

Expanded View Figures

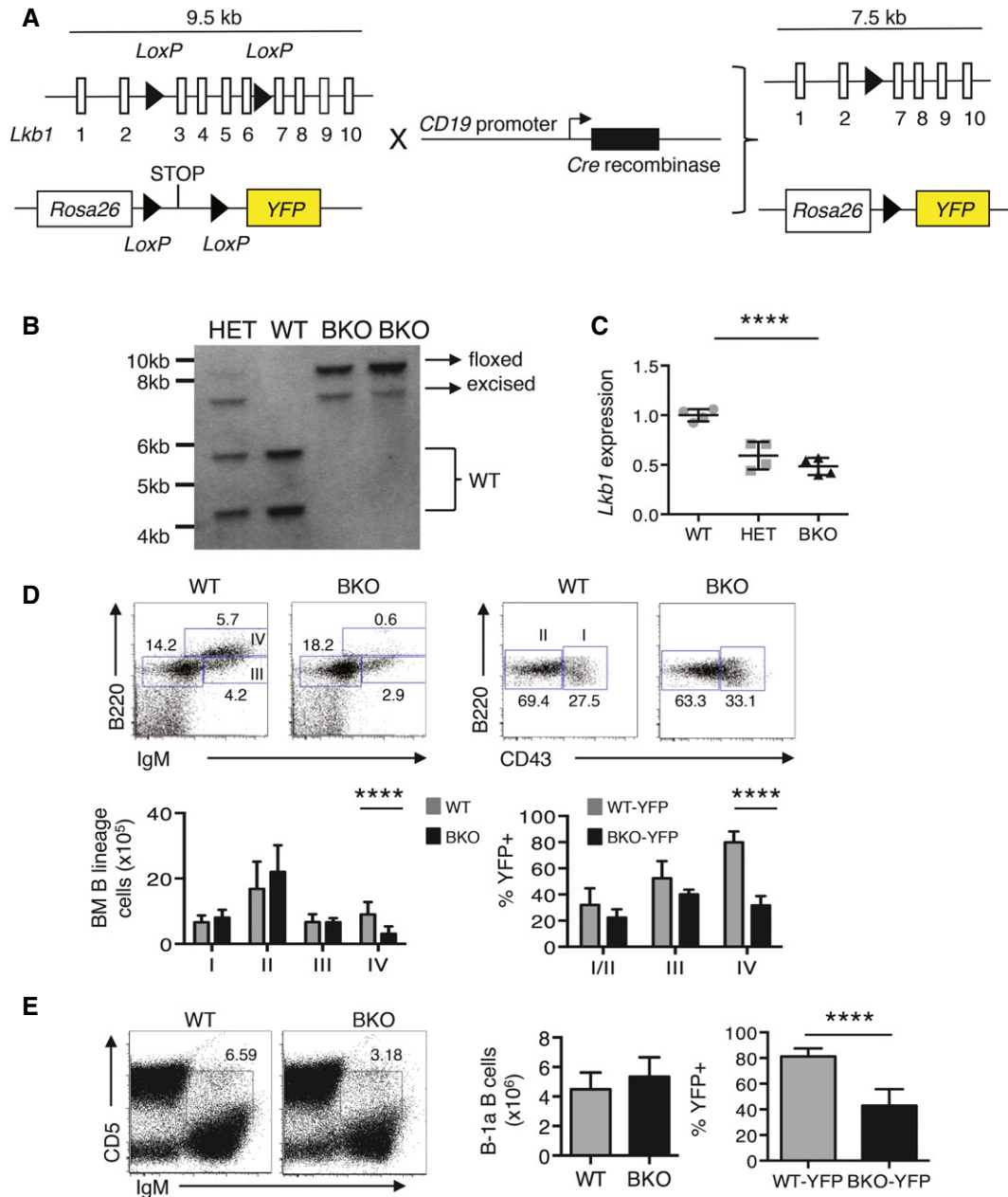


Figure EV1. Decreased recirculating B cells in the BM of BKO mice.

A Strategy for generating *Lkb1* B-cell lineage-specific knockout (BKO, BKO-YFP) mice.

B Southern blot of CD43-depleted splenocytes (> 85% B cells) from HET, WT, and BKO mice evaluating excision of *Lkb1* exons 3–6.

C *Lkb1* expression, relative to *36b4*, by qRT-PCR of CD43-depleted splenocytes from WT ($n = 4$), HET ($n = 4$), and BKO ($n = 4$) mice. Mean \pm s.d., **** $P = 0.0001$ by two-tailed, unpaired Student's t -test.

D Flow cytometry showing pro-B (I), pre-B (II), immature B (III), and recirculating B (IV) cells in the BM of WT ($n = 9$) and BKO ($n = 10$) mice and the frequency of YFP⁺ B cells in each subpopulation ($n = 5$). Mean \pm s.d., **** $P = 0.0004$ by Mann-Whitney U -test (left) and **** $P = 8.8E-006$ by two-tailed, unpaired Student's t -test (right), respectively.

E Flow cytometry showing splenic B-1a cells in WT ($n = 6$) and BKO ($n = 9$) mice and the frequency of YFP⁺ B-1a cells ($n = 9$ and 8, respectively). Mean \pm s.d., **** $P = 1.8E-05$ by two-tailed, unpaired Student's t -test.

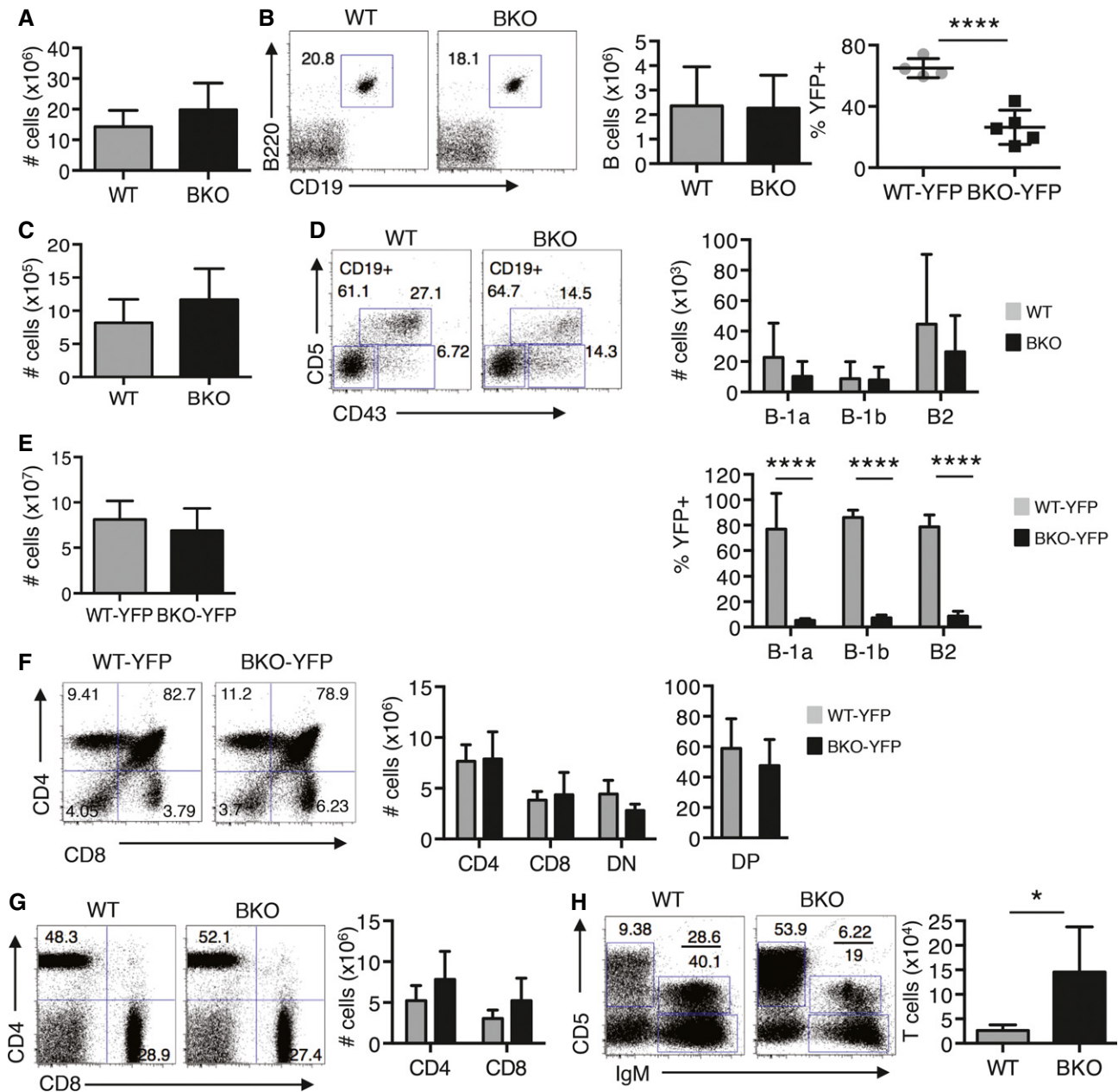


Figure EV2. Reduced LKB1⁻ B cells in lymphoid tissues, but increased T cells in the peritoneum of BKO mice.

A Lymph node cellularity in WT ($n = 7$) and BKO ($n = 10$) mice.

B Left and center panels: Flow cytometry of B220⁺CD19⁺ B cells in lymph nodes of WT ($n = 7$) and BKO ($n = 10$) mice. Right panel: Flow cytometry for YFP⁺ expression in B220⁺CD19⁺ B cells in lymph nodes of WT-YFP ($n = 4$) and BKO-YFP ($n = 5$) mice. **** $P = 0.0005$ by two-tailed, unpaired Student's t -test.

C Peritoneal cavity cellularity in WT ($n = 5$) and BKO ($n = 5$) mice.

D Upper panels: Flow cytometry of B cells in the peritoneal cavity of WT ($n = 5$) and BKO ($n = 5$) mice. Lower panel: Frequency of YFP⁺ cells in total B-1a, B-1b, and B2 B-cell subsets in the peritoneal cavity of WT-YFP ($n = 8$) and BKO-YFP ($n = 8$) mice. **** $P = 0.0002$, $9.8E-012$, and $6.7E-009$, respectively, by two-tailed, unpaired Student's t -test.

E Thymus cellularity in WT-YFP ($n = 5$) and BKO-YFP ($n = 5$) mice.

F Flow cytometry for CD4⁺ and CD8⁺ expression in thymocytes of WT-YFP ($n = 5$) and BKO-YFP ($n = 5$) mice.

G Flow cytometry for CD4⁺ and CD8⁺ T cells in lymph nodes from WT ($n = 7$) and BKO ($n = 10$) mice.

H Flow cytometry for CD5⁺ T cells in the peritoneum of WT ($n = 5$) and BKO ($n = 5$) mice. * $P = 0.016$ by two-tailed, unpaired Student's t -test.

Data information: Mean \pm s.d.

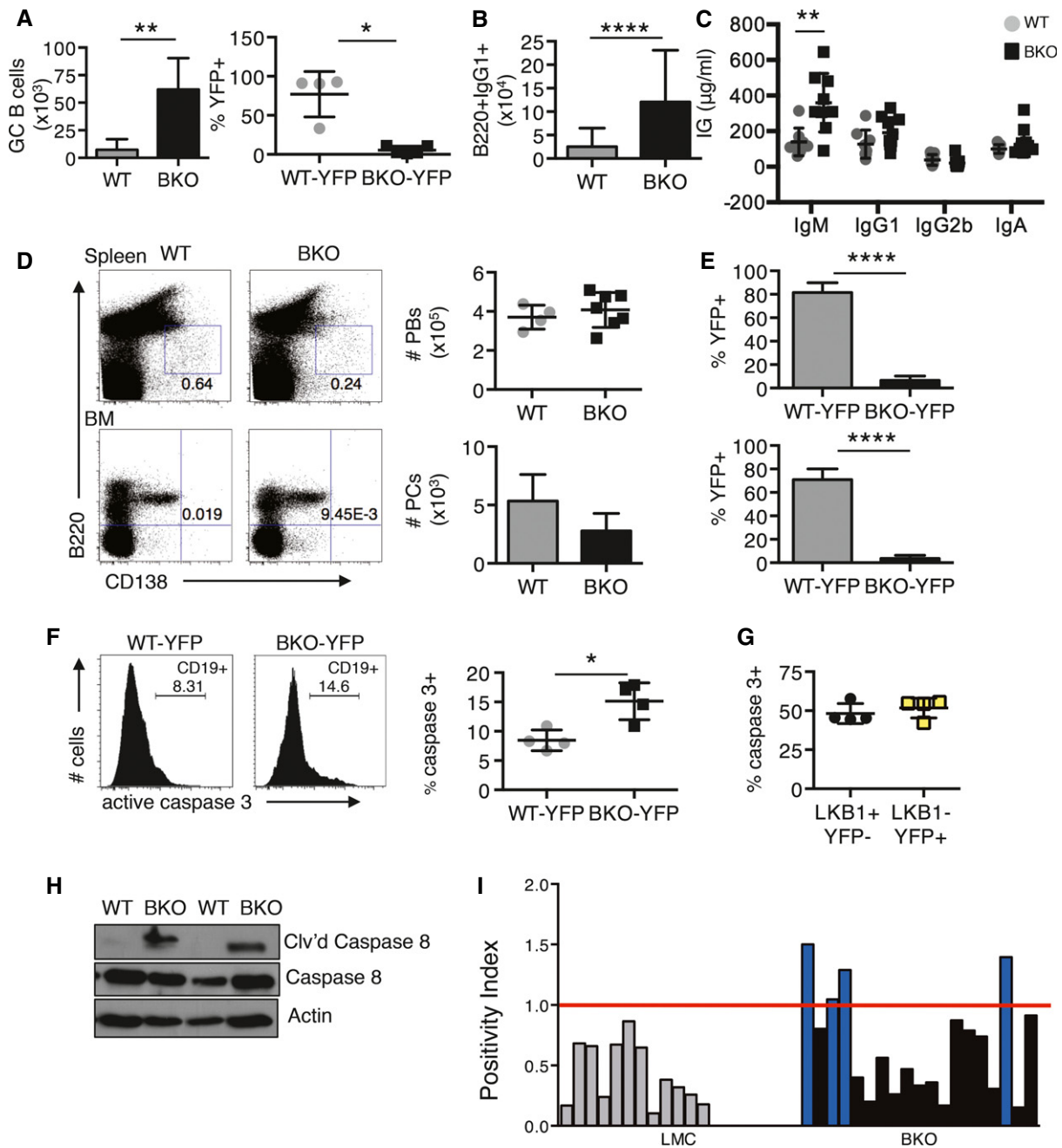


Figure EV3. BKO mice contain abundant isotype-switched and apoptotic GC B cells.

- A Number of B220⁺GL7⁺FAS⁺ GC B cells from WT (*n* = 5) and BKO (*n* = 8) lymph nodes and the frequency of YFP⁺ GC B cells from WT-YFP (*n* = 4) and BKO-YFP (*n* = 5) lymph nodes. ***P* = 0.006 by Mann–Whitney *U*-test and **P* = 0.015 by two-tailed, unpaired Student's *t*-test.
- B Number of B220⁺IgG1⁺ B cells from WT (*n* = 5) and BKO (*n* = 9) lymph nodes. *****P* = 0.0004 by Mann–Whitney *U*-test.
- C Serum Ig isotypes from un-immunized WT (*n* = 7) and BKO (*n* = 10) mice. ***P* = 0.01 by two-tailed, unpaired Student's *t*-test.
- D Flow cytometry and number of B220^{low}CD138⁺ plasmablasts (*n* = 4 and 7, respectively) and B220[−]CD138⁺ plasma cells (*n* = 5 and 5, respectively) from the spleen and BM of WT and BKO mice.
- E Percentage of YFP⁺ to total plasmablasts (*n* = 8) (top) and plasma cells (*n* = 5) (bottom) from the spleen and BM of WT and BKO mice. *****P* = 2.4E-05 and 2.7E-05, respectively, by two-tailed, unpaired Student's *t*-test.
- F Flow cytometry and percentage of CD19⁺ B cells with cleaved caspase 3 expression from spleens of WT-YFP (*n* = 4) and BKO-YFP (*n* = 4) mice. **P* = 0.016 by two-tailed, unpaired Student's *t*-test.
- G Percentage of cleaved caspase 3⁺ CD19⁺YFP⁺ and CD19⁺YFP[−] B cells in spleens of BKO (*n* = 4) mice.
- H Representative Western blot analysis of CD43-depleted splenic B cells from WT and BKO mice for presence of cleaved caspase 8.
- I Anti-nuclear antibodies from the serum of WT or HET littermate controls (LMC) (*n* = 12) and BKO (*n* = 19) mice; blue bars represent animals scoring positive for ANAs.
- Data information: (A–G) Mean ± s.d.

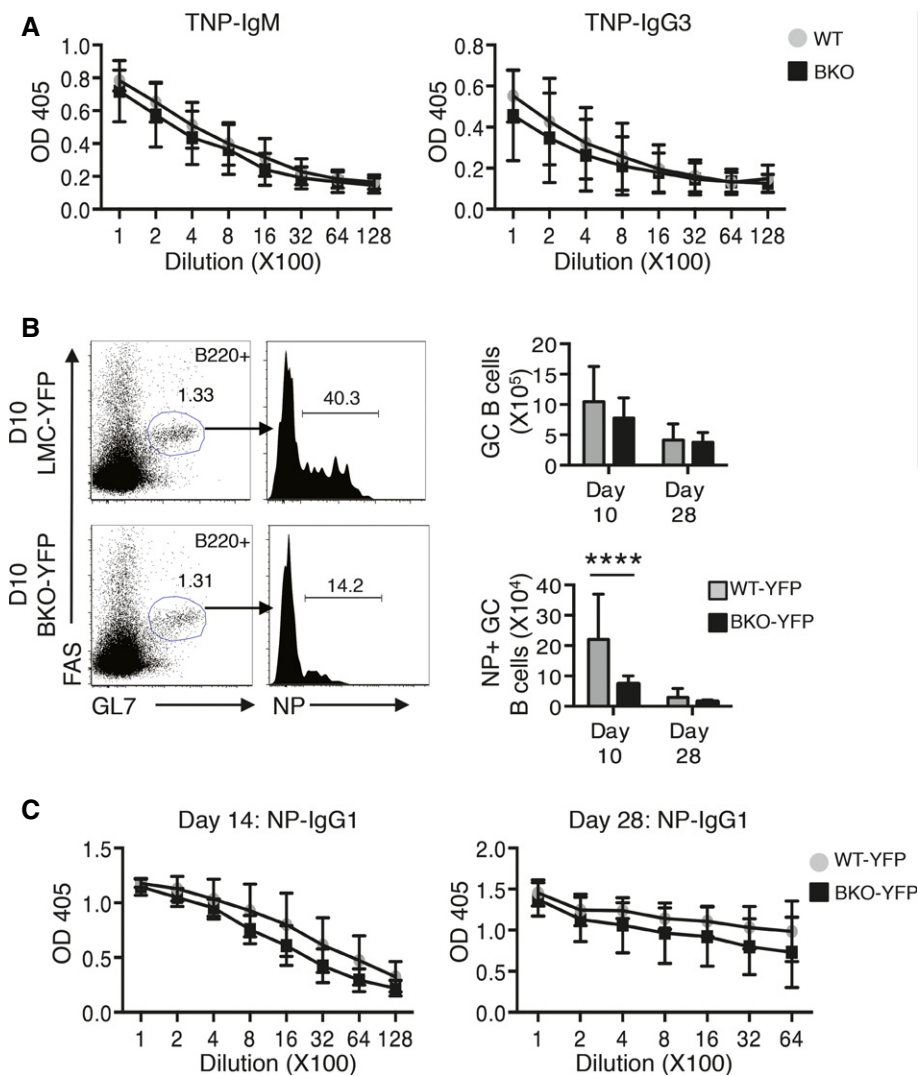


Figure EV4. BKO mice respond to T-cell-independent and T-cell-dependent antigens.

A α TNP-IgM and IgG3 serum responses by ELISA to TNP-AECM-FICOLL 7 days after immunization of WT ($n = 8$) and BKO ($n = 8$) mice.

B Representative flow cytometry for B220, GL7, FAS, and NP expression in splenocytes on day 10 post-immunization from WT-YFP and BKO-YFP mice. Number of total GC and NP-specific GC B cells in response to NP-CGG 10 ($n = 8$ and 8) and 28 days ($n = 7$ and 7) after immunization of WT-YFP and BKO-YFP mice. **** $P = 0.0003$ by Mann-Whitney U -test.

C α NP-IgG1 serum response by ELISA to NP-CGG 14 ($n = 4$ and 4) and 28 days ($n = 6$ and 6) after immunization of WT-YFP and BKO-YFP mice.

Data information: Mean \pm s.d.

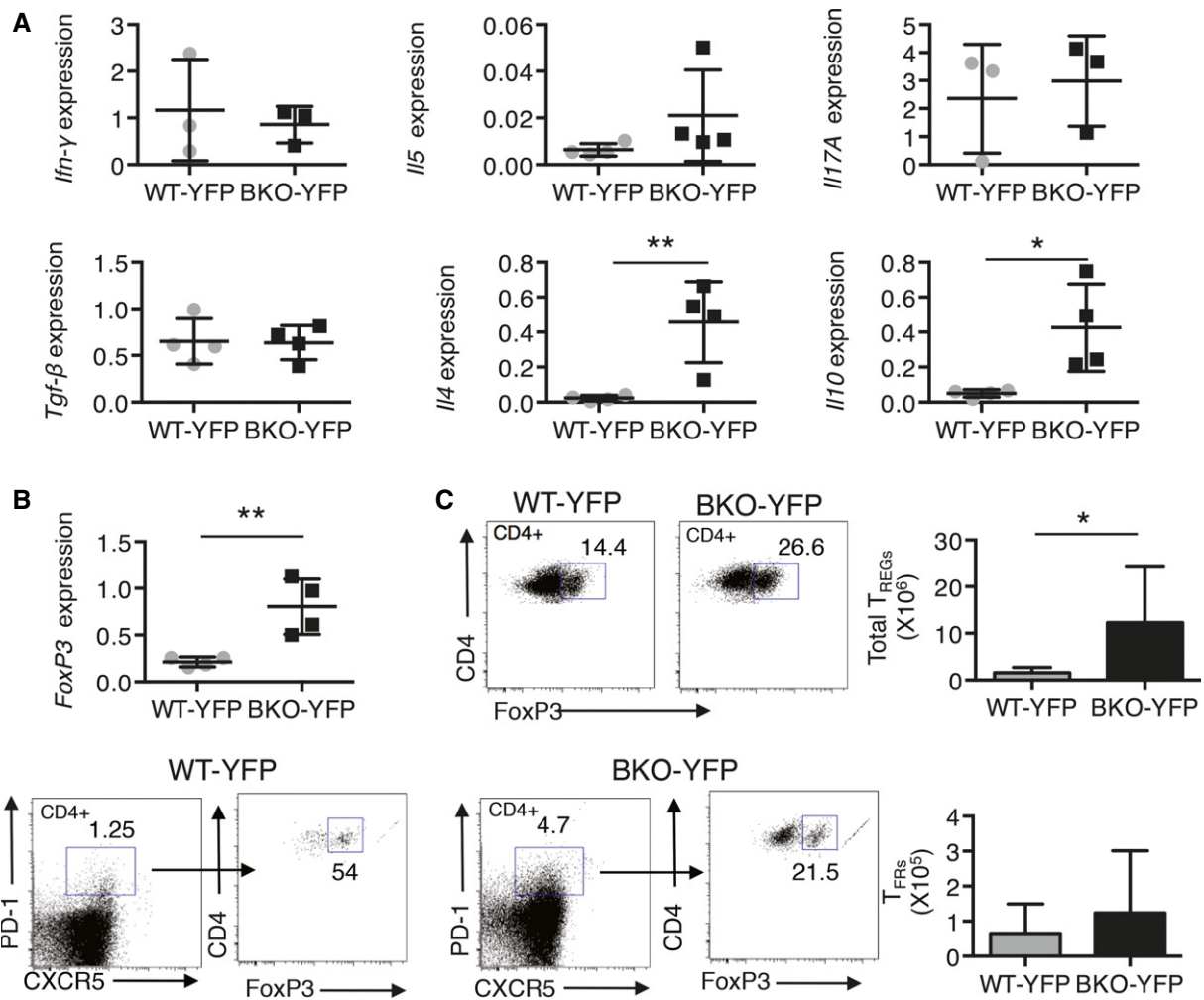


Figure EV5. Activated T cells from BKO spleens are made up of T_{FH} and T_{regs}.

A qRT-PCR for *Ifn-γ*, *Il4*, *Il5*, *Il17A*, *Tgf-β*, and *Il10* expression, relative to *36b4* expression, in splenic CD4⁺ T cells from WT-YFP (*n* = 4) and BKO-YFP (*n* = 4) mice.

***P* = 0.01 and **P* = 0.02 by two-tailed, unpaired Student's *t*-test.

B qRT-PCR for *FoxP3* expression, relative to *36b4* expression, in splenic CD4⁺ T cells from WT-YFP (*n* = 4) and BKO-YFP (*n* = 4) mice. ***P* = 0.008, two-tailed, unpaired Student's *t*-test.

C Flow cytometry analysis for CD4⁺FoxP3⁺ T_{REG} and T_{FR} effector cell populations in WT-YFP (*n* = 6) and BKO-YFP (*n* = 6) mice. **P* = 0.04 by Mann-Whitney *U*-test.

Data information: Mean ± s.d.