antibodies (12–14). Conversely, the over-expression of miR-155 resulted in B cell lymphoproliferative disorders in transgenic mice (15).

Despite growing evidence of biological roles for a limited

retroviral vector that co-expresses scrambled miR mimic and GFP or with a retroviral vector that co-expresses miR-125b mimic and GFP. A day after infection, cells were cultured in the presence of different doses of LPS or CpG. At 4 days post-infection, cells were analyzed by ow cytometry for expression of GFP, CD19 and CD138 (Syndecan-1).

Search for miRNA target genes and analysis of miRNA clusters

Publicly accessible miRNA sequences were obtained from miRBase (Release 11.0) (21). Two prediction programs TargetScan (22) and RNAhybrid (23) were used to nd out the possible targets. To analyze the miRNA clusters, we used the genomic sequences of human (Homo sapiens) (assembly: NCBI Build 36.2, Sep 2006) and mouse (Mus musculus) (assembly: NCBI m36, Dec 2005) from Ensembl genome browser. The homologous sequences were determined using BLASTn algorithm. The sequence alignments were performed using the CLUSTALW program and the alignments were inspected manually to maximize similarity. To nd out the possible transcription promoter, we used PROSCAN

Both miR-125a and miR-125b possess the seed sequence (CCCUGAG) that is predicted to be able to bind to BLIMP-1 and IRF-4 3# UTR target sequence (GGGACUC) that is conserved in humans and mice (Fig. 1A and B). Since miRNAs often exist within clusters (25, 26), we conducted an in silico search for neighbours of the miR-125a and miR-125b genes in human and mouse genomic sequences. This analysis indicated that miR-125a and miR-125b are present in clusters, wherein the neighbouring miRNA genes are located within a few kilobases (Fig. 1C). A similarity search using the BLASTn program revealed that the clustering patterns for miR-125a and miR-125b genes are identical in the human and mouse genomes (Fig. 1C). The miR-125a cluster on chromosome 19 of humans includes three miRNA genes: miR-125a, miR-let-7e and miR-99b; the paralogous miR-125b cluster on chromosome 21 also includes three miRNA genes: miR-125b, miR-let-7c and miR-99a. The orthologous miR-125a and miR-125b clusters in the mouse are located on chromosomes 17 and 16, respectively. The genomic organization of both miR-125a and miR-125b clusters thus is highly

centroblasts may re ect their shared transcriptional orientation and close proximity within the transcription unit of a non-protein-coding RNA gene (NCRNA 00085) (Supplementary Figure 1 is available at International Immunology Online), given that miRNA genes located in the transcription unit with the same transcription orientation are often co-transcribed with the host gene (27, 28). In contrast, a potential independent promoter and a c-Myc-binding site were identi ed 12 Kb upstream of the miR-125b gene in humans. Transcription factors, including c-Myc and TP53, have been shown to regulate the expression of miRNAs through binding to their promoters, thereby contributing to a feedback loop wherein transcription factors regulate miRNA gene expression and the miRNAs regulate transcription factors post-transcriptionally by mRNA binding (29, 30). The differential expression patterns observed for the 125a and 125b miRs indicate that these closely related miRs could inhibit the differentiation of GC centroblasts, with a dominant role for miR-125b being favored by its much higher levels of expression.

Analysis of miR-125b versus miR-125a binding to BLIMP-1 and IRF-4 transcripts

Because both miR-125a and miR-125b possess the seed sequence that is predicted to be able to bind to BLIMP-1 and IRF-4 3# UTR target sequences, we used a luciferase reporter assay to test whether miR-125a and miR-125b could bind to the 3# UTR of BLIMP-1 and IRF-4. This analysis con rmed the binding of miR-125b to the 3# UTR of BLIMP-1 and IRF-4 mRNAs (31) but did not af rm the predicted binding of miR-125a (Fig. 3A and B). Moreover, when expressed together, miR-125a did not enhance or compete with miR-125b binding to the 3# UTR of BLIMP-1 and IRF-4 mRNAs (Fig. 3A and B). Ectopic miR-125b expression achieved by transfection of an miR-125b duplex into HEK-293T cells suppressed the activity of a Renilla luciferase construct containing the miR-125b miRNA response element (MREs) of mouse BLIMP-1 by ~50% ($P < 0.01$) and the activity of a R. luciferase construct containing the miR-125b MREs of mouse IRF-4 by ~20% ($P < 0.05$). In contrast, miR-125a had a negligible effect alone or when combined with miR-125b (Fig. 3A and B). Thus, while miR-125a and miR-125b are unique among the members of their respective clusters in their predicted ability to bind to BLIMP-1 or IRF-4 transcripts, the actual binding to the 3# UTR was veri able only for miR-125b.

BLIMP-1 and IRF-4 inhibition by miR-125b over-expression in B and myeloma cell lines

Since BLIMP-1 and IRF-4 transcripts are potential targets for miR-125b, we re-examined their expression levels during B lineage differentiation. Consistent with previous reports (32–34), we found in this analysis of the different stages of B lineage cells isolated from human tonsils that BLIMP-1 and IRF-4 transcripts are selectively expressed by plasma cells (Fig. 4A and B). To evaluate the effects of miR-125b on BLIMP-1 expression, we introduced precursors of miR-125b into the murine Bcl1.3B3 B lymphoma and the human U266 multiple myeloma cell lines. Stable lentiviral vector-mediated expression of an miR-125b mimic resulted in reduced expression of the BLIMP-1 protein in both cell lines, ndings consistent with our observations in transient transfection experiments (Fig. 5A–C). Introduction of the synthetic miR-125b mimic into the U266 human myeloma cell line led to reduced expression of Syndecan-1 (CD138), a cell surface682(with)-684

shown to regulate antibody production in these cell lines, we examined the effect of miR-125b by retroviral vector-mediated over-expression in primary B cells from the spleen, which were then stimulated with either LPS or CpG. In keeping with our observations in the B cell line, retroviral-mediated over-expression of an miR-125b mimic in murine primary splenic B cells impaired terminal differentiation as re ected by a reduction in the numbers of LPS-induced $CD19^{lo}$

B cell differentiation into plasma cells without demonstrable effect on B cell viability.

Discussion

The coordination of antigen-induced B cell proliferation, somatic V region hypermutation and Ig isotype switching in GC is essential for optimal humoral responses in higher vertebrates. Progression through the distinctive phases in GC differentiation requires a nely tuned program of gene regulation during which extrinsic signals derived from T cell interaction and cytokines modulate the up-regulation or down-regulation of speci c transcription factors, including BCL6 and BLIMP-1. Aberrations in the complex gene expression patterns that coordinate the GC proliferation and differentiation programs may lead to B cell malignancies and autoimmune diseases (35–37). The results of our studies suggest that miR-125b serves an important checkpoint role to guard against premature GC B cell differentiation in humans by binding and sequestering BLIMP-1 and IRF-4.

expressed by centroblasts (38, 44). These miRs could poten-

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