AS transcripts in GC B cell biology will require careful investigation.

Pkm and Tymys are but two examples of c-MYC-regulated genes that are subject to PTBP1-dependent AS. Inhibition of Tymys by 5-flurouracil blocks the proliferation of primary T cells and B cells in vitro (data not shown). Splicing of the retained intron upstream of exon 2 from the full-length Tymys transcript is necessary for increased Tymys mRNA expression in cultured cells. Reduced amounts of Tymys could be one limiting factor for the proliferation of positively selected GC B cells. Other events regulated by PTBP1 must also promote GC B cell proliferation, and our results indicate that the regulation of Pkm activity is important for B cell proliferation. PTBP1, by regulating the AS of Pkm, might limit glycolytic flux and thereby contribute to biosynthetic pathways through the accumulation of glycolytic intermediates. Consistent with that, Pkm1 is growth inhibitory when it is expressed in cancer xenograft tumor models, and B cell proliferation in vitro was inhibited by activators of Pkm2.

Published studies have reported a function for PTBP2 in antibody class-switch recombination. We did not detect PTBP2 expression in GC B cells unless Ptbp1 was deleted, and secretion of antigen-specific IgG1 was unaffected in mice with Ptbp2-deficient B cells. Therefore, it is unlikely that PTBP2 promotes class-switch recombination in GC B cells. Nonetheless, the increase in the frequency of IgG1-positive GC B cells among Ptbp1-deficient GC B cells might indicate that, if expressed, PTBP2 might indeed promote antibody class-switch recombination.

There might be additional PTBP1-mediated post-transcriptional processes of importance to the GC reaction. Changes in APA analyzed with the DaPars tool found that deletion of Ptbp1 affected the APA of four genes in LZ B cells and seven genes in DZ B cells (data not shown), suggestive of a more limited role for PTBP1 in regulating APA in GC B cells than its roles controlling AS and mRNA abundance. However, full elucidation of the roles of PTBP1 in APA will require analysis of RNA-based next-generation sequencing libraries specifically targeted at capturing 3′ ends. In the present study, we were unable to measure the effect of PTBP1 on the tempo of translation, such as through internal-ribosomal-entry-site-mediated regulation, within GC B cells, and this must await improved techniques for measuring translational regulation in rare cell populations.

In summary, we observed that the regulation of gene expression in B cells by PTBP1 was necessary for GC B cell proliferation. At the cellular level, PTBP1 functioned in GC B cells to promote the rapid progression through the late S phase of the cell cycle. At the molecular level, we identified the role of PTBP1 in regulating the quantitative and qualitative changes in the transcriptome that were part of the c-MYC-dependent gene-expression program. Post-transcriptional regulation by PTBP1 acted in concert with transcription factors such as c-MYC to integrate anabolic metabolism and cell-cycle progression and to drive the production of high-affinity antibodies.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41590-017-0035-5.

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**Author contributions**
E.M.-C., C.W.J.S. and M.T. designed experiments; E.M.-C., M. Screen, M.D.D.-M., S.E.B. performed and analyzed experiments; E.M.-C. and M.T. wrote the manuscript with input from the co-authors.

**Competing interests**
The authors declare no competing financial interests.

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Methods

Mice. All mice were on a C57BL/6 background. For bone marrow chimera experiments, B6.SJL were used as recipients. Conditional-knockout mice used in this study derive from crossing the following transgenic strains: Ptpn6tm1Slek (Cd79aCre/Fl), Ptpn6tm2Slxt (Cd79aCre/Fl), Ptpn6tm1Msol (Cd79bCre/Fl), and Cd79atm1Fwa (Cd79bCre/Fl). Mice expressing the floxed Aicda and Cd79a transgenes were generated as previously described14 using two different conjugation methods.

RT7b rats were used for the generation of monoclonal antibodies to SRBCs. B6.SJL mice with 3 × 10^6 bone marrow cells derived from B6.SJL mice at a 1:1 ratio with 25 mg NP-KLH (Gibco) or 25 mg NP-KLH (Biosearch Technologies) intraperitoneally. Mice immunized with SRBCs received 2 × 10^6 SRBCs intraperitoneally. For in vivo BrdU incorporation experiments, mice received 2 mg BrdU intraperitoneally 1 h before they were culled. Bone marrow competitive chimeras were generated by reconstitution of lethally irradiated (300 rads, twice) B6.SJL mice with 3 × 10^6 bone marrow cells from B6.SJL mice at a 1:1 ratio with 25 mg NP-KLH (Gibco) or 25 mg NP-KLH (Biosearch Technologies) intraperitoneally. All experiments were approved by the Babraham Institute’s Animal Welfare and Experimentation Committee and the UK Home Office and are in compliance with all relevant ethical regulations. Mice were housed in a specific pathogen-free environment. Animals were checked daily for signs of distress and were culled if necessary. Before immunoprecipitation of PTBP1, cells were treated with Turbo DNase (Ambion) and small amounts of RNAse A (1.5 to 3 units) from Ambion (Cat. # AM2294). Immunoprecipitates were separated by SDS-PAGE and RNA-protein complexes were transferred to a nitrocellulose membrane. Protein–RNA complexes were isolated from the nitrocellulose membrane after cutting only the areas (from ~75 to ~120 kDa) where PTBP1 was expected to be cross-linked to long RNAs. After protein digestion, RNA was isolated and cDNA was synthesized by reverse transcription. At this time bar-coded primers were used that allow the identification of cDNAs generated from the same RNA molecule (with a random unknown four-nucleotide barcode), which allows the discrimination of PCR duplicates, and then multiplexing of several samples together. Amplification of iCLIP cDNA libraries was done with 20–27 PCR cycles. We carried out five replicates. Multiplexed iCLIP cDNA libraries were sequenced on an Illumina HiSeq 2000 platform on a 50-bp single-ended mode. Negative controls (immunoprecipitation of PTBP1 from non–UV-cross-linked lysates and immunoprecipitation with a mouse IgG1 negative isotype-matched control antibody (MOPC-1, Sigma, Cat. # 015K4806) resulted in very little isolated RNA, from which no cDNA library could be generated.

Computational processing of iCLIP data. Identification of transcriptome-wide PTBP1 binding sites was done as previously described34. In brief, mapping of cDNAs to the mouse genome (mm10) was carried out with Bowtie, and those reads that mapped to the same location and had the same random four-nucleotide barcode were considered PCR duplicates and collapsed as a single cDNA molecule. A PTBP1-binding site (or X-link site) is the nucleotide before the first nucleotide of a mapped cDNA molecule. An FDR value, which determines the probability of a X-link site to appear by chance, was calculated (as described34) for each X-link site. iCLIP is highly dependent on RNA abundance and therefore, is not an absolute measurement of RNA-protein interactions. For this reason we pooled the five replicates together before calculating FDRs for each X-link site.

Generation of mRNAsq libraries. LZ and DZ GC B cells were sorted by flow cytometry from C57/6tm1Slek or C57/6tm2Slxt mice immunized with alum-NP KLH 7 days before. Single-cell suspensions were prepared from spleens of immunized mice. Erythrocytes were lysed and GC B cells were enriched before sorting by flow cytometry by depleting of cells stained with biotinylated anti-IgD (1:260, 2a, Southern Biotech), anti-CD3e (145-2C11, eBioscience), and anti-Gr1 (RB6-8C5, Thermofisher) and anti-Ter119 (TER-119, eBioscience) by MACs. Flow cytometry replicates were performed under conditions of standard biological replicate. GC B cell–enriched splenocytes from three to five mice of the same genotype and sex were pooled together before sorting by flow cytometry. LZ and DZ GC B cells were sorted from the same GC B cell–enriched samples. LZ and DZ GC B cells from C57/6tm1Slek or C57/6tm2Slxt mice were sorted on the same day.

Analysis of mRNAsq libraries generated in this study. Trimming of libraries was carried out with Trimgalore (v0.4.2) using default parameters. After that, reads were mapped to the mouse Mus_musculus.GRCm38 genome with Hisat2 using the HTSeq count command. The ends of mapped reads were annotated with sashimi plots using Cufflinks (Agilent). RNA integrity numbers ranged from 9.1 ng to 10.2 ng of total RNA was used to generate cDNA from polyadenylated transcripts using the SMART-Seq v4 Ultra low input RNA kit from Clontech (Cat. # 638488). cDNA quality was analyzed on a 2100 Bioanalyzer (Agilent). cDNA libraries were prepared to the mRNAsq libraries with eight cycles of PCR using the Ultra Low library preparation kit v2 from Clontech (Cat. # 634899). Compatible barcoded libraries were multiplexed and sequenced across three lanes on an Illumina HiSeq 2500 platform on a 100-bp paired-end mode.

Analysis of mRNAsq libraries generated in this study. Trimming of libraries was carried out with Trimgalore (v0.4.2) using default parameters. After that, reads were mapped to the mouse Mus_musculus.GRCm38 genome with Hisat2 using the HTSeq count command. The ends of mapped reads were annotated with sashimi plots using Cufflinks (Agilent). RNA integrity numbers ranged from 9.1 ng to 10.2 ng of total RNA was used to generate cDNA from polyadenylated transcripts using the SMART-Seq v4 Ultra low input RNA kit from Clontech (Cat. # 638488). cDNA quality was analyzed on a 2100 Bioanalyzer (Agilent). cDNA libraries were prepared to the mRNAsq libraries with eight cycles of PCR using the Ultra Low library preparation kit v2 from Clontech (Cat. # 634899). Compatible barcoded libraries were multiplexed and sequenced across three lanes on an Illumina HiSeq 2500 platform on a 100-bp paired-end mode.
complementary to all annotated exons of a particular gene in the RNAseq data. Information on the genotype and sex of the animals was included in the design formula in order to control for variation in the data due to the sex differences in the samples. DESeq2 results were only considered for genes that are expressed at least 1 FPKM in any of the conditions. Significant differentially abundant genes are those that have an adjusted P value of <0.1.

Differential AS was analyzed with rMATS (v3.2.2.3). rMATS uses an exon-centric approach to discover both annotated and unannotated AS events in a gene. Transcriptomic conservation matrices were compared across AS between two conditions. rMATS calculates first inclusion levels (defined as the proportion of transcripts containing that particular AS segment) for five different types of AS events: skipped exons (SE), mutually exclusive exons (MXE), alternative 5′ splice sites (A5SSs) alternative 3′ splice sites (A3SSs) and retained introns in each of the two conditions (Supplementary Fig. 4e). Subsequently, rMATS calculates inclusion level differences for each of these inclusion levels from 16 condition two (Supplementary Fig. 4f). The version of rMATS used accepts only mapped reads of a particular length. For this reason, libraries trimmed with Trimgalore as described above were further trimmed with Trimomatic v0.35 so that all reads had a length of 98 bp. Reads shorter than 98 bp were discarded. These reads of only 98 bp were then mapped to the mouse genome using Hisat2 as described above. rMATS was run on a paired mode (-analysis P) to analyze differential AS between two conditions using the Mus_musculus.GRCm38.84.gtf annotation. Only results obtained with reads that map to exon–exon junctions were used for further analysis. Genes that have less than 1 FPKM in one of the conditions are discarded. Significantly differentially spliced events are those that have an FDR < 0.05. A cut-off of an inclusion-level difference greater than 10% (0.1) was introduced for significant differentially alternatively spliced events.

We assigned PTBP1 binding to the vicinity of a differentially alternatively spliced event if we found at least a significant PTPB1-binding site in our iCLIP data in the flanking constitutive exons (SE) or alternatively spliced exons (MXE) were bound by PTBP1 if a binding site was identified on the SE, on the intronic 500 nucleotides upstream and downstream of the SE. 3′ splice site (SS) and 5′ SS, respectively; on the upstream flanking constitutive exon and the intronic 500 nucleotides downstream of its 5′ SS or on the downstream flanking constitutive exon and the intronic 500 nucleotides upstream of its 3′ SS. Mutually exclusive exons (MXEs) were bound by PTBP1 if a binding site was found on any of the MXE and the intronic 500 nucleotides upstream and downstream of their 3′ SS and 5′ SS, respectively; on the upstream flanking constitutive and the intronic 500 nucleotides downstream of its 5′ SS or on the downstream flanking constitutive exon and the intronic 500 nucleotides upstream of its 3′ SS. Alternative 5′ splice sites (A5SSs) and retained introns were determined by the rMATS method and the mapped reads of the different RNAseq libraries described above. GO analysis. Gene ontology (GO) term–enrichment analysis was carried out with GOrilla. A background list of genes (genes expressed with at least 1 FPKM in the conditions analyzed) was included in the analysis. For visualization purposes, when several related terms were significantly enriched, the term with a higher percentage of significant genes with different mRNA abundance or AS was chosen to be presented in Supplementary Fig. 5.

RNaseq data visualization. Sashimi plots, which show RNAseq coverage and reads mapping across exon–exon junctions, were generated using the IGV genome browser.

Analysis of Jh4 mutations. Mutations in the Jh4 intronic region were analyzed as previously described. In brief, genomic DNA was isolated from GC B cells sorted by flow cytometry from the spleen of mice immunized with alum-NP-KLH 7 d before GC. B cells from two to four mice of the same genotype and sex were pooled together. Jh4 intronic regions were amplified with the primers Jh4-intron Forward and Jh4-intron Reverse in using the Phusion Ultra II (2×) kit using the Agilent Technologies (PCR with 35 cycles, 57°C annealing temperature and 15 s extension time at 72°C. PCR amplified Jh4 intronic regions were cloned using the Zero Blunt TOPO PCR Cloning Kit (ThermoFischer scientific). Mutation frequencies were calculated by dividing the total number of mutations identified in each replicate by the total length of amplified DNA (565 bp per clone analyzed, which is the genomic region amplified by PCR without taking into account the regions complementary to the primers).

Generation of monoclonal antibody to PTBP3. RT7b rats were immunized with a GST–PTBP3 fusion protein containing amino acids 279–359 of the full-length mouse PTBP3 generated in Escherichia coli. After several immunizations, spleens were isolated and fused to the IR938F rat myeloma cell line. Hybridomas were screened by immunoblot using HKT1 cell lysates expressing mouse PTBP3, PTBP1 or PTBP2-GFP fusion proteins. Two hybridomas secreted IgG2a antibodies specific for PTBP3: MAC454 and MAC455. Monoclonal antibodies secreted by these two hybridomas were purified by affinity chromatography using protein G.

Immunoblot analysis. In order to analyze PKM1 and PKM2 expression, 12 μg of proteins extracted from B cells isolated as described above from individual mice and stimulated or not with LPS for 4 h were separated by 10% SDS-PAGE and transferred to a nitrocellulose membrane. PKM1 detection was carried out with an HRP-conjugated goat anti-PKM1 (clone D30G6, Cell Signaling) and PKM2 was detected with a rabbit monoclonal antibody (clone D78A4, Cell Signaling). Visualization of anti-PKM1 and anti-PKM2 was carried out with an HRP-conjugated goat anti-rabbit (Cat #2020-10, Dako). Immunoblot analysis of the different datasets was carried out with 15 μg of proteins extracted from B cells isolated as described above from individual mice and transferred to a nitrocellulose membrane. Mouse monoclonal primary antibodies were detected with TrueBlot HRP-conjugated anti-mouse (eBioscience, Cat. #18-8817-31). Rat monoclonal primary antibodies were detected with HRP-conjugated goat anti-rat (Cat. #P0450, Dako). Rabbit primary antibodies were detected with HRP-conjugated goat anti-rabbit (Cat. #2020-10, Dako).

Quantification and statistical analysis. Flow-cytometry data were analyzed using Flowjo (versions 10.0.8r1 or 9.8.3). Analysis and quantification of RNAseq
and iCLIP experiments is detailed in the following sections of methods: Analysis of mRNAseq libraries generated in this study, Analysis of mRNAseq libraries previously published, Calculation of FPKM and Computational processing of iCLIP data. Statistical significance of flow cytometry data was assessed using Prism (versions 7 or 6). The details of the tests used in different experiments can be found in the figure legends.

Life Sciences Reporting Summary. Further information on experimental design and reagents is available in the Life Sciences Reporting Summary.

Data availability. mRNAseq and iCLIP data that support the findings of this study have been deposited in GEO with the accession code GSE100969.

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Experimental design

1. Sample size

Describe how sample size was determined.

A priori the effect size and standard deviation of our tested groups are unknown, thus no specific sample sizes were calculated. In exploratory experiments a sample size of 3 to 6 animals per group was used. This was sufficient to detect relevant differences.

2. Data exclusions

Describe any data exclusions.

In Figure 1b, data from one mouse was excluded because the fluorescence intensity for the PTBP3 intracellular staining was 1.5-fold higher than the mean for both non-GC and GC B cells. The reason why this data point was excluded is because this was seen only once in one out three experiments. In the experiment shown in Figure 3 one mouse had a expansion of non lymphoid cells. Data collected from this mouse was excluded. In Supplementary Figure 3c data from two mice were excluded from the graphs showing absolute numbers because they had splenomegaly. Rarely, the fluorescence intensity of the BrdU staining in GC B cells was not sufficient to allow proper gating of cells in early/late S-phase. Data from mice where this occurred were excluded from the analysis. Occasionally control or conditional KO mice did not respond at all to the immunisation protocols. The data from these mice is not included in the figures.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All replication experiments were successful.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

For experiments with mice, samples were randomly analyzed with out separating mice by genotype. For mRNaseq experiments, four biological replicates were used per condition. In each biological replicate, GC B cell-enriched splenocytes from 3 to 5 animals of the same genotype and sex were pooled together before FACS sorting. LZ and DZ GC B cells were sorted from the same GC B cell enriched samples. LZ and DZ GC B cells from control CD79a+/+Ptbp1fl/fl and CD79acre/+Ptbp1fl/fl cKO mice were sorted on the same day. Two biological replicates per condition were from females and two from males.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Blinding was used in immunizations of mice. Blinding was not used when data were collected and analyzed from the test and control groups to ensure alternate measurements for control and test samples.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.
6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

- n/a
- Confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- A statement indicating how many times each experiment was replicated
- The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
- A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted
- A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

7. Software

Describe the software used to analyze the data in this study.

To analyze mRNAseq libraries the following software was used: Trimgalore (v0.4.2), Hisat2 (v2.0.5), HTSeq, DESeq2 (v1.12.1, v1.4.5, v1.8.1), rMATS (v3.2.2), Trimomatic (v0.35), R, DaPars (v0.9.1), TopHat2(v2.0.12) and Cuffnorm option of Cufflinks (v2.2.1), GOrilla, IGV genome browser.

Flow cytometry data were analyzed with FlowJo (versions 10.0.8r1 or 9.8.3).

Statistical analysis was carried out with Prism (versions 7 or 6), except for genome wide data which was carried out with the software listed above.

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

Mouse strains are subject to MTAs as follows: Ptbp1tm1Msol Michele Solimena TU Dresden, Germany; Ptbp2tm1.1Dbk Douglas L. Black, UCLA MIMG, Los Angeles, USA; Cd79atm1(cre)Reth allele Michael Reth University of Freiburg, Germany; AicdaTg-cre (Tg(Aicda-cre)9Mbu Meinrad Busslinger, IMP Vienna, Austria; and Myctm1Slek, B.P Sleckman, Washington University, St Louis, USA.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Novel anti-PTBP3 monoclonal antibodies are reported in this study and their validation by immunoblot of proteins derived from mouse primary B cells is shown in Supplementary Figure 2a.

Other antibodies also listed in Supplementary Table 6 used were:
PTBP1 (mouse IgG1, CLONE 1) ThermoFisher Scientific 32-4800
PTBP2 (mouse IgG2a, S43) Solimena Laboratory
PTBP3 (rat IgG2a, MAC454) Turner Laboratory
B220 (RA3-6B3) BUV395 BD Biosciences 563793
B220 (RA3-6B3) biotin eBioscience 48-0452-82
B220 (RA3-6B3) PE-Cy7 eBioscience 25-0452-82
B220 (RA3-6B2) FITC TONBO biosciences 35-0452
CD19 (6D5) FITC BioLegend 152404
CD19 (6D5) BV785 BioLegend 155543
CD19 (1D3) BUV737 BD Biosciences 564296
CD19 (6D5) BV421 BioLegend 115538
CD95 (Jo2) BV786 BD Biosciences 563646
CD95 (Jo2) BV786 BD Biosciences 740906
CD86 (GL-1) BV421 BioLegend 105031
CD86 (GL-1) biotin BD Biosciences 553690
CD38 (90) PerCP/Cy5.5 BioLegend 102722

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials
10. Eukaryotic cell lines
   a. State the source of each eukaryotic cell line used. No eukaryotic cell lines were used.
   b. Describe the method of cell line authentication used. No eukaryotic cell lines were used.
   c. Report whether the cell lines were tested for mycoplasma contamination. No eukaryotic cell lines were used.
   d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use. No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals
   Provide details on animals and/or animal-derived materials used in the study.
   All mice used in this study were on a C57BL/6 background. For bone marrow chimera experiments, B6.SJL were used as recipients. Conditional knockout mice used in this study derive from crossing the following transgenic strains: Ptbp1fl/fl (Ptbp1tm1Msol)30, Ptbp2fl/fl (Ptbp2tm1.1Dblk)49, Cd79acre(Cd79atm1(cre)Reth)50 and AicdATg-cre (Tg(Aicda-cre)9Mbu)51 as specified in the results section. GFP-c-MYC reporter mice Myctm1Slek 52 and Rag2-/- knockout mice (Rag2tm1Fwa)53 were also used in this study. Male and female mice were used in this study. RT7b rats were used for the generation of anti-PTBP3 monoclonal antibodies.

Policy information about studies involving human research participants

12. Description of human research participants
   Describe the covariate-relevant population characteristics of the human research participants.
   The study did not involve human research participants.
ChIP-seq Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data deposition

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

2. Provide all necessary reviewer access links. The entry may remain private before publication.
   The data has been deposited in GEO: GSE100969 accession number The token efercweczvizhgd gives access to it, until it is made publicly available

3. Provide a list of all files available in the database submission.
   The Fastq files for the five PTBP1 iCLIP replicates is available (GSM2698371 PTBP1 iCLIP replicate 1, GSM2698372 PTBP1 iCLIP replicate 2, GSM2698373 PTBP1 iCLIP replicate 3, GSM2698374 PTBP1 iCLIP replicate 4 and GSM2698375 PTBP1 iCLIP replicate 5).
   In addition to the raw data, the following files are available: GSE100969_PTBP1_B_LPS_All_peaks_id77424_lowFDR.bed.gz contains the processed significant PTBP1 binding sites from all the replicates together.
   GSM2698371_PTBP1_B_LPS_1_peaks_id77154_lowFDR.bed.gz contains the processed significant PTBP1 binding sites from replicate 1.
   GSM2698372_PTBP1_B_LPS_2_peaks_id77159_lowFDR.bed.gz contains the processed significant PTBP1 binding sites from replicate 2.
   GSM2698373_PTBP1_B_LPS_3_peaks_id77150_lowFDR.bed.gz contains the processed significant PTBP1 binding sites from replicate 3.
   GSM2698374_PTBP1_B_LPS_4_peaks_id77151_lowFDR.bed.gz contains the processed significant PTBP1 binding sites from replicate 4.
   GSM2698375_PTBP1_B_LPS_5_peaks_id77155_lowFDR.bed.gz contains the processed significant PTBP1 binding sites from replicate 5.

4. If available, provide a link to an anonymized genome browser session (e.g. UCSC).

Methodological details

5. Describe the experimental replicates.
   5 biological replicates were carried out.

6. Describe the sequencing depth for each experiment.
   iCLIP cDNA libraries were carried out with 20 to 27 PCR cycles. Random barcodes were used that allow unique reads identification. 267211, 300341, 62683, 325568, unique reads mapping to the sense strand were obtained in the 5 biological replicates. 8192, 8983, 5354, 13861 and 8963 unique reads mapping to the antisense strand were found in the 5 biological replicates.
   Libraries were sequenced on an Illumina HiSeq 2000 platform on a 50 bp single-end mode.

7. Describe the antibodies used for the ChIP-seq experiments.
   Anti-PTBP1 antibody (Thermofisher Catalog#: 32-4800), was used for iCLIP experiments. This antibody was validated by Western Blot (Supplementary
8. Describe the peak calling parameters.

Peak calling was carried out as in König, J. et al. iCLIP reveals the function of hnRNP particles in splicing at individual nucleotide resolution. Nat. Struct. Mol. Biol. 17, 909–915 (2010).

9. Describe the methods used to ensure data quality.

Control experiments such as RNA digestion high RNAse concentrations, immunoprecipitation without UV-light cross-linking and negative control isotype experiments were included.

10. Describe the software used to collect and analyze the ChIP-seq data.

Analysis of the iCLIP and software used to process the raw reads are described in Online Methods.
Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

▶ Data presentation

For all flow cytometry data, confirm that:

- 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- 2. 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).
- 3. All plots are contour plots with outliers or pseudocolor plots.
- 4. A numerical value for number of cells or percentage (with statistics) is provided.

▶ Methodological details

5. Describe the sample preparation.
   Single cell suspensions were prepared from tissues by passing the tissues through cell strainers with 70 and 40 μm pore sizes.

6. Identify the instrument used for data collection.
   BD LSRFortessa cytometers from BD Biosciences.

7. Describe the software used to collect and analyze the flow cytometry data.
   FlowJo versions 10.0.8r1 and 9.8.3.

8. Describe the abundance of the relevant cell populations within post-sort fractions.
   Purity of FACS-sorted samples was analysed by flow cytometry.
   Purity of the samples was >89%.

9. Describe the gating strategy used.
   Supplementary Figure 1e shows an exemplifying complete gating strategy of germinal centre B cells. Gates indicating boundaries between "positive" and "negative" are shown through out the figures with exemplifying plots.

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