Expanded View Figures

Figure EV1. Analysis of the hypomorphic nature of the *Bbs4*^{GT} allele.

A The exon inclusion in *Bbs4* transcript in the brain of *Bbs4*$^{+/+}$ (WT), *Bbs4*$^{GT/GT}$, and *Bbs4*$^{KO/KO}$ mice was analyzed by end-point RT–PCR with primers specific to exons 5, 6, 7, 8, or 10 (forward) and exon 11 (reverse). A single mouse of each genotype was analyzed.

B The relative expression of *Bbs4* exon 5–6, exon 6–7, exon 7–8, exon 8–10, exon 10–11 in the brain and kidney of *Bbs4*$^{+/+}$ (n = 2 mice), *Bbs4*$^{GT/GT}$ (n = 1), and *Bbs4*$^{KO/KO}$ (n = 1) mice was analyzed using RT–qPCR. Melting curve analysis for the amplicon representing exon 5–6 is shown for *Bbs4*$^{+/+}$ and *Bbs4*$^{GT/GT}$ mice. The average from two animals is shown for *Bbs4*$^{+/+}$.

C Results of Sanger sequencing of the amplicon representing *Bbs4* exon 5–6 of the *Bbs4*$^{GT/GT}$ mice. The sequence shown in red originates from the *Bbs4* GT cassette (En2 exon) and is absent in *Bbs4* WT RNA.

D C-terminally FLAG-tagged full-length *Bbs4* WT and *Bbs4* GT cDNA was transiently expressed in HEK293T cells. The expression of BBS4 was detected by anti-FLAG antibody via immunoblotting. Re-probing the membrane for actin served as a loading control. The same actin staining (30 s exposure) is shown for both exposures of anti-FLAG staining. The arrow indicates a weak band with low apparent molecular weight which was present only in cells transfected with *Bbs4* GT cDNA. Representative experiment out of two biological replicates is shown.

E The expression of properly spliced *Bbs4* (primers annealed to exon 5/6 boundary and exon 7) in the brains of *Bbs4*$^{+/+}$ and *Bbs4*$^{GT/GT}$ mice was analyzed by RT–qPCR. The melting curve analysis of the amplicon is shown. The experiment shows two technical replicates (the tissue was divided into halves before RNA isolation) using a single animal of each strain.

Source data are available online for this figure.

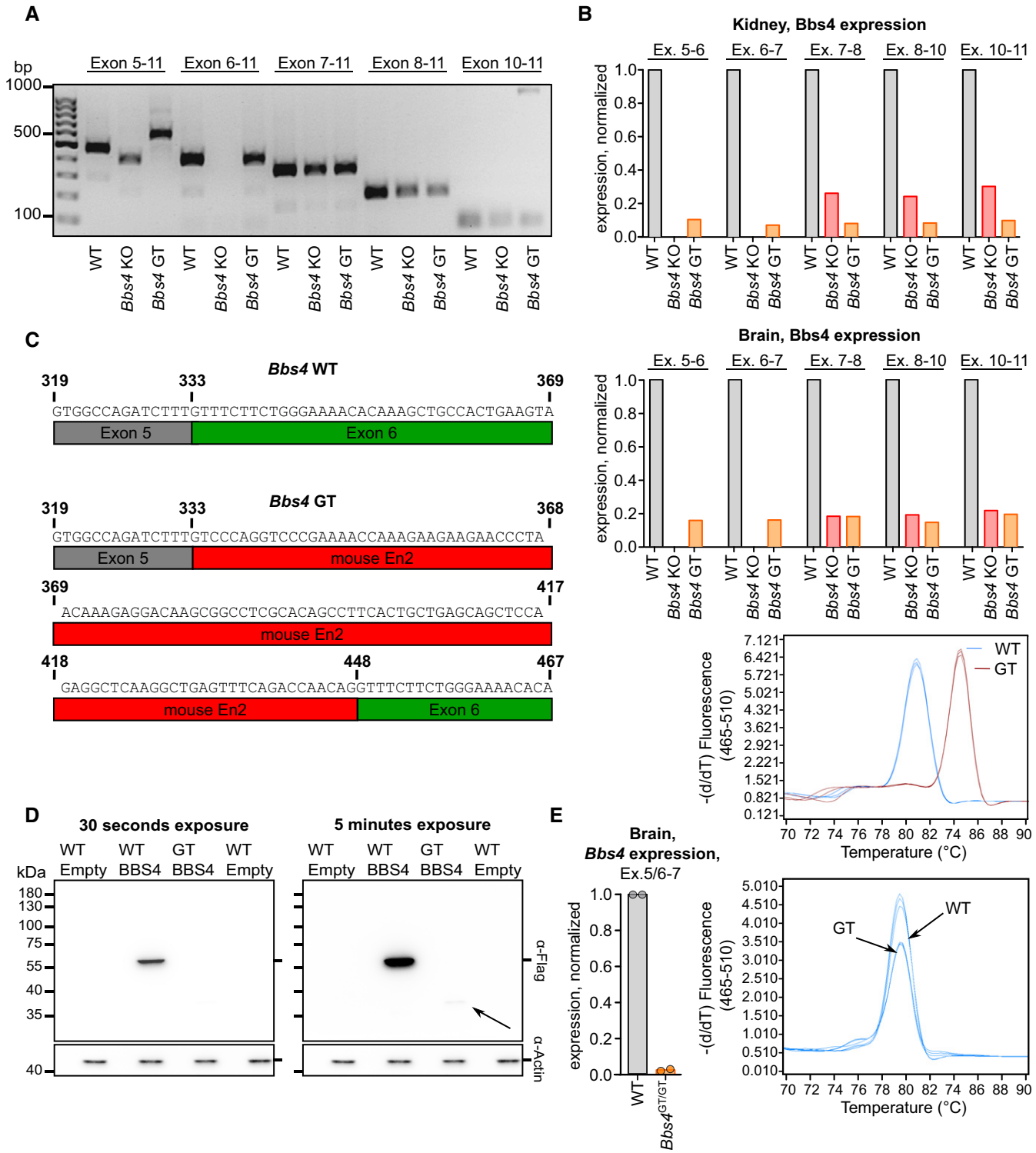

Figure EV1.

Figure EV2. T-cell compartment is mildly affected in *Bbs4*$^{KO/KO}$ mice and unaffected in *Bbs4*$^{GT/GT}$.

A, B Cells isolated from thymi of 6 weeks old *Bbs4*$^{+/+}$ (WT) (n = 5 mice) and *Bbs4*$^{GT/GT}$ (n = 4) littermates were analyzed by flow cytometry in three independent experiments. Representative staining is shown. (B) Thymic cell populations: double-positive (DP) ($CD4^+$ $CD8^+$), CD4 single-positive (SP) ($CD4^+$, $CD8^-$), CD8 SP ($CD4^-$ $CD8^+$), double-negative (DN) ($CD4^-$ $CD8^-$) cells.

C, D Cells isolated from lymph nodes (LN) and spleens (SPL) of *Bbs4*$^{+/+}$ (n = 9 mice) and *Bbs4*$^{GT/GT}$ (n = 10) littermates, or *Bbs4*$^{+/+}$ (n = 9) and *Bbs4*$^{KO/KO}$ (n = 8) littermates were analyzed by flow cytometry, and percentage of $TCR\beta^+$, or $CD8^+$, $CD4^+$ cells was determined. Seven independent experiments for *Bbs4*$^{GT/GT}$, six independent experiments for *Bbs4*$^{KO/KO}$. Mean ± SEM. Statistical significance was calculated using two-tailed Mann–Whitney test.

E Representative experiment showing $CD4^+$ and $CD8^+$ T-cell populations in spleens of *Bbs4*$^{GT/GT}$, *Bbs4*$^{KO/KO}$, and their WT littermates.

F Cells isolated from lymph nodes (LN) and spleens (SPL) of *Bbs4*$^{+/+}$ (n = 8 mice) and *Bbs4*$^{GT/GT}$ (n = 9) littermates, or *Bbs4*$^{+/+}$ (n = 9) and *Bbs4*$^{KO/KO}$ (n = 8) littermates were analyzed by flow cytometry, and percentage of $CD8^+$ $CD44^+$ cells was determined. Six independent experiments were performed for each strain. Representative staining of T cells from spleen is shown. Mean ± SEM. Statistical significance was calculated using two-tailed Mann–Whitney test.

Source data are available online for this figure.

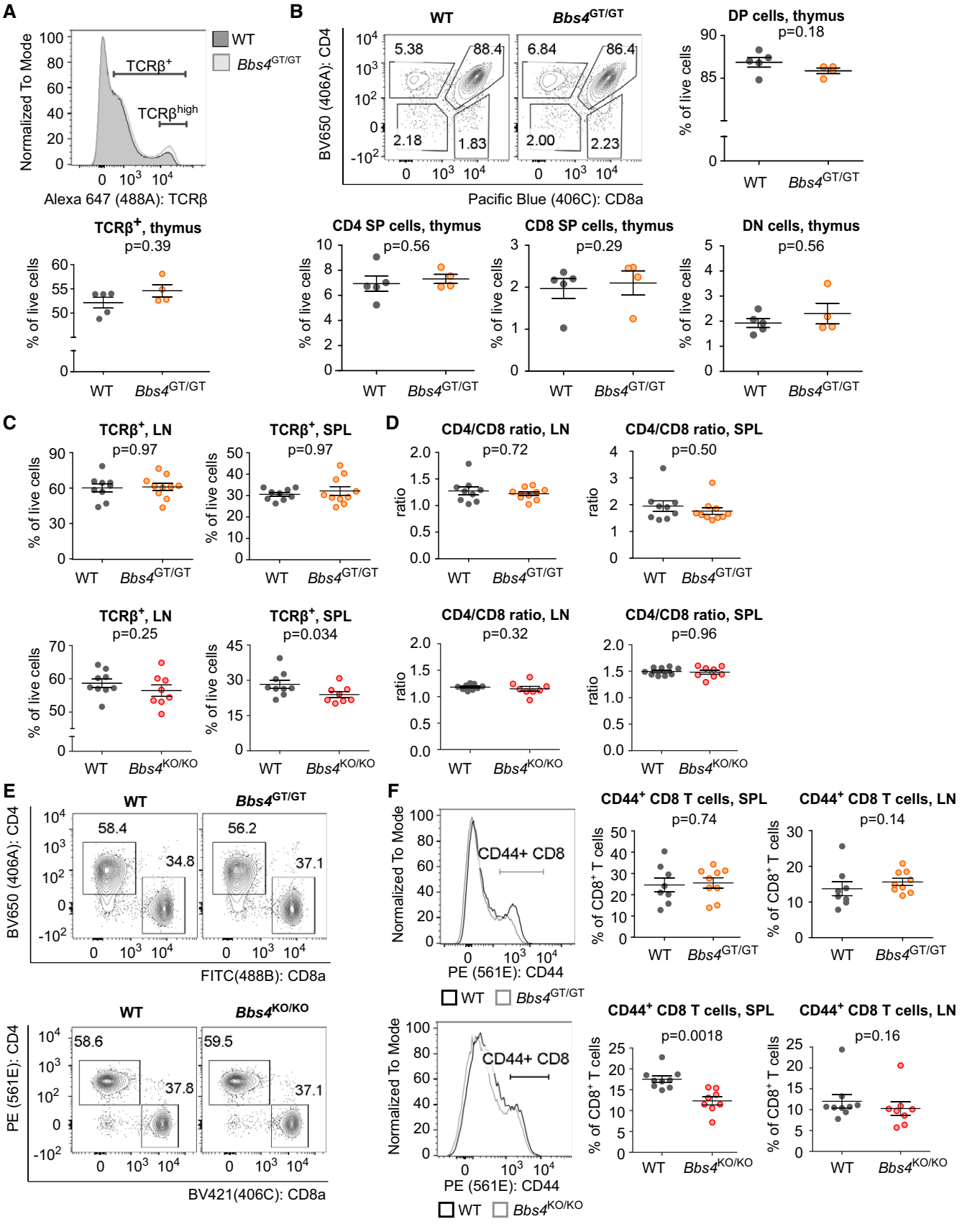

Figure EV2.

Figure EV3. *Bbs4* deficiency in mice leads to B-cell compartment alterations.

A Percentage of $B220^+$ cells in the bone marrow (BM) of $Bbs4^{GT/GT}$ (n = 10 mice), $Bbs4^{KO/KO}$ (n = 10) and their $Bbs4^{+/+}$ (WT) littermates (n = 9, n = 11, respectively) was determined by flow cytometry. For *Bbs4* GT strain, a representative experiment out of six in total is shown. For *Bbs4* KO strain, a representative experiment out of eight in total is shown. Statistical significance was calculated using two-tailed Mann–Whitney test. Medians are shown.

B Splenocyte count in $Bbs4^{+/+}$ (n = 9 mice) and $Bbs4^{KO/KO}$ (n = 7) mice. Medians are shown. Statistical significance was calculated using two-tailed Mann–Whitney test.

C Percentage of B cells ($CD19^+$) in the spleen (SPL) of $Bbs4^{GT/GT}$ (n = 8 mice), $Bbs4^{KO/KO}$ (n = 10) and their $Bbs4^{+/+}$ littermates (n = 9 and n = 11, respectively) was determined. Six independent experiments for $Bbs4^{GT/GT}$ and eight experiments for $Bbs4^{KO/KO}$ were performed. Statistical significance was calculated using two-tailed Mann–Whitney test. Medians are shown.

D Percentage of splenic (SPL) late mature (IgM^- IgD^+) B cells (Late matB) among viable $CD19^+$ cells in $Bbs4^{+/+}$ (n = 9 mice) and $Bbs4^{GT/GT}$ (n = 8) mice. Representative experiment out of six in total is shown. Statistical significance was calculated using two-tailed Mann–Whitney test. Medians are shown.

E Percentage of late mature (IgM^- IgD^+) B cells (Late matB) among viable $CD19^+$ cells in lymph nodes (LN) from $Bbs4^{GT/GT}$ (n = 8 mice) and $Bbs4^{+/+}$ littermates (n = 9), or $Bbs4^{KO/KO}$ (n = 10) mice and $Bbs4^{+/+}$ controls (n = 11). Representative experiments are shown. Statistical significance was calculated using two-tailed Mann–Whitney test. Medians are shown.

F Schematic representation of the *Bbs18* KO mouse model. *Bbs18* reference sequence: ENSMUSG00000084957 (Ensembl database).

G Genotypic ratio of $Bbs18^{+/+}$, $Bbs18^{+/KO}$, or $Bbs18^{KO/KO}$ at weaning from mating of $Bbs18^{+/KO}$ parents. Binomial test was used for statistical comparison of the observed distribution to the expected Mendelian ratio.

H, I Comparison of $Bbs18^{KO/KO}$ mice (n = 2 mice) mice with pooled $Bbs18^{+/+}$ (n = 1, dark gray square) and $Bbs18^{+/KO}$ (n = 3, light gray square) controls. All six mice were littermates and were analyzed side by side in a single experiment. (H) Percentage of B-cell precursors (IgM^- IgD^-) in the bone marrow (BM). Gated on viable $B220^+$ cells. Medians are shown. (I) Percentage of splenic MZ B cells ($CD23^-$ $CD1d^+$) in $Bbs18^{KO/KO}$ mice and their control littermates was determined. Gated on viable $CD19^+$, IgD^- IgM^+, $CD138^-$ cells. Medians are shown.

Source data are available online for this figure.

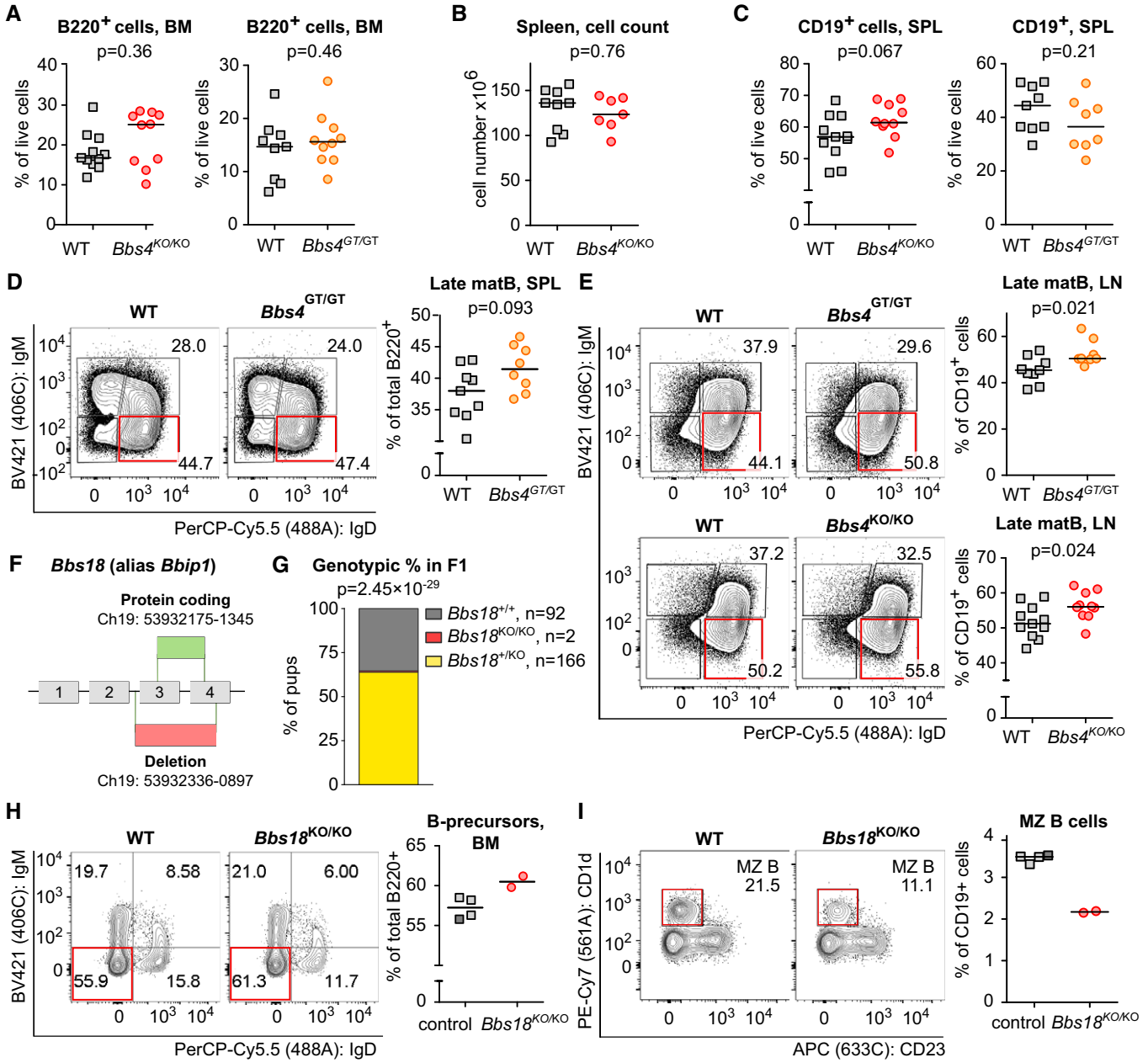

Figure EV3.

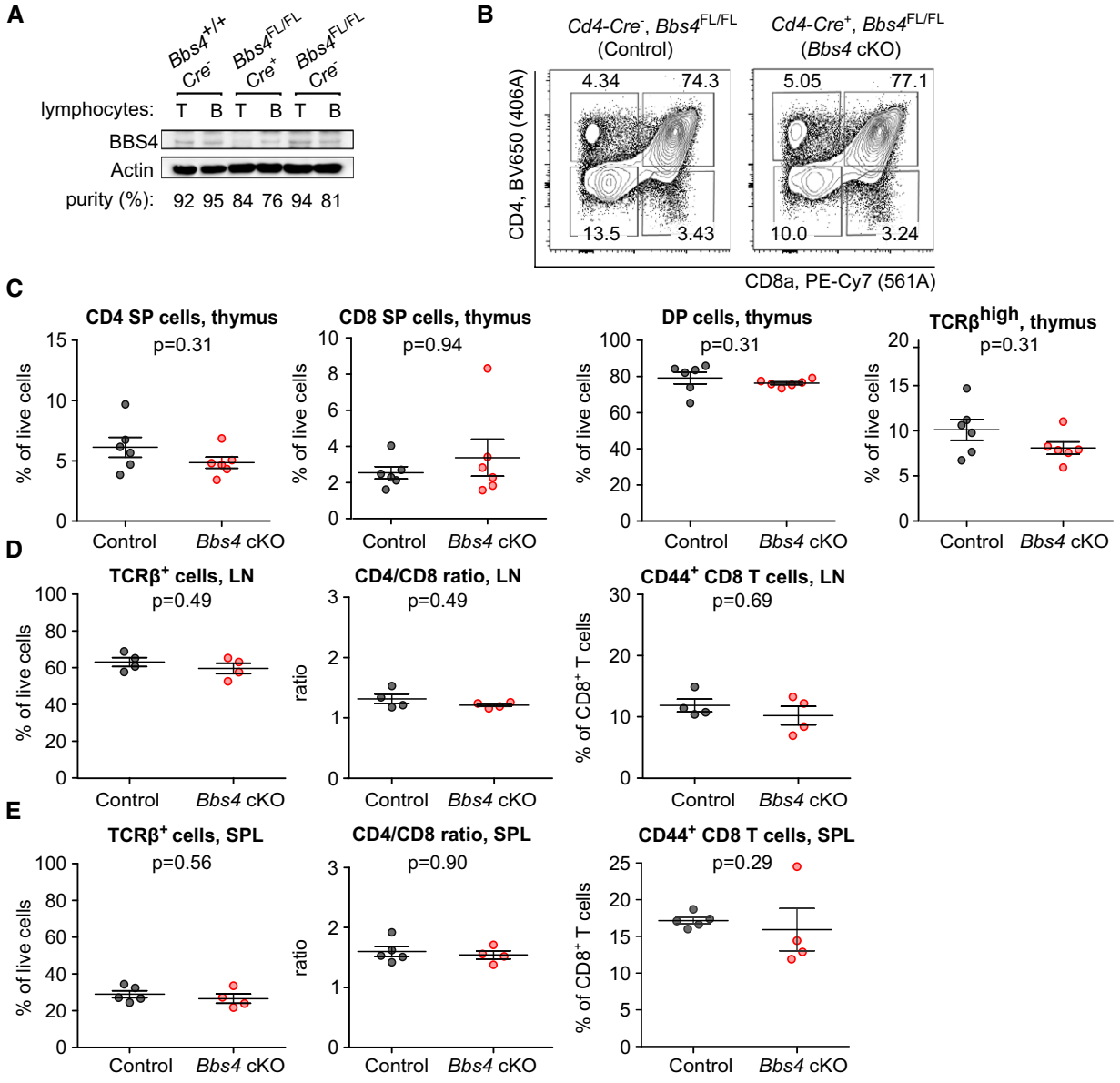

Figure EV4. BBS4 has no intrinsic role in T-cell development.

A The immunoblot analysis of the BBS4 expression in enriched T or B cells taken from lymph nodes and spleens of *Bbs4*$^{+/+}$ *Cd4-Cre*$^{-}$, *Bbs4*$^{FL/FL}$ *Cd4-Cre*$^{+}$, and *Bbs4*$^{FL/FL}$ *Cd4-Cre*$^{-}$ mice. The purity of the enriched populations is indicated. β-actin staining served as a loading control. Part of the identical immunoblot is shown in Fig 1B. A representative experiment out of three biological replicates in total is shown.

B–E Cells isolated from thymi (B, C), lymph nodes (LN) (D), and spleens (SPL) (E) of *Bbs4*$^{FL/FL}$ *Cd4-Cre*$^{+}$ (*Bbs4* cKO) mice were analyzed by flow cytometry. *Bbs4*$^{+/+}$ *Cd4-Cre*$^{+}$ and *Bbs4*$^{FL/FL}$ *Cd4-Cre*$^{-}$ mice were used as controls interchangeably. (B, C) Thymic cell populations: CD4 single-positive (SP) (CD4^{+}, CD8^{-}), CD8 SP (CD4^{-} CD8^{+}), double-positive (DP) (CD4^{+} CD8^{+}), TCRβ^{high} cells. n = 6 mice per group, four independent experiments. Mean ± SEM, two-tailed Mann–Whitney test. (D) T-cell populations in lymph nodes, n = 4 mice per group, three independent experiments. Mean ± SEM, two-tailed Mann–Whitney test. (E) T-cell populations in spleens of control (n = 5 mice) and *Bbs4* cKO (n = 4), analyzed in three independent experiments. Mean ± SEM, two-tailed Mann–Whitney test.

Source data are available online for this figure.

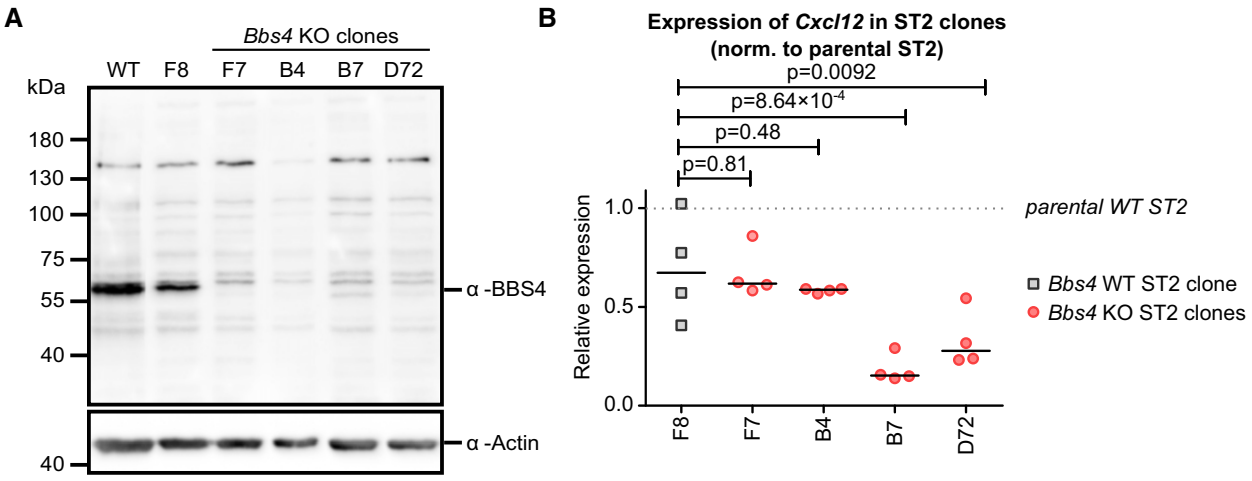

Figure EV5. BBS4 regulates Cxcl12 expression in ST2 cells.

A The expression of BBS4 in the parental ST2 line and in five *Bbs4* KO ST2 clones was analyzed by immunoblotting. The parental line and the clone F8 expresses BBS4, whereas the other clones lack BBS4. Re-probing the membrane for actin served as a loading control. These results are in agreement with the results of the sequencing of the *Bbs4* locus (Appendix Table S6). A representative experiment out of two biological replicates is shown.

B The expression of *Cxcl12* in the parental ST2 line, in one *Bbs4* WT clone (F8) and in four *Bbs4* KO ST2 clones (indicated in red) was analyzed by RT–qPCR. The expression was normalized to *Gapdh* and to the parental ST2 line for each experiment (= 1, represented as a dotted line). The statistical significance was calculated with repeated measures ANOVA ($P = 0.0007$) with Dunnett's multiple comparison post-test (indicated) for comparing each *Bbs4* KO clone to clone F8. $n = 4$ biological replicates.

Source data are available online for this figure.