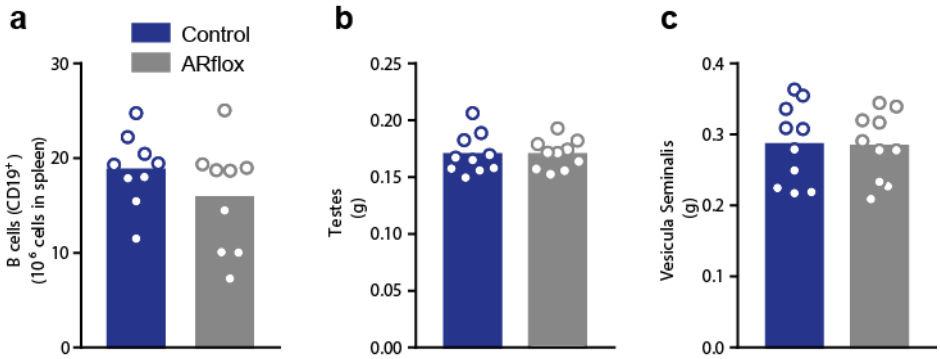
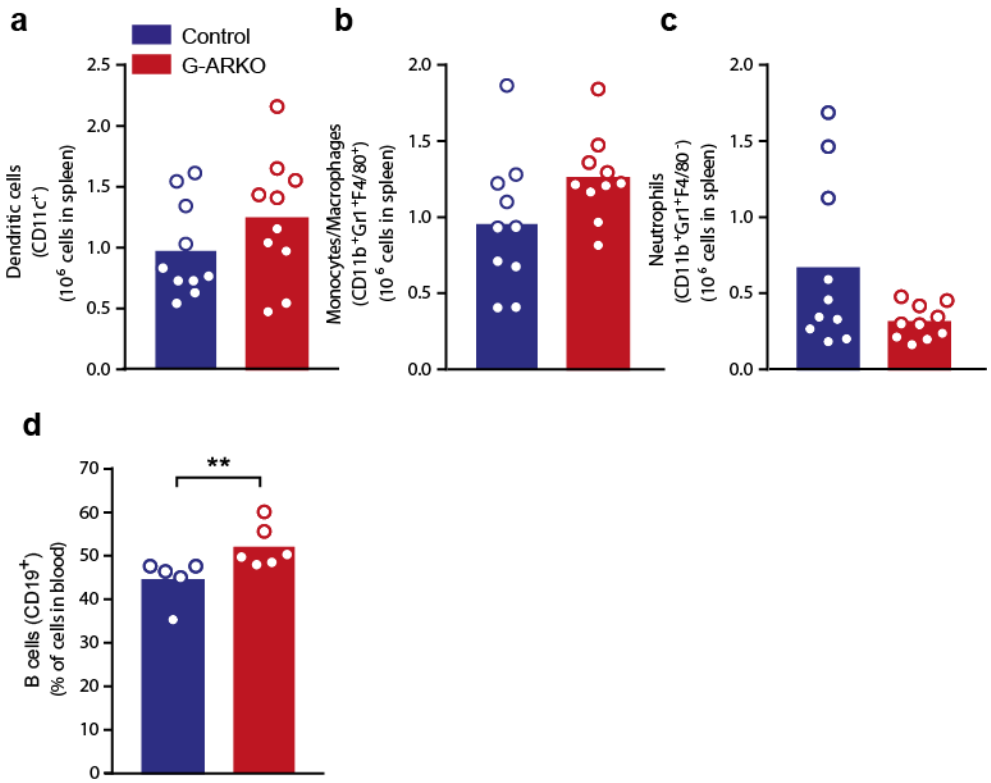
# SUPPLEMENTARY INFORMATION

**Testosterone is an endogenous regulator of BAFF and splenic B cell number**
Wilhelmson et al.

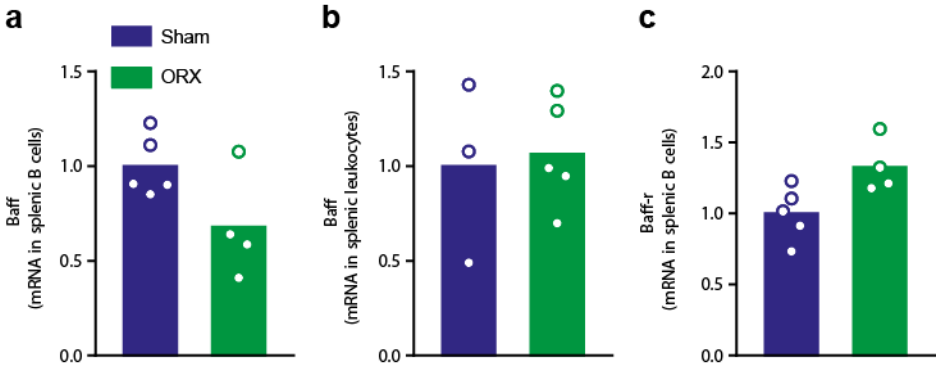
## SUPPLEMENTARY FIGURES



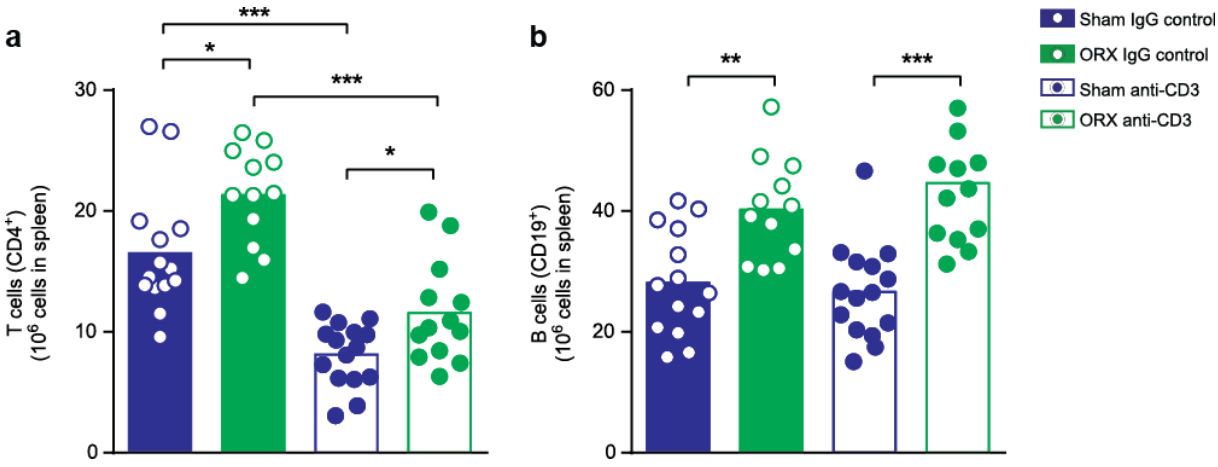

**Supplementary Figure 1. Number of splenic B cells and weights of reproductive organs in ARflox mice.** (**a**) Number of $CD19^+$ B cells in spleen from control ($Ar^+$) and $Ar^{flox}$ male mice; $n$ = 9/group. (**b-c**) Weight of testes (**b**) and seminal vesicles (**c**) in control ($Ar^+$) and $Ar^{flox}$ male mice; $n$ = 10/group. All bars indicate means; circles represent individual mice.



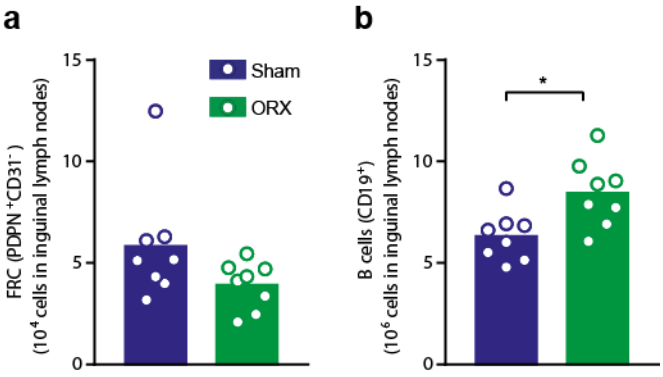

**Supplementary Figure 2. Immune cells in G-ARKO mice.** (**a**) Number of $CD11c^+$ dendritic cells, (**b**) $CD11b^+Gr1^+F4/80^+$ monocytes/macrophages, and (**c**) $CD11b^+Gr1^+F4/80^-$ neutrophils in spleen from control (*Pgk-Cre*$^+$) and general androgen receptor knockout (G-ARKO) male mice. $n = 10$/group. (**d**) Percent $CD19^+$ B cells in blood from control ($n = 5$) and G-ARKO ($n = 6$) male mice. All bars indicate means; circles represent individual mice.**$P < 0.01$ (Mann-Whitney test).



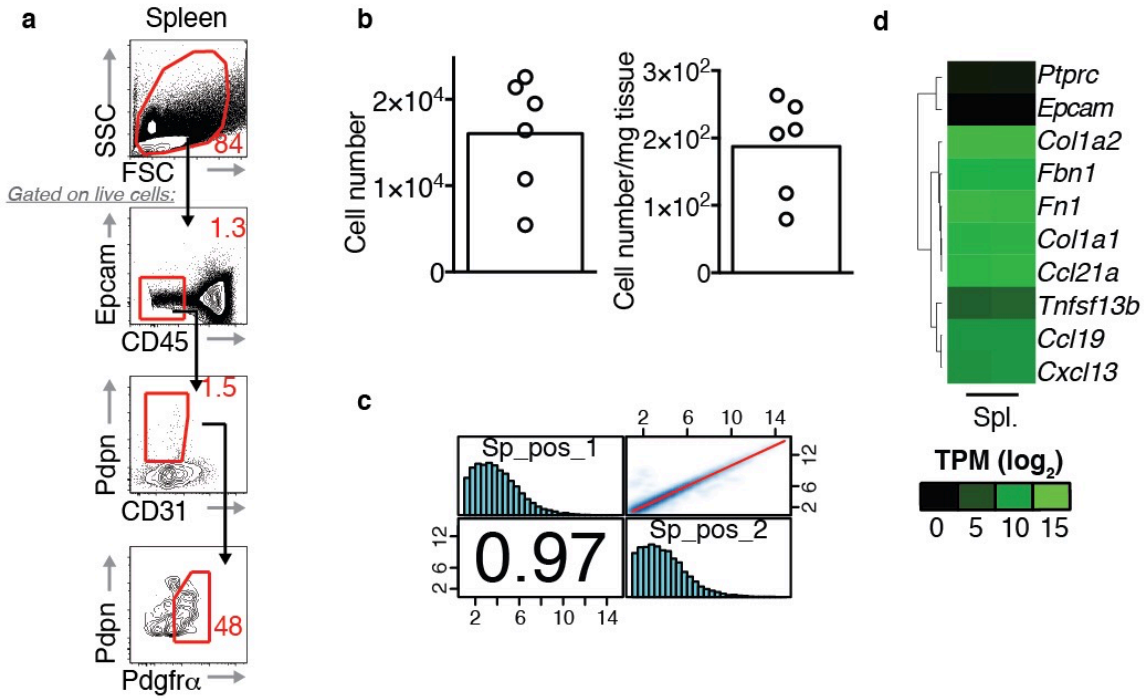

**Supplementary Figure 3. The effect of castration on splenic *Baff* and *Baff-r* mRNA.** (**a-b**) *Baff* mRNA in sorted splenic $CD19^+$ B cells (**a**) and other ($CD19^-CD45^+$) splenic leukocytes (**b**) from sham-operated ($n$ = 5 and 3, respectively) and castrated (ORX; $n$ = 4 and 5, respectively) male mice. (**c**) *Baff-r* mRNA levels in sorted splenic B cells from sham-operated controls ($n$ = 5) and castrated ($n$ = 4) male mice. All bars indicate means; circles represent individual mice.



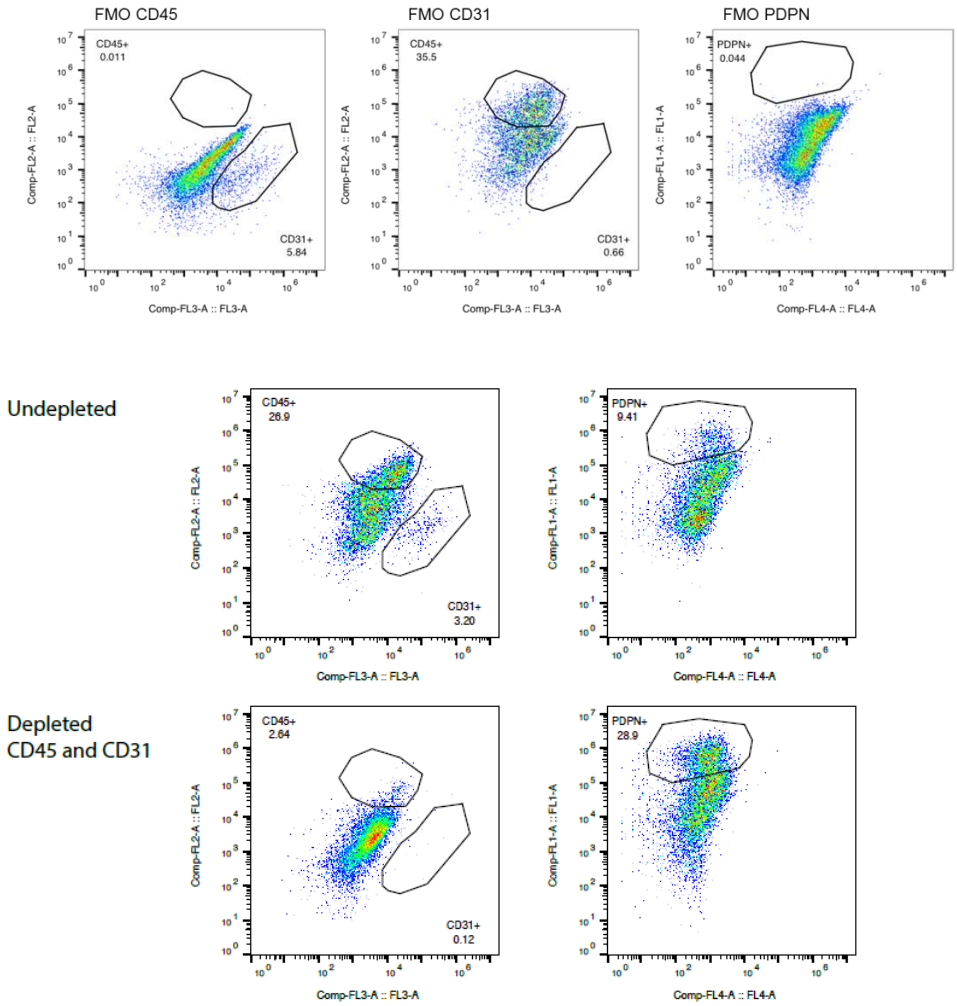

**Supplementary Figure 4. Antibody-mediated T cell depletion does not alter the effect of castration on splenic B cell number.** (**a**) T cells ($CD4^+$) and (**b**) B cells ($CD19^+$) in spleen in control IgG-treated and anti-CD3-treated sham-operated or castrated (ORX) male mice at 16 weeks of age; 2 weeks after last antibody injection. Surgery was performed at 4 weeks of age and antibody injections given from 5 weeks of age, with 3-week intervals. $n$ = 12–15/group. All bars indicate means; circles represent individual mice. *$P$ < 0.05, **$P$ < 0.01, ***$P$ < 0.001 (Kruskal-Wallis followed by Mann-Whitney test).



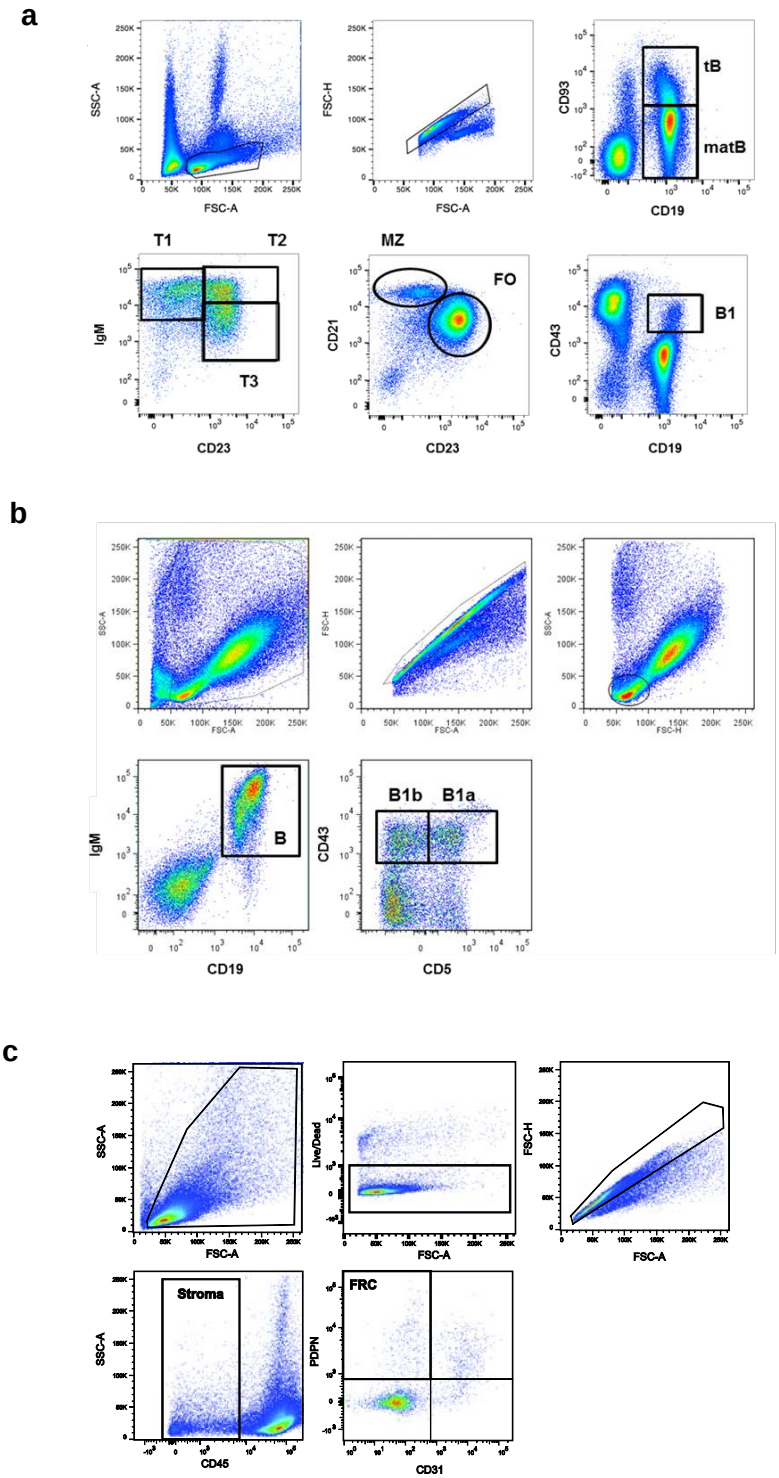

**Supplementary Figure 5. The effect of castration on numbers of FRCs and B cells in lymph nodes.** Number of (**a**) fibroblastic reticular cells (FRCs; $CD45^{-}PDPN^{+}CD31^{-}$) and (**b**) B cells ($CD19^{+}$) in inguinal lymph nodes from sham-operated and castrated (ORX) male mice. $n$ = 8/group. All bars indicate means; circles represent individual mice. *$P < 0.05$ (Mann-Whitney test).




**Supplementary Figure 6. Gene expression in splenic FRCs.** (**a**) Gating strategy and representative flow cytometry plots used to sort splenic fibroblastic reticular cells (FRC); numbers in red denote frequency of gated cells. (**b**) Number of live, $CD45^{-}CD31^{-}PDPN^{+}$ FRCs from spleen and number of FRCs per milligram of tissue. Data are from two experiments. Each dot represents one mouse. (**c**) Similarity matrix between RNAseq samples. Lowly expressed genes were removed (below 1 transcripts per million, TPM) and (TPM + 1) values were then log2 transformed. Pairwise Spearman correlation statistics were calculated among all samples and reported here (lower left). (**d**) Expression of fibroblast and FRC genes in sorted splenic FRC. Corresponding data on adrenergic receptor expression presented in Fig. 6a.




**Supplementary Figure 7. Splenic fibroblastic reticular cell (FRC) culture *in vitro*.** Spleens from wild-type male mice were digested and after culture for 6 days, cells were enriched for nonhematopoietic and nonendothelial cells with anti-CD31 and anti-CD45 microbeads. The stromal cell composition was assessed by flow cytometry for CD31, CD45, and PDPN before and after enrichment.




**Supplementary Figure 8. Gating strategies.** (**a**) Splenic B cells (data in Fig. 1b-c, 1e-j, 2a-d, 3h-k, 6h). Nucleated cells were gated to exclude red blood cells; smaller fragments and nonsingle cells were excluded. Transitional B cells (tB) were identified as $CD19^{+}CD93^{+}$ and mature B cells (matB) as $CD19^{+}CD93^{-}$. These were further divided into T1–3 cells by IgM and CD23 expression and follicular (FO) and marginal zone (MZ) B cells by CD21 and CD23 expression. B1 cells were identified as $CD19^{+}CD43^{+}$. (**b**) Peritoneal B cells (data in Fig. 1d). Nucleated cells were gated to exclude red blood cells; smaller fragments and nonsingle cells were excluded. Lymphocytes were selected and gated for IgM. $CD19^{+}$ B cells were identified as $CD19^{+}IgM^{+}$, B1a cells as $CD43^{+}CD5^{+}$, and B1b cells as $CD43^{+}CD5^{-}$. (**c**) Splenic FRCs (data in Fig. 5d-f, 6b). Smaller fragments, dead cells, and nonsingle cells were excluded. Stromal cells were identified as $CD45^{-}$ and of these FRCs (fibroblastic reticular cells) were identified as $PDPN^{+}CD31^{-}$.

## SUPPLEMENTARY TABLES

**Supplementary Table 1.** Characteristics of eugonadal and hypogonadal men

| **Characteristics** | **Eugonadal (*n* =114)** | **Hypogonadal (*n* = 12)** | ***P* value** |
|---|---|---|---|
| Age (years) | 36 ± 8 | 42 ± 8 | 0.006 |
| Body mass index (kg/m$^2$) | 25 ± 3 | 27 ± 4 | 0.022 |
| Total testosterone (nmol/L) | 19 ± 5 | 10 ± 6 | <0.0001 |
| Free testosterone (nmol/L) | 0.38 ± 0.08 | 0.18 ± 0.03 | <0.0001 |

Values are means ± s.d. P-values are from t-test.

**Supplementary Table 2.** Primers used for genotyping

| Gene | Type | Primer sequence (5'-3') |
|---|---|---|
| *Ar* (exon 2 locus) | F | AGCCTGTATACTCAGTTGGGG |
| | R | AATGCATCACATTAAGTTGATACC |
| *Cre* | F | AACATGCTTCATCGTCGG |
| | R | TTCGGATCATCAGCTACACC |
| *Zfy* | F | AAGATAAGCTTACATAATCACATGGA |
| | R | CCTATGAAATCCTTTGCTGCACATGT |

We assessed *Ar*[1] and *Cre* genotypes using PCR amplification of genomic DNA. Male sex (presence of the Y chromosome) was confirmed by detection of the *Zfy* gene[1].

## REFRERENCES

1. De Gendt, K., *et al.* A Sertoli cell-selective knockout of the androgen receptor causes spermatogenic arrest in meiosis. *Proc Natl Acad Sci U S A* **101**, 1327-1332 (2004).