

Figure S1. Reconstitution of functional B cells *in vivo* from *iR9X2-ESC*, Related to Figure 1. (A) Schematic diagram of establishing the *iR9X2-ESC* cell line. A CAG Pr-GFP-PGK Pr-PuroR cassette was inserted into the *Hipp11* locus of mouse ESC by homologous recombination to form GFP-ESC clones which were selected by puromycin (1 μ g/ml). Next, a CAG Pr-rtTA-3 \times Stop-TRE-Runx1-p2a-Hoxa9-t2a-Lhx2-pA-PGK Pr-HygroR cassette was inserted into the *Rosa26* locus of GFP-ESC to form *iR9X2-ESC* clones which were selected by hygromycin B (150 μ g/ml). (B) Q-PCR analysis of the expression of *Runx1*, *Hoxa9*, and *Lhx2* in *iR9X2-ESC* after doxycycline induction. (C) *iR9X2-ESCs*-derived GFP⁺CD45⁺Mac1⁺ myeloid cells and GFP⁺CD45⁺CD19⁺ B cells in the PB of μ MT mice were analyzed by flow cytometry 4 weeks and 8 weeks after transplantation. Five million iHECs-derived hematopoietic progenitors were transplanted into each sublethally irradiated μ MT mouse. The mice were fed with water containing doxycycline (1 mg/mL) to induce the generation of B lymphocytes. Two representative mice from five independent experiments are shown.

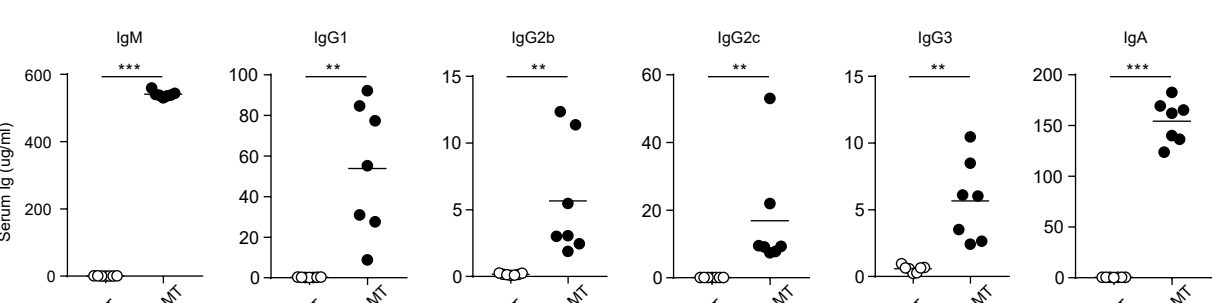


Figure S2. Long term serum antibodies levels in iB- μ MT mice, Related to Figure 2. Sera were collected from iB- μ MT mice (18 to 40 weeks after transplantation) and μ MT mice (n=7 per group). The different isotypes of antibodies (IgM /IgG1 /IgG2b /IgG2c /IgG3 /IgA) were measured by ELISA. Each symbol represents an individual mouse; small horizontal lines indicate the mean. *P < 0.05, **P < 0.01, and ***P < 0.001 (Independent-sample student t test and Mann-Whitney U test).

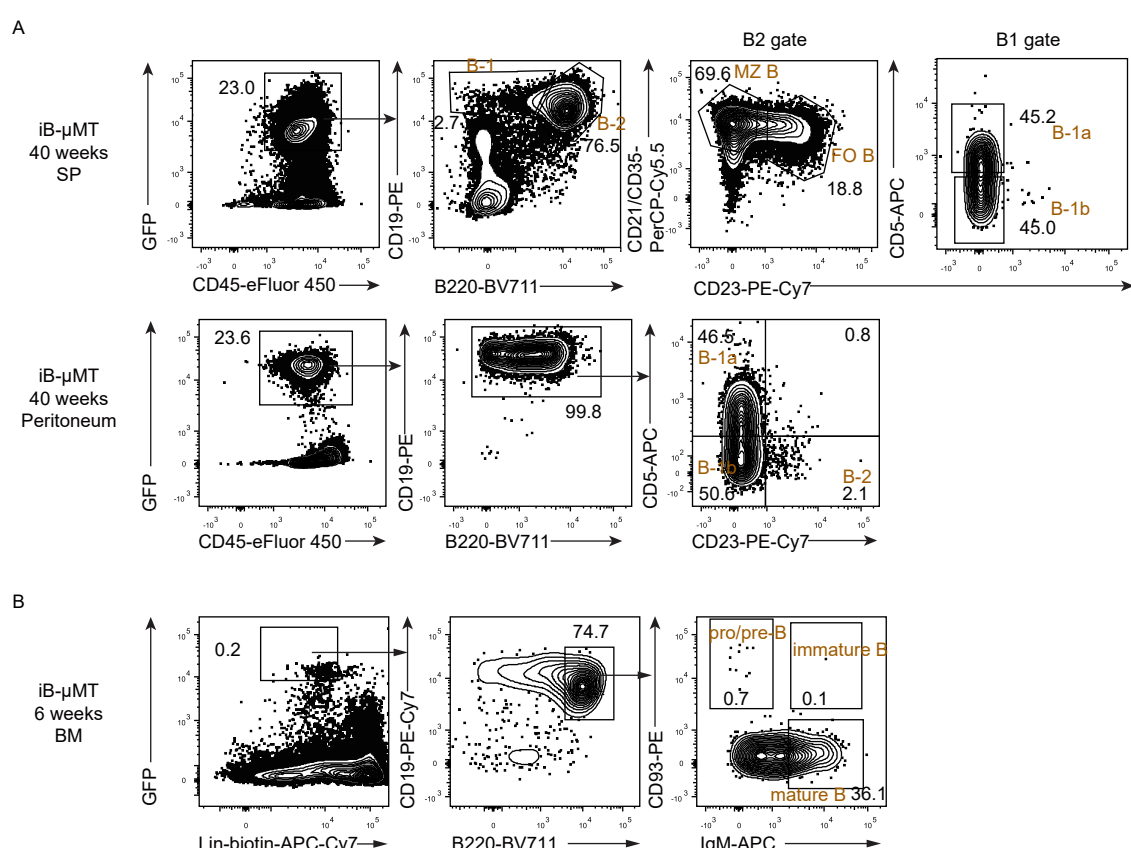


Figure S3. The regenerative B cell subsets in iB- μ MT mice at week 6 and week 40 after transplantation, Related to Figure 3. (A) All major mature B cell subsets were analyzed by flow cytometry at week 40 after transplantation. Immuno-phenotypic plots of B-1a, B-1b, FO B, and MZ B cells in the spleen of iB- μ MT mice were depicted. And immune-phenotypic plots of B-1a, B-1b, and B-2 cells in the peritoneal cavity of iB- μ MT mice were depicted. Data from one representative mouse are shown. (B) Bone marrow cells were isolated from iB- μ MT mice 6 weeks after transplantation, and flow cytometry plots of pro/pre-B cells, immature B cells, and mature B cells were depicted. Data from one representative mouse are shown. Lin⁻ was defined as Ter119⁻ Mac1⁻ Gr1⁻ NK1.1⁻ CD3⁻ CD4⁻ CD8⁻.

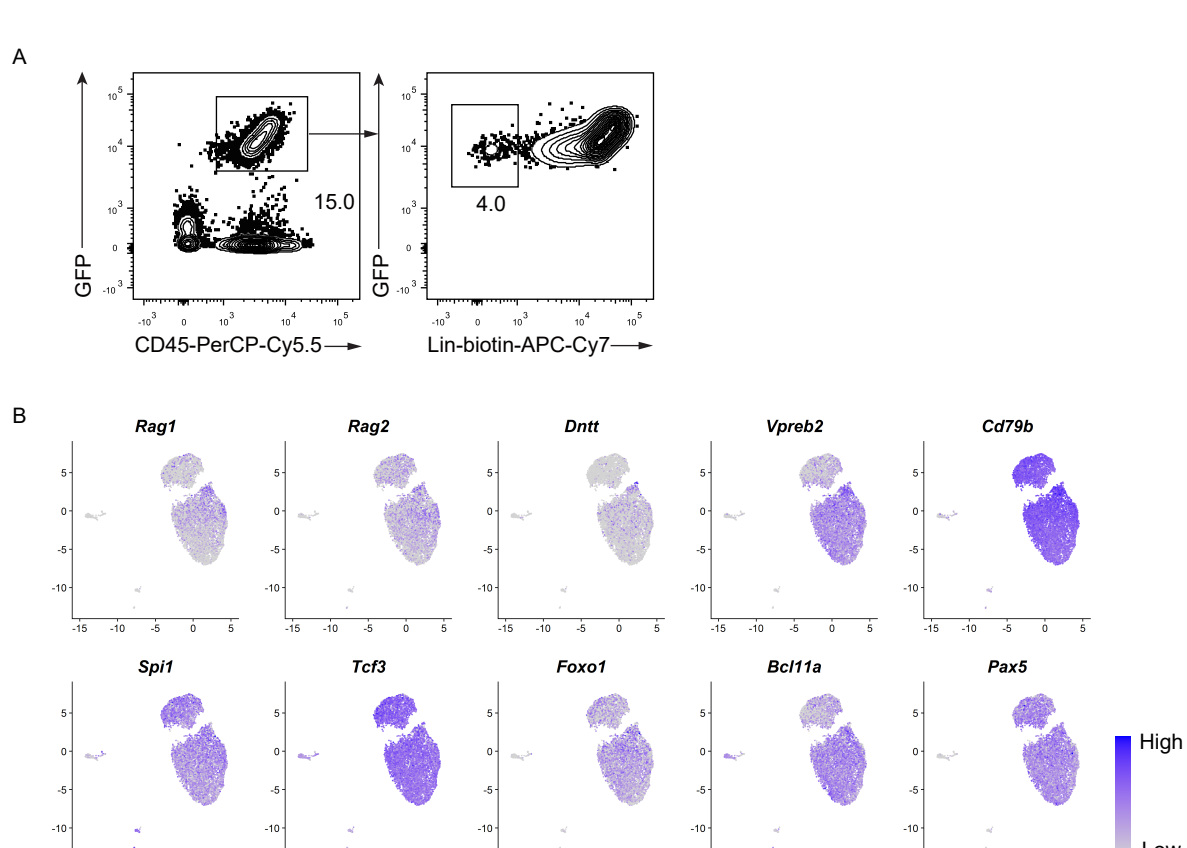


Figure S4. Sorting gates of bone marrow regenerative progenitors and UMAP analysis of the expression patterns of selected early B progenitors related genes, Related to Figure 4. (A) Bone marrow cells were sorted based on GFP⁺CD45⁺Lin⁻ surface markers for single cell sequencing at day7.5 after transplantation, Lin⁻ was defined as CD3⁻ CD4⁻ CD8⁻ Ter119⁻ Mac1⁻ Gr1⁻ NK1.1⁻. (B) Single-cell RNA-seq (7977 cells) was using UMAP plots of VDJ recombination (*Rag1*, *Rag2*, and *Dntt*), pre-BCR complex related genes (*Vpreb2* and *Cd79b*), and early B cell development related transcription factors (*Spi1*, *Tcf3*, *Foxo1*, *Bcl11a*, and *Pax5*). The expression value of each gene was converted by log₂ and illustrated by FeaturePlots of Seurat3.

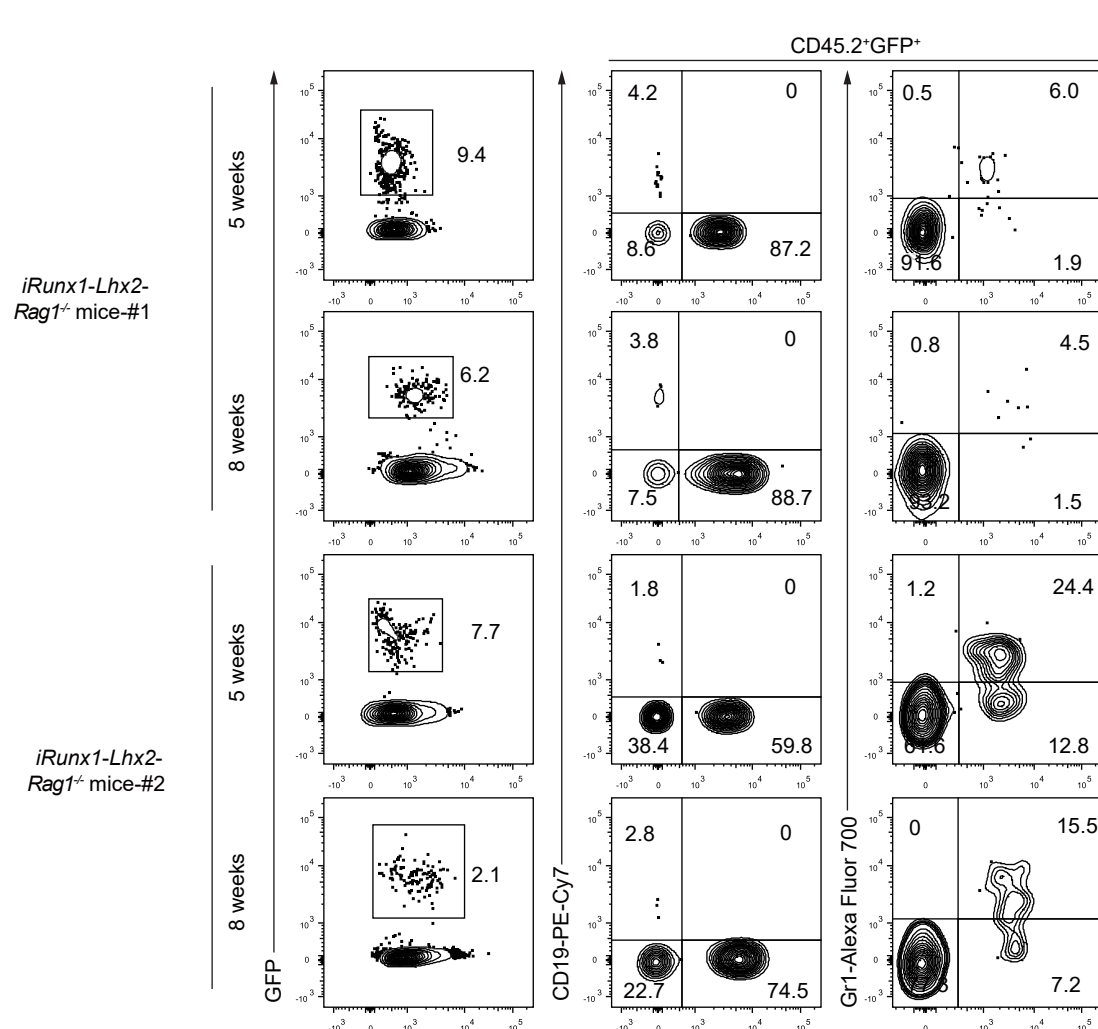


Figure S5. Flow cytometry analysis of hematopoietic cells derived from *iRunx1-Lhx2-ESC* in the peripheral blood (PB) of *Rag1*^{-/-} mice, Related to Discussion. *iRunx1-Lhx2-ESC*-derived iHECs were co-cultured with OP9-DL1 stromal cells for 15 days to obtain enough iHPCs, then 3 million iHPCs were transplanted into each sublethally irradiated *Rag1*^{-/-} mouse (3.5 Gy). The mice were fed with water containing doxycycline (1 mg/mL). Hematopoietic cells (Mac1⁺ myeloid cells, CD3/CD4/CD8⁺ T cells, and CD19⁺ B cells) in the peripheral blood of *Rag1*^{-/-} recipients were analyzed by flow cytometry at week 5 and week 8 after transplantation. Data from two representative mice are shown.