Environmental sensing by mature B cells is controlled by the transcription factors PU.1 and SpiB

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Humoral immunity requires B cells to respond to multiple stimuli, including antigen, membrane and soluble ligands, and microbial products. Ets family transcription factors regulate many aspects of haematopoiesis, although their functions in humoral immunity are difficult to decipher as a result of redundancy between the family members. Here we show that mice lacking both PU.1 and SpiB in mature B cells do not generate germinal centers and high-affinity antibody after protein immunization. PU.1 and SpiB double-deficient B cells have a survival defect after engagement of CD40 or Toll-like receptors (TLR), despite paradoxically enhanced plasma cell differentiation. PU.1 and SpiB regulate the expression of many components of the B cell receptor signaling pathway and the receptors for CD40L, BAFF and TLR ligands. Thus, PU.1 and SpiB enable B cells to appropriately respond to environmental cues.

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Antibody-mediated immunity relies on the ability of B cells to respond to multiple environmental stimuli including antigen, Toll-like receptor (TLR) ligands, and T-cell-derived help, including CD40L and the cytokines interleukin-4 (IL-4) and IL-21. The survival of mature B cells and plasma cells also depends on members of the tumor necrosis factor receptor superfamily (TNFRSF), including the B-cell-activating factor receptor (BAFF-R). Mature B cells, including follicular and marginal zone (MZ) B cells, are quiescent and relatively long lived. After exposure to cognate antigen, B cells re-enter the cell cycle and undergo multiple rounds of division, as well as initiating immunoglobulin class switch recombination (CSR). Proliferating B cells have the potential to differentiate into short-lived plasmablasts that provide the immediate, but low affinity, antibody that is important early in the immune response. Alternatively, in response to antigen and T cell help, activated B cells can enter a structure termed the germinal center (GC), where they undergo clonal amplification and somatic hypermutation and differentiation into plasma cells that secrete high-affinity antibodies. GCs also produce memory B cells that can rapidly differentiate into plasma cells upon re-exposure to antigen.

A complex network of transcription factors controls aspects of the B cell response to antigen. This network includes factors that are essential for B cell proliferation and the GC response, including PAX5, BACH2, IRF4/BATF, IRF8, NFκB, E-proteins (E2A, E2-2) and Oct2/0BF1, whereas a smaller group, including high concentrations of IRF4, BLIMP-1/PRDM1, ZBTB20 and XBP1, are required for plasma cell differentiation and antibody production (reviewed in refs. 3–5). We have reported a role for a complex of the transcription factors PU.1 and IRF8 in negatively regulating plasma cell differentiation in cell culture, although the role of these factors in vivo is unclear.

The Ets family transcription factor PU.1, encoded by the Sp1 gene, is a major regulator of haematoopoiesis, controlling the expression of hundreds of genes including growth factor receptors, adhesion molecules, transcription factors and signaling components. PU.1-deficient mice lack all lymphocytes, including B cells, suggesting that PU.1 is an essential regulator of the B cell developmental pathway; however, this requirement is limited to early lymphopoiesis as conditional deletion of PU.1 in CD19-expressing B cells is compatible with normal development and function. This minimal consequence of PU.1 loss in B cells is surprising, as PU.1 is well-known to bind tens of thousands of sites in the B cell genome. One possible explanation for this discrepancy is the strong expression of Spib, the most closely related Ets family member in B cells, that binds to the identical nucleotide sequence GGAA. Indeed, Spib−/− mice have markedly reduced B-cell numbers, and inactivation of PU.1 and Spib in bone marrow B-cell progenitors results in a block in development at the pre-B-cell stage and the formation of pre-B cells addicted to CSF-1R (ALL). A similar developmental block and leukemogenesis is also observed in B cells lacking PU.1 or IRF4 or IRF4/IRF8, demonstrating that an Ets/IRF complex controls bone marrow B-lymphopoiesis.

To better define the function of PU.1 and Spib in mature B cells, we here utilize the Cd23-Cre strain to inactivate PU.1 in Spib−/− mice at the transitional B-cell stage in the spleen, allowing us to bypass the developmental block that occurs when both PU.1 and Spib are deleted in immature B cells. We find that PU.1/Spib reductively control mature B cell survival and differentiation in response to a diverse range of stimuli. PU.1/Spib-deficient B cells cannot signal efficiently through the B-cell receptor (BCR) and thus mutant mice lack antigen-specific B-cell responses entirely. PU.1 and Spib directly control many components of the BCR pathway as well as important receptors for cell-derived signals and microbial products. In keeping with this observation, PU.1/Spib-deficient B cells are not very responsive to these stimuli in vitro, but plasma cell lineage differentiation is increased, a cellular stage in which Spib is lowly expressed and the Spib gene is silenced. These findings highlight PU.1 and Spib as cell intrinsic regulators of B cell responsiveness to environmental cues, a critical process for humoral immunity.

Results

PU.1 and Spib control follicular B cell homeostasis. To investigate the function of PU.1 and Spib in mature B cells we have generated mice that carry floxed alleles of Spib, and a BAC transgene where Cre recombination is brought under the control of the Cd23 (Fcer2) regulatory sequences (Spib−/−Cd23-CreT/+), hereafter termed PU.1 Spib double knockout (DKO). Cd23-Cre is active from the transitional stage of B-lymphopoiesis, which results in highly efficient gene inactivation in both follicular and MZ B cells. The frequency and phenotype of splenic B cells from the PU.1 Spib DKO mice was compared to control (Cd23-Cre), PU.1 conditional knockout (cKO; Spib−/−Cd23-Cre T/+), and Spib knockout (KO; Spib−/−Cd23-Cre+/+ genotypes. Although the singular loss of either PU.1 or Spib did not substantially alter the proportion, total number or subset make up of the splenic B cell compartment, the proportion and number of follicular B cells was decreased 2-fold to 3-fold in PU.1 Spib DKO spleens (Fig. 1a, b), whereas the number of MZ B cells remained unaltered. In keeping with a previous report, PU.1 and Spib were redundantly required for cell surface expression of CD23, which was significantly down-regulated in the absence of Spib (Supplementary Fig. 1) and more so in the absence of both factors (Fig. 1a). As a consequence, follicular B cells were defined as B220+IgD−IgMint (Fig. 1a). Taken together, these data suggest that PU.1 and Spib are redundantly required for follicular B cell survival.

Increased plasma cells without PU.1 and Spib. In contrast to the relative loss of follicular B cells, the combined deficiency of PU.1 and Spib resulted in a significantly increased frequency and proportion of plasma cells (B220+CD138+CD98+ in both the spleen and bone marrow (Fig. 2a, b). PU.1 cKO mice displayed a normal plasma cell frequency while Spib KO mice harbored significantly more plasma cells in the spleen, with a similar but non-significant increase in the bone marrow. Analysis of the serum immunoglobulin titers and isotype make up of the plasma cell compartment revealed a significant increase in IgM and IgG3 in Spib KO and PU.1 Spib DKO sera and decreased IgA and IgG2b (Supplementary Fig. 2). Curiously, PU.1 cKO mice displayed increased titers of IgG2b and decreased titers of IgG2c that were not seen in the PU.1 Spib DKO mice. In keeping with the increase in IgM and IgG3, isotypes that arise from extrafollicular sources, such as MZ B cells, immunofluorescence on the spleens of naive PU.1 Spib DKO mice revealed a marked increase in IgM + plasma cells in the red pulp (Fig. 2c). Taken together these data demonstrate that PU.1 and Spib act both individually and redundantly to negatively regulate plasma cell homeostasis.

The germinal center reaction requires PU.1 and Spib. Previous studies found that Spib was dispensable for the establishment of a GC, but that the GC could not be sustained beyond 4 weeks, while mice lacking a single allele of Spib and both copies of Spib throughout B-cell development generated few mature B cells that could not initiate a GC reaction. However, in neither study was the fate of the antigen-specific B cells tracked. Analysis of control mice 14 days after immunization with the T cell dependent antigen NP-KLH in alum revealed robust production of NP-binding B cells that had undergone CSR to
Fig. 1 PU.1 and SpiB regulate follicular B cell numbers. a Flow cytometric analysis of splenocytes from control (Cd23^+/+), PU.1 cKO (Spi1^{fl/fl} Cd23^+/+), SpiB KO (Spi1^{fl/fl} Cd23^+/+ Spib^-/-) and PU.1 SpiB DKO (Spi1^{fl/fl} Cd23^+/+ Spib^-/-) mice showing the abbreviated gating strategy for the detection of follicular (Fo) B cells (B220^+ IgD^+ IgM^{int}) and MZ (B220^+ IgM^+ IgD^{low} CD21^+) B cells. As the loss of PU.1 and SpiB leads to the downregulation of CD23 expression, follicular B cell numbers were based on the gate for B220^+ IgD^+ IgM^{int}. The full gating strategy is shown in Supplementary Fig. 8A. Data are representative of 7–10 mice per genotype. b Absolute cell numbers are graphed as the mean ± s.d. P-values compare the indicated samples (two tailed t-test). Each circle represents the results from an individual mouse. *P < 0.05, **P < 0.01, ****P < 0.0001
IgG1 and near uniformly upregulated the GC regulator Bcl6. (Fig. 3a, b). As expected SpiB KO B cells responded similarly to controls at this time point. In contrast, immunization of PU.1 SpiB DKO mice elicited virtually no response, generating neither IgG1+ nor Bcl6+ GC B cells (Fig.3a, b, d). PU.1 cKO, in contrast to our previous studies using Cd19-Cre14, displayed an intermediate phenotype, with reduced NP+IgG1+ and NP+ Bcl6+ GC B cells compared to the control mice. Immunofluorescence studies of spleen sections from immunized mice confirmed the presence of GC structures in control mice and those singly deficient in PU.1 or SpiB, but their absence in PU.1 SpiB DKO mice (Fig. 3e). Similarly, ELISpot analysis of the NP-specific antibody response revealed a normal frequency of NP-binding IgG1 antibody-secreting cells at day 14 post immunization in mice lacking SpiB, a non-significant reduction in mice lacking PU.1 and very few NP reactive cells in the DKO spleen (Fig. 3c, d). Together these data indicate that PU.1 and SpiB are redundantly required for the GC reaction and the production of high-affinity antibody.

**PU.1 and SpiB negatively regulate plasma cell differentiation.**

Our previous studies using Cd19-Cre to inactivate PU.1 revealed that PU.1 and IRF8 acted together to repress plasma cell differentiation in vitro6. It that model, the singular deficiency of PU.1 resulted in a relatively mild enhancement of plasma cell formation after stimulation with CD40 L and IL-4, a finding that agreed with a second study in which inactivation of a miRNA binding site in the 3′ untranslated region of Spi1 resulted in an increased concentration of PU.1 in activated B cells and impaired plasma cell formation25.

To address the combined importance of PU.1 and SpiB for B cell differentiation in vitro we cultured follicular B cells of the four genotypes, generated exclusively from lymph nodes to exclude any possible MZ B cell contamination, in CD40 L + IL-4, conditions that promote B cell proliferation, CSR to IgG1 and plasma cell differentiation26. Although B cells of each genotype proliferated similarly in CD40 L + IL-4, as assessed by CTV dilution (Fig. 4a), the yield of cells was consistently reduced in the PU.1 cKO and PU.1 SpiB DKO cultures (Fig. 4b). Analysis of the
frequency and total number of CD138+ plasma cells and IgG1+ B cells displayed an inverse relationship in that PU.1 cKO and PU.1 SpiB DKO cultures had increased plasma cells and reduced IgG1+ B cell numbers compared to the control and SpiB KO cultures.

**Identification of PU.1 and SpiB dependent genes in B cells.** To determine how the singular and combined loss of PU.1 and SpiB affected B cell differentiation, we isolated RNA from lymph node follicular B cells of the four genotypes before and after 48 h of culture in the presence of CD40 L + IL-4 and performed RNA-
seq. Analysis of the frequency of the inactivation of the floxed Spi1 exon 5 in follicular B cells displayed complete inactivation in PU.1 cKO B cells, but marked retention of exon 5 in PU.1 SpiB DKO B cells (Supplementary Fig. 3A). This may reflect a reduction in the expression of Cre, which is under the control of the Cd23 promoter in SpiB-deficient cells (Supplementary Fig. 1) or a requirement by follicular B cells to retain a minimal level of either PU.1 or SpiB for survival. Differential expression (DE) analysis revealed that PU.1 and SpiB regulated 547 and 332 genes, respectively, in naïve follicular B cells (Supplementary Fig. 3B), with a clear redundancy observed in the absence of SpiB and a partial deletion of PU.1 (1306 DE genes). Pathway analysis revealed association of genes whose expression required either PU.1 or SpiB with a follicular B-cell signature (Supplementary Fig. 3C, D), while genes repressed by SpiB were uniquely associated with other cell lineages including plasmacytoid dendritic cells (pDC), macrophages and CD8 T cells (Supplementary Fig. 3C). Tnfrsf13c, encoding the BAFF-R, which is essential for peripheral cell survival and homeostasis was significantly downregulated at both the transcript (Supplementary Fig. 4A) and protein level (Supplementary Fig. 4B, C).

Given the partial retention of PU.1 observed in naïve follicular B cells, we turned our attention to the RNA-seq data that was generated in activated B cell cultures. At this early time point following activation, we observed minimal differentiation into CD138+ plasmablasts (<1%; Supplementary Fig. 5A) and a modest increase in proliferation in PU.1 cKO and PU.1 SpiB DKO B cells as judged by CTV labeling (Supplementary Fig. 5B). Importantly, exon 5 of Spi1 was completely excised regardless of the presence or absence of SpiB (Fig. 5a), confirming that neither factor was absolutely essential for B cell proliferation and survival following activation in vitro. A similarly high deletion efficiency was obtained in activated B cell cultures stimulated with LPS + IL-4 (Supplementary Fig. 6C), where enhanced B cell differentiation into plasmablasts was also observed (Supplementary Fig. 6A, B).

Analysis of the RNA-seq data derived from CD40L + IL-4 activated B cells revealed that the loss of PU.1 alone or in combination with SpiB resulted in 1009 and 1443 DE genes, respectively (Fig. 5b and Supplementary Data). In contrast, SpiB deficiency had minimal impact on the transcriptome of activated B cells (17 DE genes, Fig. 5B and Supplementary Data), potentially due to the rapid downregulation of SpiB expression during B-cell differentiation. Our re-analysis of ChIPseq data of PU.1 binding in LPS activated plasmablasts found that PU.1 bound in 4826 genes27. Comparison with the gene expression data revealed a highly significant enrichment of PU.1 binding in genes that required PU.1 or PU.1 and SpiB for their expression (Fig. 5c and Supplementary Data). These data demonstrate that PU.1 functions predominantly as an activator of gene expression during B cell terminal differentiation.

To investigate how PU.1 and SpiB interact with the gene regulatory networks controlling late B cell and plasma cell differentiation, we interrogated our RNA-seq data from activated B cells for the expression of the transcriptional regulators known to be important in these processes5. Although all components of the B cell network were expressed in the absence of PU.1 and SpiB, indicating that the network is intact at a global level, we observed partial reduction in several key regulators, including Pax5 and Bcl6 in PU.1 cKO and PU.1 SpiB DKO B cells (Fig. 5d). PU.1 and SpiB also appeared to redundantly regulate the expression of Bach2, a critical negative regulator of Blimp1 and plasma cell differentiation28. In contrast the expression of other important regulators, including Irf4, Irf8, and Pou2af1 (Obf1) was similar in all genotypes. Curiously, we observed a small but significant increase in Tcf3 (E2A) and a similar decrease in Tcf4 (E2-2) in PU.1 SpiB DKO B cells (Fig. 5d). Similar results were also observed in activated B cell cultures following stimulation with LPS + IL-4 (Supplementary Fig. 6D).

Analysis of the transcriptional regulators of the plasma cell fate identified premature de-repression of Prdm1, El2, Irf4 and Celbp without PU.1 or PU.1 and SpiB and Xbp1 in the absence of both factors (Fig. 5d). As no bona fide plasma cells are observed at this early time point (Supplementary Fig. 3), we interpret the de-repression of these genes to be early markers of the increased propensity of the PU.1 cKO and PU.1 SpiB DKO B cells to undergo terminal differentiation. Notably, we also observed a robust increase in Aicda, encoding Activation induced cytidine deaminase (AID) in PU.1 cKO and PU.1 SpiB DKO B cells, making it unlikely that a deficiency in this enzyme is the underlying cause of the defective CSR observed in vitro and in vivo without PU.1 and SpiB (Fig. 5d, Supplementary Fig. 6D).

Gene ontology analysis of the DE genes revealed enrichment for “Metabolic pathways” in each gene set. More informatively the pathways downregulated in PU.1 cKO and PU.1 SpiB DKO largely overlapped and included several gene sets associated with lymphocyte signaling, including the BCR signaling pathway (Fig. 5e). Genes upregulated showed a strong increase in gene frequency in the DKO comparison and included the “protein processing” gene set that contains many genes involved in the unfolded protein response, which is critical for high level antibody secretion by plasma cells (Fig. 5e).

Given the strong deficiency we observed in PU.1 SpiB DKO mice after T-dependent immunization and the existing literature in this area, we decided to focus on the B cell receptor (BCR) signaling pathway. Strikingly, although the core constituents of the BCR (Igh, Igk, Igl, Cd79a, and Cd79b) were normally expressed, many downstream components of the BCR signal transduction cascade were significantly downregulated (most between 2-fold and 3-fold) in the absence of PU.1 and SpiB (Fig. 6a). A similar trend, albeit with a lower fold change was also observed in PU.1 cKO B cells. Western analysis confirmed these findings, with modest reductions in protein expression for several BCR signaling components including Btk, Rasgrp3, and Card11 (Fig. 6c) in the absence of PU.1 or PU.1 and SpiB. Analysis of the PU.1 ChIPseq data identified the presence of PU.1 binding in the proximal promoter of most of the PU.1 and PU.1/SpiB dependent components of the BCR pathway (Fig. 6b). A similar trend, in
several BCR signaling components was also observed following activation with LPS + IL-4 (Supplementary Fig. 7).

To determine if these quantitative changes in the expression of multiple components of the BCR resulted in impaired signaling and cellular responses, we cultured follicular B cells of the four genotypes in the presence of anti-IgM + IL-4 for 3 days. Proliferation was similar between the control, PU.1 cKO and SpiB KO B cells, however in keeping with the gene expression data, PU.1 SpiB DKO B cells displayed extremely low viability after BCR engagement (Fig. 6d). To assess more proximal signaling events after BCR crosslinking we could not use the traditional cell source of naive B cells as the inactivation of Spi1 is incomplete in PU.1 SpiB DKO follicular B cells (Supplementary Fig. 3A). To circumvent this problem, we first cultured naive follicular B cells for 48 h in CD40 L + IL-4, a time point where exon 5 of Spi1 was absent (Fig. 5a), rested them for one hour and then exposed the cells to anti-IgM. This approach showed clear inhibition in the BCR response, as assessed by calcium mobilization, in the absence of PU.1, and to a lesser extent SpiB (Fig. 6e). These data demonstrate that PU.1 and SpiB are redundant regulators of multiple components of the BCR signaling pathway.

**Regulation of immune receptors by PU.1 and SpiB.** In addition to the regulation of the genes encoding members of the BCR signaling pathway, it was striking that PU.1 or PU.1 and SpiB were required for the normal expression of several other immune receptors critical for the ability of a B cell to sense its immunological environment (Fig. 7a, Supplementary Fig. 7B). This included Tnfrsf13c, encoding the BAFF-R, essential for peripheral B cell survival and homeostasis1 and Cd40, a critical component of T cell help29. Flow cytometric analysis confirmed the down-regulation of both the BAFF-R and CD40 in the absence or PU.1, with a further reduction in expression in the absence of both PU.1 and SpiB (Fig. 7b, c). Analysis of the PU.1 ChIP-seq data again showed the presence of PU.1 binding in the proximal promoter of both of these targets (Fig. 7d).

This quantitative reduction in Tnfrsf13c is likely to be responsible for the 2-fold to 3-fold reduction in follicular B cells in PU.1 SpiB DKO mice (Fig. 2b), while the deceased CD40 expression may be responsible for the lower cell yields in cultures containing CD40 L and IL-4, despite the increased propensity to differentiate into ASCs (Fig. 4b). In contrast, cytokine receptors, such as the IL4ra and Il5r were marginally upregulated in the absence of PU.1 and SpiB (1.6-fold each).

Several important microbial sensors were also directly regulated by PU.1 and SpiB including Tlr4 and Tlr9, with each having clear proximal promoter-binding sites for PU.1 (Fig. 7a, d, Supplementary Fig. 7B). To examine the relevance of the reduced TLR4, we cultured naive lymph node follicular B cells of each genotype in LPS. Although all genotypes robustly proliferated, as assessed by CTV dilution, there was a decrease in cell yields without PU.1 and SpiB (Fig. 7e). LPS induced a strong
differentiation response to CD138+ plasma cells in control B cells that was augmented as a proportion of live cells at day 4, in the PU1 SpiB DKO cells (Fig. 7e), in a manner similar to that observed for CD40 L + IL-4 cultures (Fig. 5a). However, after adjustment for the different cell numbers obtained from the various genotypes, it was apparent that the total number of CD138+ cells was reduced without PU.1 and SpiB (Fig. 7e). LPS-induced CSR to IgG3 was dependent on PU.1 (Fig. 7e), in a manner similar to that observed for CD40 L + IL-4 cultures (Fig. 5a).
manner similar to what we observed for IgG1. Taken together these data demonstrate that PU.1 and SpiB redundantly control the receptors for several major pathways by which B cells sense and respond to environmental cues.

Discussion
Humoral immunity is underpinned by the ability of B cells to sense and integrate signals from their environment and respond with the appropriate intrinsic differentiation program. These environmental cues include the cognate antigen, T cell help and survival factors such as BAFF. In this study, we show that the Ets family transcription factors, PU.1 and SpiB, well-known regulators of early lymphopoiesis, are redundantly required for the normal expression and function of virtually all pathways by which B cells sense and respond to the surrounding milieu. In contrast plasma cell development was either unaffected or accelerated by the loss of both factors. Thus PU.1 and SpiB act redundantly to balance the proliferative and differentiation response of B cells that is essential for protective humoral immunity.

Within the Ets family, PU.1 and SpiB are the genes most highly related to each other. They bind to the same consensus DNA recognition site, either on their own or in complex with IRF4 or IRF8. Both factors are constitutively expressed throughout B cell development, with SpiB expression rapidly extinguished upon plasma cell differentiation, while PU.1 remains expressed at a low concentration and can, at least in culture, still occupy many thousands of binding sites in the genome. The similarity of this pair of transcription factors has long complicated the interpretation of their function in B cells. PU.1 null embryos die in late gestation and lack all lymphocytes. Removal of PU.1 in adult hematopoietic stem cells ablates B-cell development. Surprisingly, conditional inactivation of PU.1 in committed B cells using Cd19-Cre, which is first active during B cell development in the bone marrow, results in pronounced defects in B cell production or function beyond a role in B1 cell formation. Loss of SpiB is also compatible with normal B cell development, however SpiB KO mice have defects in the GC reaction at later times points after immunization. We observed normal numbers of peripheral B cell subsets in mice lacking PU.1 or SpiB, as well as functional GC, at the peak time post immunization (day 14). In contrast to our previous studies using Cd19-Cre, we observed a partial impairment in GC responses in vivo and enhanced plasma cell differentiation in vitro in the absence of PU.1. The major difference between the studies is that here we have utilized Cd23-Cre that is active from the transitional stage in the spleen, sparing the bone marrow development process entirely. This, as discussed below may impact on the ability of SpiB to compensate for the loss of PU.1.

Previous attempts to investigate the degree of functional compensation between PU.1 and SpiB have been hampered by the complete absence of B cells in PU.1-deficient mice. As a compromise, B cell development was examined in Spi1−/−Spib−/− mice, which show a 9-fold decrease in splenic follicular B cells. The residual B cells in those mice, many of which were MZ B cells, appeared to be functionally defective, unable to contribute to GCs and impaired in their signaling responses through the BCR and TLRs. More recently, conditional inactivation of PU.1 on a SpiB KO background using Cd19-Cre, revealed a dramatic loss of follicular B cells and an expansion of the pre-B-cell compartment that ultimately generated B-ALL at high frequency. By utilizing Cd23-Cre our study has bypassed the important function of these proteins at the pre-B-cell stage and allowed the first robust analysis of the functionality of PU.1 SpiB DKO B cells. Although we found a 2-fold to 3-fold reduction in the frequency of follicular B cells in the DKO mice, these data should be interpreted cautiously, as we observed strong retention of the floxed exon 5 of Spi1 in B cells only in the absence of Spib, suggesting that these two proteins are redundantly essential for follicular B cell survival. Interestingly, the gene encoding the BAFF-R, Tnfrsf13c, is a direct PU.1/SpiB target gene providing a likely mechanism for the reduced follicular B cell numbers.

The redundancy between PU.1 and SpiB was also apparent on the molecular scale, with both the number of DE genes and the magnitude of change being increased in the PU.1 SpiB DKO activated B cells. Importantly, exon 5 of Spi1 was efficiently excised in the DKO B cells after in vitro activation for 48 h, allowing a full examination of the transcriptional consequences of gene inactivation at this stage. As with the cellular data, redundancy between PU.1 and SpiB was observed. This agrees well with ChIPseq studies that show that the vast majority of SpiB occupied sites in the B cell genome also bind PU1. Pathway analysis of these data revealed a striking role for PU.1 and SpiB in directly regulating the expression of many components of the BCR signaling pathway, including the previously characterized targets, Blnk and Btk. Although the reduced expression of the individual target genes was in each case modest, combined reductions resulted in grossly impaired in vitro responsiveness to anti-IgM and a total ablation of the antigen-specific immune response. The function of PU.1 and SpiB was not restricted to the BCR pathway, as the ability of the double mutant follicular B cells to respond to antigen-independent simulation, either via T cells derived signals such as CD40 L or microbial stimuli such as LPS was also diminished, again likely due to the direct regulation of these key signaling pathways by PU.1 and SpiB.

In contrast to the essential function of PU.1 and SpiB to allow B cell responsiveness to environmental cues, both factors act as negative regulators of plasma cell development, in steady-state conditions in vivo and after activation in vitro. The increased
plasma cell frequency in vivo was predominantly IgM secreting cells, with a milder increase in IgG production, isotypes that often derive from MZ B cells. In keeping with this conclusion, MZ B cell frequency was unaltered in PU.1 SpiB DKO and mildly increased in Spi1+/−SpiB−/− spleens. The preferential retention of MZ B cells is potentially also due to the role of PU.1 and SpiB in activating the expression of key components of the BCR pathway, as impaired BCR signaling is known to favor MZ over follicular B cell fate. The splenic MZ B cells from Spi1+/−SpiB−/− mice have been shown to be impaired in their responses to several microbial products, including LPS, because of reduced *Nfkb1* expression. In contrast our RNA-seq and ChIPseq data derived from in vitro activated lymph node follicular B cells pointed to defective *Tlr4* expression as the underlying cause of the reduced responsiveness to LPS in PU.1 Spib DKO B cells.

PU.1 cKO and PU.1 Spib DKO B cells also produced markedly more plasma cells after in vitro activation with CD40L+IL-4 or LPS+IL-4. This is in keeping with our recent report of increased plasma cell differentiation in the absence of PU.1 and IRF8 but occurred despite the lower overall cell yield without PU.1 and SpiB. PU.1/SpiB loss resulted in reduced expression of key B cell program regulators such as *Pax5*, *Bcl6*, and *Bach2*, while plasma cell promoting factors such as *Prdm1*, *Xbp1*, *Ell2*, and *Cebpb* were increased in expression. As with PU.1/IRF8 DKO B cells, *Aicda* expression was increased in the absence of PU.1 and SpiB suggesting that reduced expression of this enzyme was not the

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**Fig. 6** PU.1 and SpiB maintain expression of multiple BCR components. **a** Gene expression (from Fig. 5) for the indicated genotype is plotted as the mean fold change (log2 from 2 experiments) relative to the expression in the control genotype. *Indicates genes that are DE relative to controls (defined as log2 fold change >0.6-fold, log2 RPKM (reads per kilobase of exon model per million mapped reads) >0, *P* < 0.15). **b** PU.1 ChIPseq identifies promoter-binding sites for PU.1 in many components of the BCR signaling pathway. Gene structures are shown below. Gene names in red. **c** Lysates were generated from activated B cells (cultured for 48 h in CD40 L + IL-4) of the indicated genotype. Western blotting was performed for the indicated proteins. Actin serves as a control for protein loading. Data in **b**, **c** are representative of two experiments each. **d** Loss of PU.1 and SpiB leads to decreased viability in response to BCR signaling. Resting lymph node B cells from mice of the indicated genotype were labeled with CTV and cultured with CD40 L + IL-4 for 48 h before being washed and rested for one hour. Cells were then loaded with Indo-1 AM before flow cytometric analysis. The number of live cells is plotted for each genotype examined. Data are the mean ± s.d. for three independent experiments. *P*-values compare the indicated samples (two tailed t-test). **** *P* < 0.0001. **e** Resting B cells of the indicated genotype were cultured with CD40 L + IL-4 for 48 h before being washed and rested for one hour. Cells were then loaded with Indo-1 AM before basal fluorescence was measured for 30 s. Anti-IgM was then added and fluorescence changes recorded for an additional 2.5 min. Results are presented as arbitrary units of changes in fluorescence ratio over time. Data are representative of two experiments.
underlying cause of the decreased CSR in PU.1 SpiB DKO B cells. Collectively these data suggest that PU.1 and SpiB act to balance B cell proliferation with the need to produce sufficient numbers of plasma cells, with PU.1 and SpiB deficiency tipping the balance away from clonal expansion and towards terminal differentiation.

Fig. 7 PU.1 and SpiB are required to maintain the expression of several immune sensors. a Gene expression (from Fig. 5) for the indicated genotype is plotted as the mean fold change (log2, from 2 experiments) relative to the expression in the control genotype. *Indicates genes that are DE relative to controls (defined as log2 fold change >0.6-fold, log2 RPKM (reads per kilobase of exon model per million mapped reads) >0, P < 0.15). b Flow cytometric analysis showing the expression of BAFF-R or CD40 on activated B cells of the indicated genotype (black line), relative to control B cells (solid gray), 48 h after stimulation with CD40 L + IL-4. c Mean fluorescence intensity (MFI) is graphed as the mean ± s.d. Each circle represents the results from an individual mouse. P-values compare the indicated samples (two tailed t-test). *P < 0.05, **P < 0.01. Data in b, c are from 3 experiments. d ChIPseq identifies proximal promoter-binding sites for PU.1 for the genes shown in a. Gene structures are shown below. Gene names in a, d with PU.1 peaks (P < 10^-6) are indicated in red. Data are representative of two experiments. e Impaired survival of PU.1 SpiB DKO B cells in response to the TLR4 agonist LPS. Resting lymph node B cells from mice of the indicated genotype were labeled with CTV and cultured in the presence of LPS for 4d before flow cytometric analysis. Numbers in the boxes are the proportion of CD138+ antibody-secreting cells (ASC). Data are representative of three experiments. P-values compare the indicated samples (two tailed t-test). *P < 0.05, **P < 0.01. The full gating strategies are shown in Supplementary Fig. 9A and B, respectively.
There is to date only limited understanding of the unique function(s) of SpiB, although it is of note that Spib expression is restricted to B cells and pDCs, lineages that have a distinctly low rate of PU.1 expression. Our finding of increased expression of genes associated with pDCs, macrophages and T cells in naïve B cells lacking SpiB is reminiscent of similar observations of PU.1 repression of natural killer cell associated genes in pro-B and pro-T cells and suggests a previously unappreciated function of SpiB to repress lineage inappropriate gene expression in B cells that warrants closer examination in a future study. PU.1, in contrast, has a broad expression pattern and has been intensively studied in many blood cell lineages. One of the recurrent themes of PU.1 function in haematopoiesis is its pivotal role in activating a range of receptors, including the genes encoding M-CSFR, GM-CSFR, IL3R, IL7R, and Flt3 and many homing molecules such as integrins. The data presented in this study provide a novel example of this paradigm, with PU.1 controlling the interplay between the extrinsic signals that control B cell homeostasis and responsiveness and intrinsinc programs that govern differentiation events essential for humoral immunity.

**Methods**

**Mice and immunizations.** The Spib<sup>fl/fl</sup>, Spi−/−, and Cd23<sup>cre</sup> mouse strains have been previously reported. All mice were maintained on a C57Bl/6 genetic background and analyzed between 6–20 weeks of age. Animals were aged and sex matched within individual experiments. The genotypes analyzed were control mice (Cd23<sup>+/+</sup>), PU.1<sup>Cre</sup> mice (Spib<sup>fl/fl</sup>, Cd23<sup>cre</sup>),SpiB KO mice (Spib<sup>fl/fl</sup> Cd23<sup>+/+</sup> Spib−/−) and PU.1 Spib<sup>Cre</sup> DKO mice (Spib<sup>fl/fl</sup> Cd23<sup>cre</sup> SpiB−/−). Mice were immunized at 6–20 weeks of age with 4 (hydroxy-3-nitrophenyl) acetyl (NP)-keyhole limpet hemocyanin (KLH) made at a molar ratio of 1:1 (NP/KLH). Antigen was precipitated in alum at a concentration of 1 mg/ml and delivered by i.p. injection (100 μg/mouse). Mice were sacrificed 14 d post immunization and post immunization splenocyte suspensions were made from the spleen and bone marrow for analysis. All animal experiments were conducted according to the protocols approved by the Walter and Eliza Hall Institute animal ethics committee.

**Antibodies and flow cytometry.** Single-cell suspensions were stained with mAb against mouse B220 (RA3-6B2; BD Pharmingen; 1 : 200), IgM (33-2581; 1 : 800), IgD (1126 C; 1 : 800), Gr-1 (RB6-8C5; 1 : 800), CD21 (7G6; 1 : 2000), CD23 (B3B4; Biologend; 1 : 200), IgG1 (X56; BD Pharmingen; 1 : 300), IgG3 (pooled antisera; Southern Biotechnic; 1 : 250), CD138 (281-2; BD Pharmingen; 1 : 600), FcgR (2.4G2; 1 : 800), Cd23 (B3B4; BD Pharmingen; 1 : 100), goat anti-hamster IgG (DyLight 594 or 649; Biolegend; 1 : 50 and 1 : 100). Cells were analyzed on ow cytometers (BD Biosciences). Intracellular staining for BCL6 (7D1) was performed using the FoxP3 staining buffer set (eBiosciences). All Abs were produced in-house unless otherwise indicated. Antibodies and cytokines were from the indicated suppliers.

**Cell culture.** Naïve lymph node B cells were purified by negative selection using the B cell isolation kit (130-090-862; Miltenyi Biotec). Cultures were seeded at 1–2 × 10<sup>6</sup> with optimal concentrations of recombinant CD40 D (1163-CL; R&D Systems; 50 ng/ml) and IL-4 (404-ML; R&D Systems; 10 ng/ml), or LPS (11264; 20 μg/ml) 4–5 days before analysis. Slides were made in Mowiol (Sigma-Aldrich) containing 2.5% DARCO (Sigma). Images were acquired using a Zeiss LSM780 confocal microscope, and analyzed with the Fiji software.

**Calcium flux.** Naïve resting B cells were isolated as described above and cultured for 4 h in CD40 L + IL-4, to ensure complete deletion of the SpiB allele in PU.1 Spib DKO B cells. Cells were then washed extensively and rested for one hour before being resuspended in loading buffer (KDS-BSA containing 0.1% glucose and 0.1% FCS) containing Indo-1 acetoxyethyl (Molecular Probes). Following an incubation for 40 min at 37°C, loaded cells were washed extensively and then resuspended in the loading buffer indicated above. Calcium flux was measured by flow cytometry. Baseline fluorescence was established for 30 s before anti-IgM (10 μg/ml; Jackson Immunoresearch) was added and the subsequent calcium flux followed for the indicated times.

**Whole transcriptome analysis.** RNA was isolated from either naïve or activated (48 h in CD40 L + IL-4 or LPS + IL-4) lymph node derived B cells using the RNeasy Plus Mini kit (74,134; Qiagen). Two biological replicates were generated and sequenced for each sample. mRNA was subjected to transcriptome re-sequencing on an Illumina NextSeq sequencer. Between 15 and 90 million reads were analyzed per sample. Each sample’s read set was aligned to the GRCh38/mm10 build of the Mus musculus genome using the Subread aligner. Only uniquely mapped reads were retained. Gene expression was calculated using edgeR and featureCounts. Overlapping exons in annotation build 38.1 of NCBI RefSeq database were included. Genes were filtered from downstream analysis if they failed to achieve a CPM (counts per million mapped reads) value of at least 0.5 in at least two libraries.

**PU.1 binding analysis.** The PU.1 ChIPseq dataset from LPS activated plasmablasts was downloaded from the Gene Expression Omnibus (GEO) database (GSM143342). Sequence reads were mapped to mouse genome mm10 using Subread aligner. Only uniquely mapped reads were kept. PU.1 binding peaks were called by using MACS2 program with a false discovery rate cutoff of 0.01. Called peaks must also have at least 200 support reads in the 200 bp region centered on the binding sum. Peaks were assigned to genes if they occurred <20 kb upstream of the transcriptional start sites, within the gene body or <5 kb downstream of the end of transcription. Within these parameters multiple gene assignment was allowed.

**Western analysis.** The polyclonal antibodies against Btk (D3H5; 1:1000), Rasgpr3 (C33A3; 1:1000) and Card11 (D12; 1:1000) were from Cell Signaling Technology while the antibody against actin (I-19; 1:3000) was from Santa Cruz Biotechnology. Protein extracts corresponding to equal cell numbers were loaded onto the gel and subjected to western blotting using standard techniques.

**Statistical analysis.** Sensitivity analysis of the non-RTA-Seq data was assessed with Prism6 (GraphPad). t tests for all single comparisons were unpaired, assumed Gaussian distribution and that both populations have the same standard deviation (SD). Enrichment of PU.1 binding in DE gene lists was determined using z-tests. Bar graphs display the arithmetic mean ± s.d. unless otherwise stated.

**Data availability.** Sequence data that support the findings of this study have been deposited in GEO with the primary accession code GSE90094.
References
1. Rickert, R. C., Jellusova, J. & Miletic, A. V. Signaling by the tumor necrosis factor receptor superfamily in B-cell biology and disease. ImmunoL Rev. 244, 115–133 (2011).
2. Victor, G. D. & Nussenzenzweig, M. C. Germline centers. Annu. Rev. Immunol. 30, 429–457 (2012).
3. Igarashi, K., Ochiai, K., Itoh-Nakadai, A. & Muto, A. Orchestration of plasma cell differentiation by Bach2 and its gene regulatory network. ImmunoL Rev. 261, 116–128 (2014).
4. Nera, K. P., Kylaniemi, M. K. & Lassila, O. Regulation of B cell to plasma cell transition within the follicular B cell response. Scand. J. Immunol. 82, 225–234 (2015).
5. Nutt, S. L., Hodgkin, P. D., Tarlinton, D. M. & Corcoran, L. M. The generation of antibody-secreting plasma cells. Nat. Rev. Immunol. 15, 160–171 (2015).
6. Carotta, S. et al. The transcription factors IRF8 and PU.1 negatively regulate plasma cell differentiation. J. Exp. Med. 211, 2169–2181 (2014).
7. Xu, H. et al. Regulation of bifurcating B cell trajectories by mutual antagonism between transcription factors IRF4 and IRF8. Nat. Immunol. 16, 1274–1281 (2015).
8. Turkistani, S. A. & DeKoter, R. P. The transcription factor PU.1 is a critical regulator of cellular communication in the immune system. Arch. Immunol. Ther. Exp. 59, 431–440 (2011).
9. Daki, A. et al. PU.1 regulates the commitment of adult hematopoietic progenitors and restricts granulopoiesis. J. Exp. Med. 201, 1487–1502 (2005).
10. Iwasaki, H. et al. Distinctive and indispensable roles of PU.1 in maintenance of hematopoietic stem cells and their differentiation. Blood 106, 1590–1600 (2005).
11. Scott, E. W. et al. PU.1 functions in a cell-autonomous manner to control the differentiation of multipotential lymphoid-myeloid progenitors. Immunity 6, 437–447 (1997).
12. Scott, E. W., Simon, M. C., Anastasi, I. & Singh, H. Requirement of transcription factor PU.1 in the development of multiple hematopoietic lineages. Science 265, 1573–1577 (1994).
13. Pang, S. H. et al. PU.1 cooperates with IRF4 and IRF8 to suppress pre-B-cell leukemia. Leukemia 30, 1375–1387 (2016).
14. Polli, M. et al. The development of functional B lymphocytes in conditional PU.1 knock-out mice. Blood 106, 2083–2090 (2005).
15. Sokalski, K. M. et al. Deletion of genes encoding PU.1 and Spi-B in B cells impairs differentiation and induces pre-B cell acute lymphoblastic leukemia. Blood 118, 2801–2808 (2011).
16. Ye, M., Ermakova, O. & Graf, T. PU.1 is not strictly required for B cell development and its absence induces a B-2 to B-1 cell switch. J. Exp. Med. 202, 1411–1422 (2005).
17. Ray-Gallet, D., Mao, C., Tavitian, A. & Moreau-Gachelin, F. DNA binding specificities of Spi-1/PU.1 and Spi-B transcription factors and identification of a Spi-1/Spi-B binding site in the c-fesc/c-fps promoter. Oncogene 11, 303–313 (1995).
18. Solomon, L. A., Li, S. K., Piskorz, I., Xu, L. S. & DeKoter, R. P. Genome-wide comparison of PU.1 and Spi-B binding sites in a mouse B lymphoma cell line. BMC Genomics 16, 76 (2015).
19. Garrett-Sinha, L. A. et al. PU.1 and Spi-B are required for normal B cell receptor-mediated signal transduction. Immunity 10, 399–408 (1999).
20. Lu, R., Medina, K. L., Lancki, D. W. & Singh, H. IRF-4.8 orchestrate the pre-B-to-B transition in lymphocyte development. Genes Dev. 17, 1703–1708 (2003).
21. Kwon, K. et al. Instructive role of the transcription factor E2A in early B lymphopoiesis and germinal center B cell development. Immunology 28, 751–762 (1993).
22. Su, G. H. et al. Defective B cell receptor-mediated responses in mice lacking the Ets protein, Spi-B. EMBO J. 16, 7118–7129 (1997).
23. DeKoter, R. P. et al. Regulation of follicular B cell differentiation by the related E26 transformation-specific transcription factors PU.1, Spi-B, and Spi-C. J. Immunol. 185, 7374–7384 (2010).
24. Oliver, A. M., Martin, F., Garland, G. L., Carter, R. H. & Kearney, J. F. Marginal zone B cells exhibit unique activation, proliferative and immunoglobulin secretion responses. Eur. J. Immunol. 27, 2366–2374 (1997).
25. Lu, D. et al. The miR-155-PU.1 axis acts on Pax5 to enable efficient terminal B cell differentiation. J. Exp. Med. 211, 2183–2198 (2014).
26. Hasbold, J., Corcoran, L. M., Tarlinton, D. M., Tangye, S. G. & Hodgkin, P. D. Evidence from the generation of immunoglobulin-G-secreting cells that stochastic mechanisms regulate lymphocyte differentiation. Nat. Immunol. 5, 55–63 (2004).
27. Minnich, M. et al. Multifunctional role of the transcription factor Blimp-1 in coordinating plasma cell differentiation. Nat. Immunol. 17, 331–343 (2016).
28. Muto, A. et al. Bach2 represses plasma cell gene regulatory network in B cells to promote antibody class switch. EMBO J. 29, 4048–4061 (2010).
29. Sasaki, I. et al. The transcription factor PU.1 and Spi-B promotes Toll-like receptor-mediated splenic B cell proliferation. Mol. Cell. Biol. 35, 1619–1632 (2015).
30. Xu, L. S. et al. Regulation of B cell linker protein transcription by PU.1 and Spi-B in murine B cell acute lymphoblastic leukemia. J. Immunol. 189, 3347–3354 (2012).
31. Mackay, F., Figgitt, W. A., Saulep, D., Lepage, M. & Hibbs, M. L. B-cell stage and context-dependent requirements for survival signals from BAFF and the B-cell receptor. ImmunoL Rev. 237, 205–220 (2015).
32. Christie, D. A. et al. PU.1 opposes IL-7-dependent proliferation of developing B cells with involvement of the direct target gene broton tyrosine kinase. J. Immunol. 194, 595–605 (2005).
33. Pillai, S. & Cariappa, A. The follicular versus marginal zone B lymphocyte cell fate decision. Nat. Rev. Immunol. 9, 767–777 (2009).
34. Zhang, D. E. et al. Critical role of the transcription factor PU.1 in the development of multiple hematopoietic progenitors and their differentiation. Blood 113, 3129 (2009).
35. Yamaguchi, T. et al. PU.1 cooperates with IRF4 to repress plasma cell gene regulatory network in B cells. Blood 117, 4574–4579 (2011).
36. Takahashi, A. et al. PU.1 is critical for plasma cell dendritic cell function and development. Blood 120, 4733–4743 (2012).
37. Liao, Y., Smyth, G. K. & Shi, W. The Subread aligner: fast, accurate and scalable read mapping by seed-and-vote. Top. Microbiol. Immunol. 171, 330–342 (2016).
38. Muto, A. et al. Bach2 represses plasma cell gene regulatory network in B cells to promote antibody class switch. EMBO J. 29, 4048–4061 (2010).
39. DeKoter, R. P., Walsh, J. C. & Singh, H. PU.1 represses plasma cell gene regulatory network in B cells to promote antibody class switch. EMBO J. 29, 4048–4061 (2010).
40. Carotta, S. et al. The transcription factor PU.1 controls dendritic cell development and Flt3 cytokine receptor expression in a dose-dependent manner. Immunity 32, 628–631 (2010).
41. DeKoter, R. P., Walsh, J. C. & Singh, H. PU.1 regulates cytokine-dependent proliferation and differentiation of granulocyte/macrophage progenitors. EMBO J. 17, 4456–4468 (1998).
42. Zhang, D. E. et al. Function of PU.1 (Spi-1), C/EBP, and AML1 in early myelopoiesis: regulation of multiple myeloid CSF receptor promoters. Carcinogenesis 20, 1781–1789 (2003).
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Author contributions
S.N.W. performed most of the experiments; J.T. provided the immunofluorescence; S.T., A.L., O.K. assisted in some experiments; Y.L. and W.S. performed the bioinformatics analysis; L.A.G.-S. provided a key reagent; D.M.T. and S.L.N. supervised the research; S.N.W. and S.L.N. designed the experiments, analyzed the data and wrote the manuscript.

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