

## **SUPPLEMENTAL METHODS**

### **Animals**

C57BL/6, E $\mu$ /miR-125b, E $\mu$ /miR-125b-Cas9, CD19cre IRF4<sup>flox/flox</sup>, E $\mu$ /miR-125b-CD19cre IRF4<sup>flox/flox</sup> Tg mice were maintained in the Caltech Office of Laboratory Animal Resources (OLAR) facility (all protocols were in accordance to the rules and regulations of the Institutional Animal Care and Use Committee of California Institute of Technology). E $\mu$ /miR-125b mice were a gift from Dr. Toshio Kitamura (The University of Tokyo). CD19cre IRF4<sup>flox/flox</sup> mice were bred using IRF4<sup>flox/flox</sup> and *CD19cre* mice purchased from Jackson laboratory. Bone marrow reconstitution experiments were performed as previously described <sup>1</sup> using donor cells from appropriate mice, and with the vectors as described in the text. Transplantation of miR-125b-induced cancer cells were performed as previously described <sup>2</sup>

### **Luciferase reporter assays**

HEK293T cells were plated and then transfected with either pMG or pMG-miR-125b, a  $\beta$ -gal expression vector, and a pMiReport vector containing S1PR1 3'UTR. After 48 hr, cells were lysed with reporter lysis buffer and subjected to luciferase reporter assays according to manufacturer's instructions (Promega). The relative luciferase activity was measured and normalized to beta-galactosidase activity. The normalized luciferase activity for the samples transfected with pMG control vector was set as 100% and the relative luciferase activity for the samples transfected with pMG-miR-125b vector was then represented as a percent of the samples transfected with pMG control vector.

### **Labeling of bone marrow sinusoidal and parenchymal B cells**

Upon leaving the bone marrow parenchyma, immature B cells pass through an endothelial barrier and enter the blood sinusoids, where they are retained before finally being released into the peripheral blood. Treatment with PE-conjugated antibodies in vivo for 2 minutes allowed selective labeling of cells present within BM sinusoids but not within parenchyma. To detect bone marrow sinusoidal and parenchymal pro/pre, immature and mature B cells, E $\mu$ /miR-125b Tg mice and littermate controls were injected intravenously with 1  $\mu$ g of PE-conjugated anti-CD45.2 antibody in 200  $\mu$ L PBS for 2 min. The mice were then immediately euthanized and the cells were collected from bone marrow and analyzed by flow cytometry to enumerate the sinusoidal pro/pre B cells (B220<sup>+</sup>IgM<sup>-</sup>IgD<sup>-</sup>CD45.2<sup>+</sup>), immature B cells (B220<sup>+</sup>IgM<sup>+</sup>IgD<sup>-</sup>CD45.2<sup>+</sup>) and mature B cells (B220<sup>+</sup>IgM<sup>+</sup>IgD<sup>+</sup>CD45.2<sup>+</sup>), and parenchymal pro/pre B cells (B220<sup>+</sup>IgM<sup>-</sup>IgD<sup>-</sup>CD45.2<sup>-</sup>), immature B cells (B220<sup>+</sup>IgM<sup>+</sup>IgD<sup>-</sup>CD45.2<sup>-</sup>) and mature B cells (B220<sup>+</sup>IgM<sup>+</sup>IgD<sup>+</sup>CD45.2<sup>-</sup>).

### **Epigenetic Analysis**

The epigenetic analysis was described previously <sup>3</sup>. Cells were fixed with 1% paraformaldehyde at 37°C for 10 min and either MNase-treated for ChIP or sonicated for Polymerase II and p300 IP. Precipitated DNA fragments were processed in accordance with Illumina's protocol and sequenced on a Genome Analyzer with manufacturer's instructions. During analysis, short sequence reads were trimmed to 25 nts

and aligned to the mouse genome with either ELAND or Bowtie. Uniquely aligned reads were analyzed by SICER<sup>4</sup> with an expectation value E of 50 in a random background model. Reads on significant islands as defined by SICER were normalized to the total number of reads on islands. Downstream analysis was carried out in R.

### **Flow cytometry and cell sorting**

Respective tissue samples were collected from the appropriate mice as indicated in the text and processed as previously described<sup>1</sup>. They were subsequently stained with a combination of fluorophore-conjugated antibodies, (all from BioLegend), such as CD45, B220, CD19, CD43, CD24, IgM, CD93, CD11b, CD3e, Gr-1, Ter119, Nk1.1, Ly-6C, Sca1, cKit, Flt3, and IL7Ra. For the annexin V labeling of apoptotic cells assay, the bone marrow cells were first stained with anti-B220, anti-IgD and anti-IgM antibodies as well as AAD, and later stained with the Annexin V-PE Apoptosis Detection kit (BD Pharmingen) according to the manufacturer's recommended protocol. For BrdU labeling, mice were injected intraperitoneally once with 100  $\mu$ L BrdU solution (10mg/mL) and analyzed 48 hr later. Surface markers, and BrdU/annexin V incorporation were detected and analyzed using a MACSQuant10 flow cytometry machine (Miltenyi Biotec). Gating and analysis was performed using FlowJo software. For miR-125b expression studies on B cell progenitors and reconstitution of CRISPR/Cas9-edited HSPCs, cells were sorted on a FACSaria IIu cell sorter (BD Biosciences) at the Caltech Flow Cytometry Core Facility after surface staining. For gene and protein expression studies, B cells were purified using an EasySep<sup>TM</sup> mouse B cell isolation kit (Stemcell Technologies, Inc) following the manufacturer's instructions.

### **mRNA extraction and PCR**

RNA was extracted from cells using the RNeasy Mini Kit or RNeasy plus Micro Kit (Qiagen) according to the manufacturer's protocol. TaqMan MicroRNA Assays (Life Technologies) for miR-125b-5p, miR-125b-3p and snoRNA-202 (control) were used to perform TaqMan qPCR as per manufacturer's instructions. The expression level of miR-125b was determined via qPCR by normalizing to endogenous snoRNA202 (values were presented as x100). First-strand cDNA was synthesized from extracted total RNAs using the iScript cDNA Synthesis Kit (Bio-Rad). The expression of mouse S1PR1, S1PR2, S1PR3, E2F2 and BCL2 transcripts were analyzed by SYBR Green-based real-time quantitative RT-PCR (qRT-PCR) using specific primers listed in key resources table. Data were normalized to the expression of GAPDH and are expressed as ratios of values in B cells isolated from E $\mu$ /miR-125b Tg mice to those in B cells from littermate controls.

### **Immunoblotting**

Cells were lysed in 0.5 mL of RIPA lysis buffer (Sigma-Aldrich), supplemented with a cocktail of protease inhibitors (Sigma-Aldrich). Whole protein lysates were subjected to gel-electrophoresis on a mini-PROTEAN TGX gradient (4–15%) gel (Bio-Rad Laboratories) and then transferred onto a PVDF

membrane for immunoblotting involving specific Abs: anti-EDG1 (ab125074; Abcam), actin-HRP (sc-1616; Santa Cruz Biotechnology, Inc.) and goat anti-rabbit-IgG-HRP (sc-2004; Santa Cruz Biotechnology, Inc.).

### Surveyor assay

Mouse bone marrow cells were transduced with retroviral vector carrying gRNAs and genomic DNA was extracted using the QuickExtract-DNA extraction solution (Epicentre) following the manufacturer's protocol. The genomic region flanking gRNA target sites was PCR amplified using HiFi HotStart PCR Kit (Kapa Biosystems) and appropriate primers listed in key resources table. The surveyor assay was performed according to the manufacturer's instructions (Integrated DNA Technologies).

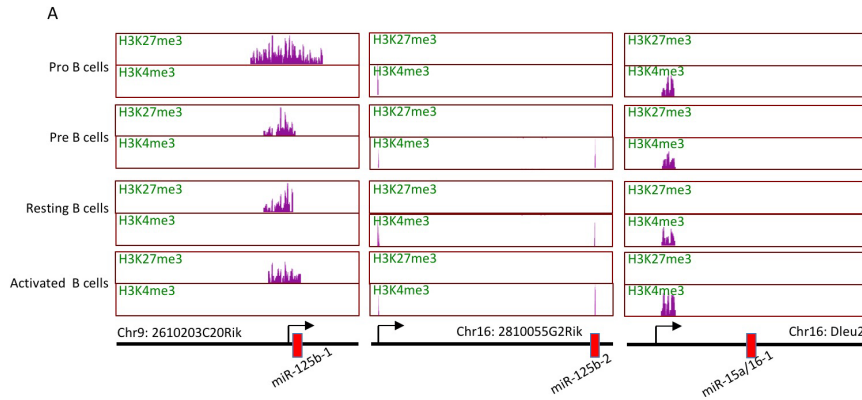
### Clonal analysis of mouse B cell tumors

Analysis of the clonality of mouse B cell lymphomas was performed as described previously {Rolink, 1993 #78;Liang, 2003 #79}. Genomic DNA from representative tumor samples was isolated and used as template for PCR reactions using primer pair (5' of D<sub>H</sub>) 5'-acaagctcaaagcacaatgcctgget-3' and (3' of J<sub>H</sub>4) 5' gggctagactctcagccggctccctcaggg-3'. PCR amplification of wild-type mouse spleen yielded a 1.7 Kb D<sub>H</sub>J<sub>H</sub> 1 fragment, a 1.45 Kb D<sub>H</sub>J<sub>H</sub> 2 fragment, a 1.1 Kb D<sub>H</sub>J<sub>H</sub> 3 fragment, and a 0.6 Kb D<sub>H</sub>J<sub>H</sub> 4 fragment. The PCR products were finally separated on a 1.5% agarose gel.

### Primers

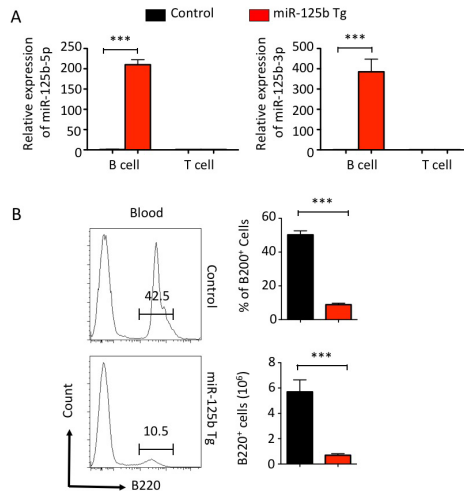
S1pr1-fwd ATGGTGTCCACTAGCATCCC	Integrated DNA Technologies
S1pr1-rev CGATGTTCAACTTGCCTGTGTAG	Integrated DNA Technologies
S1pr2-fwd ATGGGCGGCTTATACTCAGAG	Integrated DNA Technologies
S1pr2-rev GCGCAGCACAAGATGATGAT	Integrated DNA Technologies
S1pr3-fwd GCCTAGCGGGAGAGAAACCT	Integrated DNA Technologies
S1pr3-rev CCGACTGCGGGAAGAGTGT	Integrated DNA Technologies
Surveyor-fwd TCAACCAACTGGGGGAGAGT	Integrated DNA Technologies
Surveyor-revTTGCACTCACTGAACACCAGA	Integrated DNA Technologies

1. Chaudhuri AA, So AY, Mehta A, et al. Oncomir miR-125b regulates hematopoiesis by targeting the gene Lin28A. *Proc Natl Acad Sci U S A*. 2012;109(11):4233-4238.
2. So AY, Sookram R, Chaudhuri AA, et al. Dual mechanisms by which miR-125b represses IRF4 to induce myeloid and B-cell leukemias. *Blood*. 2014;124(9):1502-1512.
3. Kuchen S, Resch W, Yamane A, et al. Regulation of microRNA expression and abundance during lymphopoiesis. *Immunity*. 2010;32(6):828-839.
4. Zang C, Schones DE, Zeng C, Cui K, Zhao K, Peng W. A clustering approach for identification of enriched domains from histone modification ChIP-Seq data. *Bioinformatics*. 2009;25(15):1952-1958.



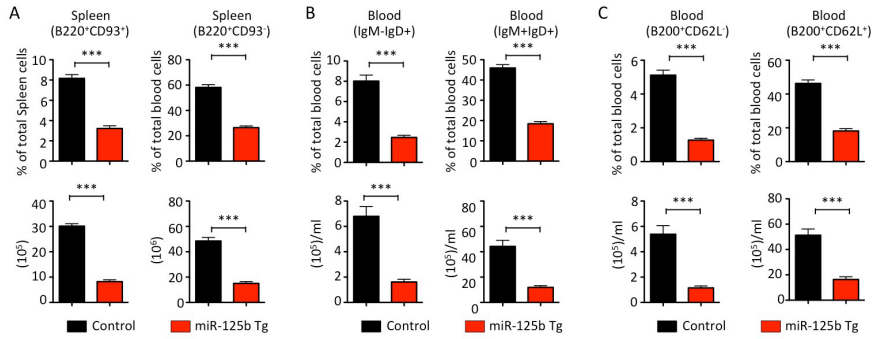
**Figure S1. Chromatin modifications associated with genomic loci encoding miR-125b and miR-15a/16-1 in different B cell subsets.**

Modification patterns of H3K4me3 and H3K27me3 in the loci encoding miR-125b and miR-15a/16-1 in pro-B, pre-B, resting and activated mature B cells.



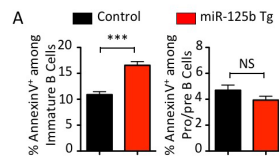
**Figure S2: Reduced number of B cells in peripheral blood of aged E $\mu$ /miR-125b Tg mice.**

(A) Expression of miR-125b-5p and miR-125b-1-3p in B cells and T cells purified from 8-week old E $\mu$ /miR-125b Tg and control mice. (B) Frequency and total number of B220<sup>+</sup> B cells in peripheral blood from 6-month old E $\mu$ /miR-125b Tg and control mice (n=6 mice per group). Data are represented as mean  $\pm$  SEM.



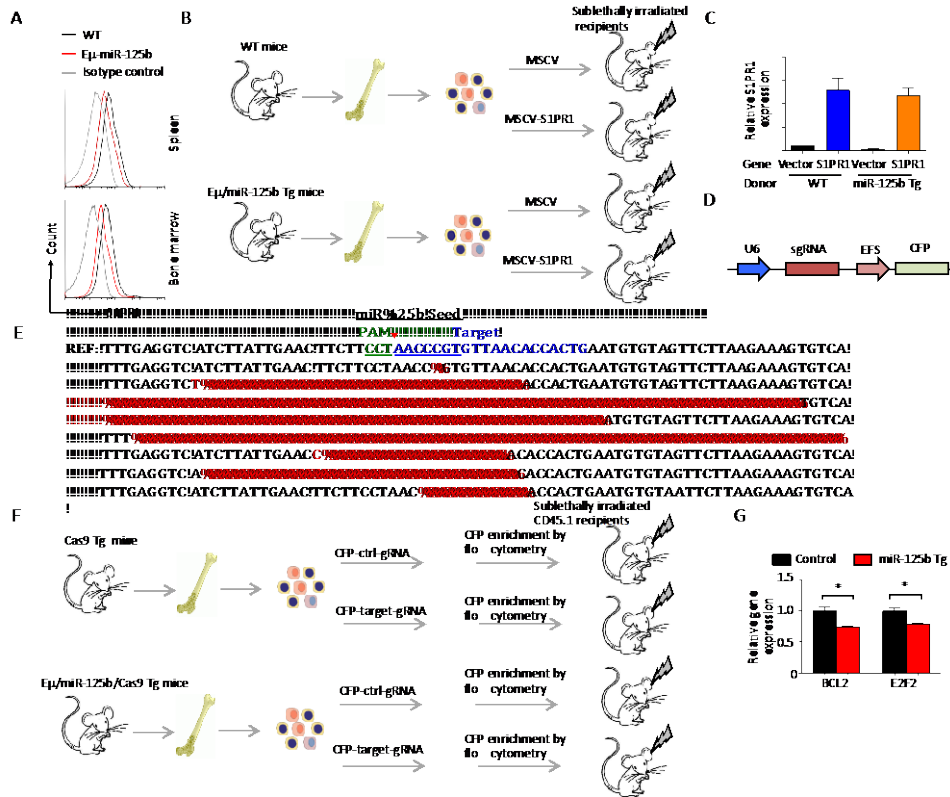
**Figure S3. Enumeration of immature B cells in the blood of E $\mu$ /miR-125b Tg mice.**

(A) The number and frequency of immature B cells (B220<sup>+</sup> CD93<sup>-</sup>) and mature B cells (B220<sup>+</sup> CD93<sup>+</sup>) in the spleens of 8-week old E $\mu$ /miR-125b Tg and control mice (n=3 mice per group). (B, C) The number and frequency of immature B cells (B220<sup>+</sup> IgD<sup>low</sup> IgM<sup>high</sup>; B220<sup>+</sup> CD62L<sup>low</sup>) and mature B cells (B220<sup>+</sup> IgD<sup>high</sup> IgM<sup>low</sup> and B220<sup>+</sup> CD62L<sup>high</sup>) in the blood of 8-week old E $\mu$ /miR-125b Tg and control mice (n=5 mice per group). Data are represented as mean  $\pm$  SEM.



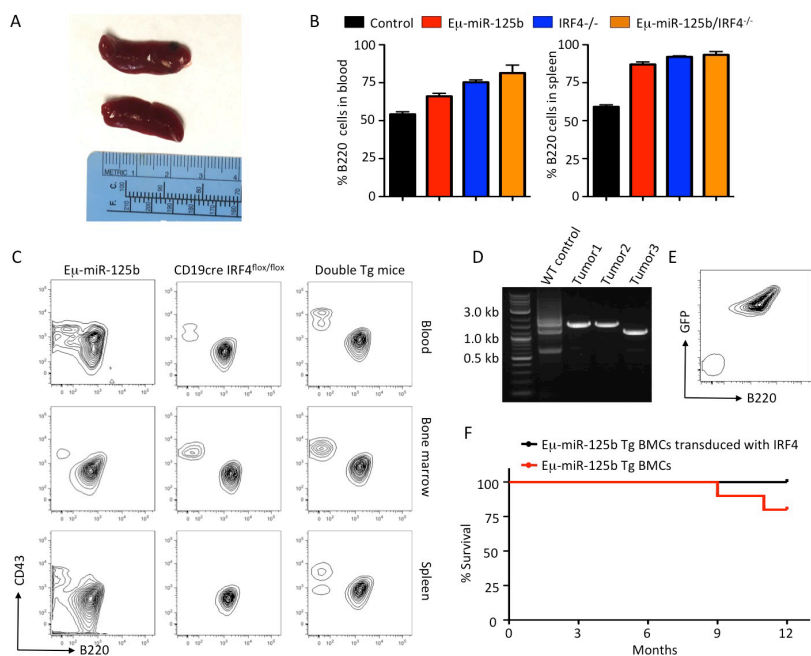
**Figure S4. Increased apoptotic immature B cell in the bone marrow of E $\mu$ /miR-125b Tg mice.**

(A) Percentage of annexin V<sup>+</sup>AAD<sup>-</sup>B220<sup>+</sup>IgD<sup>-</sup>IgM<sup>-</sup>(pro/pre) and B220<sup>+</sup>IgD<sup>low</sup>IgM<sup>high</sup>(immature) B cells in the bone marrow of 8-week old E $\mu$ /miR-125b Tg and control mice (n=5 mice per group). Data are represented as mean  $\pm$  SEM.



**Figure S5. S1PR1 overexpression and CRISPR/CAS9-mediated genome editing of miR-125b targeting site in S1PR1 3'UTR partially rescues miR-125b-mediated defect in B cell egress.**

(A) S1PR1 expression in bone marrow and splenic B cells from WT (black) and Eμ/miR-125b Tg (red) mice. (B) Schematic representation of bone marrow transplantation. Bone marrow of 5-FU pretreated donor Eμ/miR-125b Tg or control mice are harvested and infected with indicated retroviruses. Transduced HSPCs are then transplanted into the irradiated mice via retro-orbital injection. (C) Expression of S1PR1 in the bone marrow, as assayed by RT-qPCR, in mice receiving HSPCs transduced with the same constructs as in (B), is represented as fold overexpression over control. (D) Schematic representation of a gRNA expression vector. (E) Mutation patterns detected by cloning and sequencing the PCR products spanning the expected cleavage site in the S1PR1 3'UTR locus in CRISPR/Cas9 edited cells. Reference (REF) sequence is shown on top of clonal sequences and PAM (green) sequence is indicated. Red dashes denote deleted bases, and red sequences indicate mutated nucleotides. Red arrowhead indicates the predicted Cas9 cleavage site. (F) Schematic representation of bone marrow transplantation of gRNA transduced HSPCs into the irradiated CD45.1 recipient mice. (G) BCL2 and E2F2 transcript expression levels in B cells of Eμ/miR-125b Tg and control mice. Data are represented as mean ± SEM.



**Figure S6. IRF4 functions as a tumor suppressor in miR-125b induced B cell cancer.**

(A) Representative images of spleens harvested from double Tg mice when the mice were moribund and sacrificed. (B) Representative flow cytometric analysis of B cell subpopulations in the blood, bone marrow and spleen of moribund Eμ/miR-125b Tg mice, CD19cre IRF4<sup>flx/flx</sup> Tg mice. (C) Percentage of B220<sup>+</sup> B cells in the blood and spleen of control and moribund Tg mice. (D) PCR analysis of the rearrangements of the VDJ region of the immunoglobulin heavy chain locus in tumors from double Tg mice. (E) Representative flow cytometric analysis of GFP<sup>+</sup>B220<sup>+</sup> B leukemia cells in the moribund recipient mice transplanted with bone marrow cells from moribund double Tg mice. (F) Survival curve of recipient mice transplanted with Eμ/miR-125b-Tg HSPCs transduced with pMG or pMG-IRF4 (n=10 mice per group). Data are represented as mean ± SEM.