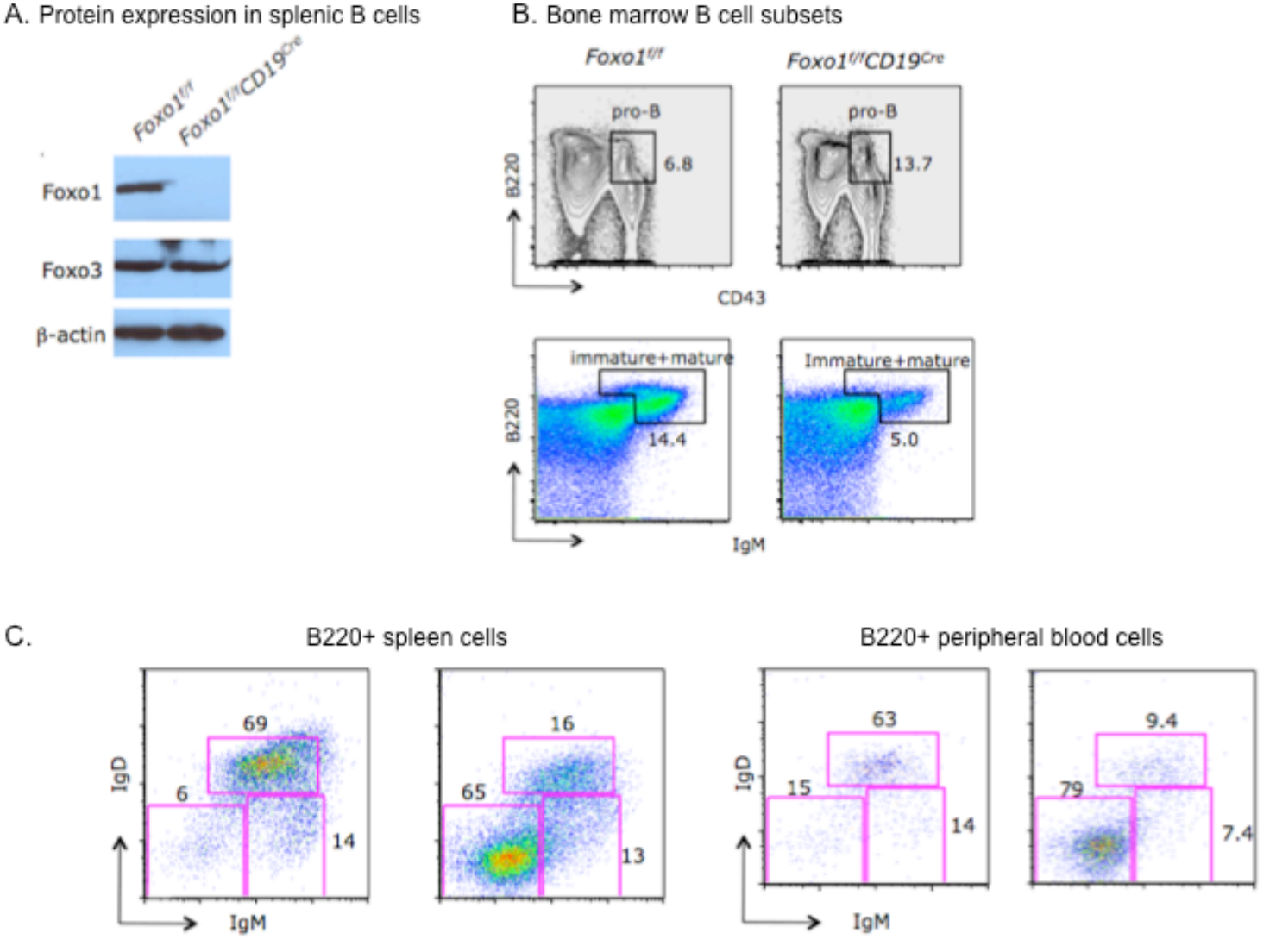
Supplementary Methods

Western blots. Primary B cells were purified from mouse spleens using MACS (Miltenyi Biotec, Auburn, CA) magnetic depletion columns and anti-CD43 beads as described [1, 2]. B cell purity was determined to be >95% by FACS analysis (FACSCalibur and CellQuest software, BD Biosciences; FlowJo software) using anti- B220 and anti-Thy1.2 antibodies. B cells were washed in 1X PBS, and whole cell lysates were prepared in ice cold RIPA buffer (10 mM Tris-HCl, pH 7.4, 50 mM NaCl, 30 mM sodium pyrophosphate, 50 mM NaF, 5 μM $ZnCl_2$, 100 μM sodium orthovanadate, 1% Triton X-100 supplemented with phosphatase inhibitor cocktails I and II (Sigma), and protease inhibitor cocktail (Sigma)) for 10 min on ice. Lysates were subsequently spun at 16000 x g for 10 min. Supernatants were mixed with 5X sample buffer and boiled for 5 min. Lysates corresponding to equal cell numbers were electrophoresed and transferred to nitrocellulose at 4°C. All antibodies were from Cell Signaling.

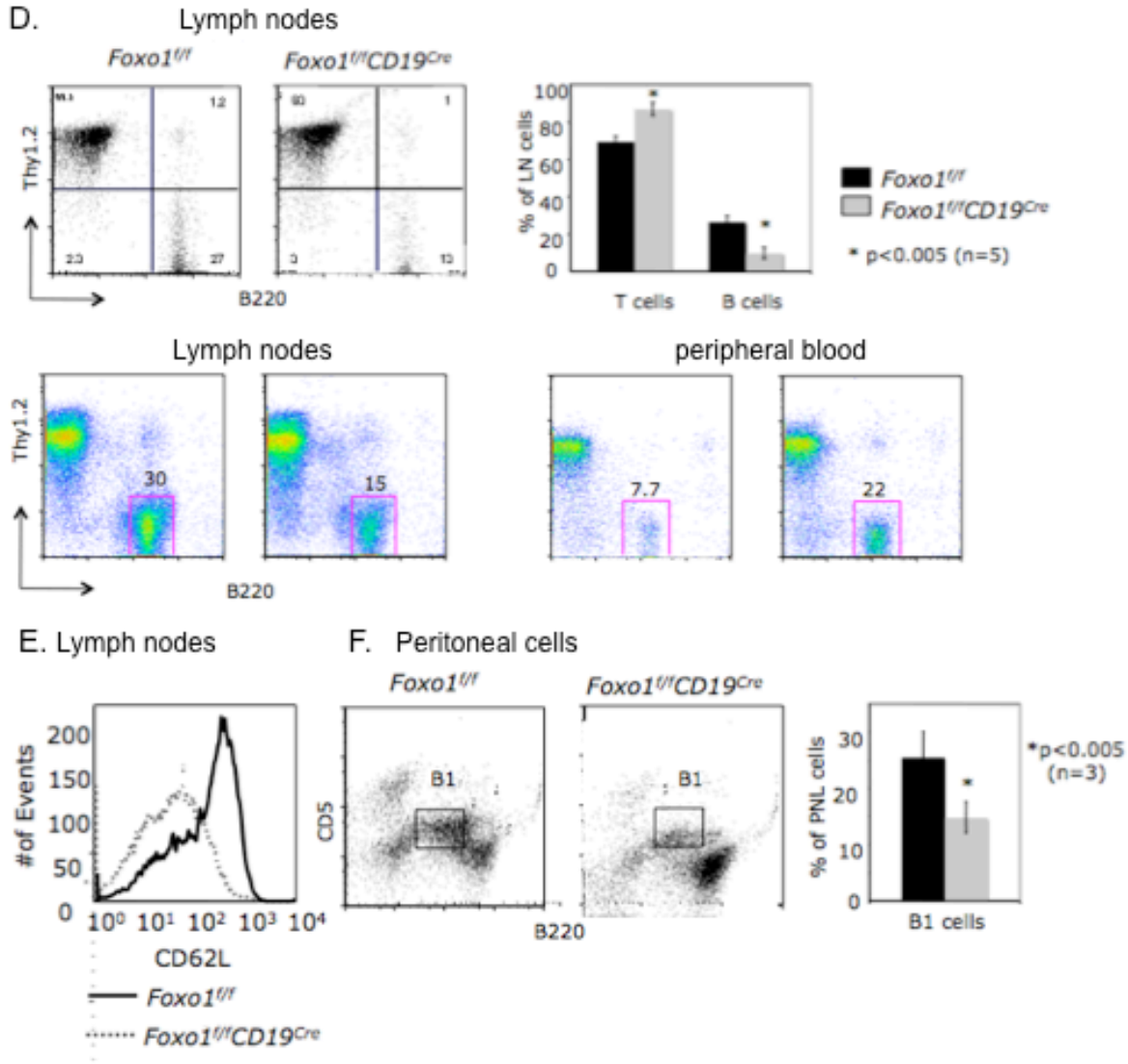
Transient transfection, luciferase assay and chromatin immunoprecipitation assay (ChIP) were carried out as we previously described [1, 2].

Supplementary Figures (below)

Suppl. Figure 1A-C

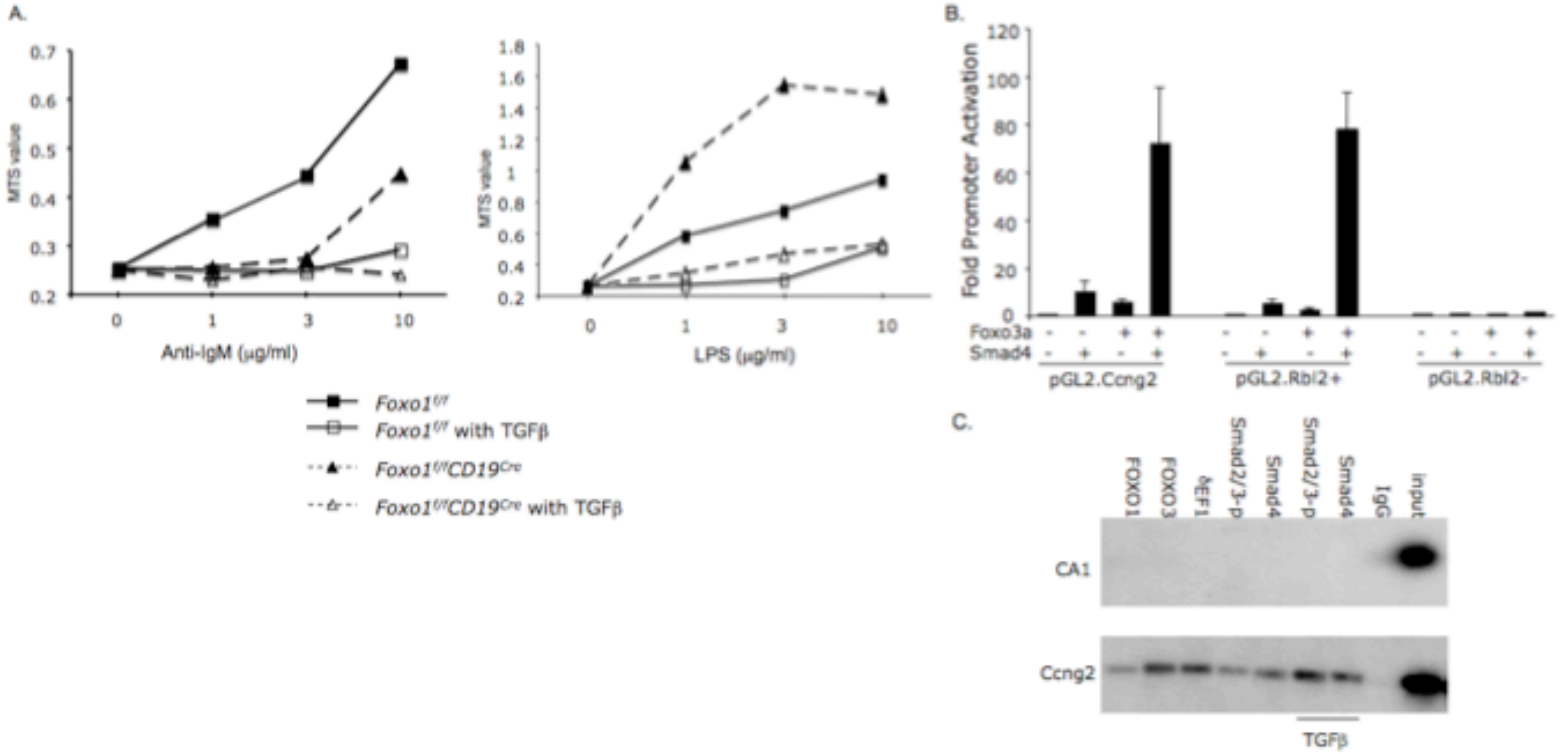


Suppl. Figure 1D-F



Supplementary Figure 1. Analysis of B cell developmental changes, guided by differences reported for another strain of mice [3]. (A) Western blot analysis of Foxo1 and Foxo3a expression in purified splenic B cells from *Foxo1*$^{f/f}$ *CD19*^{Cre} and control mice. (B-F) Flow cytometry shows (B) altered bone marrow B cell development with reduced immature/mature population; (C) prominent B220^{+}IgM^{-}IgD^{-} population in periphery; (D) reduced percentage of B cells in lymph node and increased percentage in peripheral blood; (E) reduced CD62L expression on gated B220^{+}Thy1.2^{-} B cells; (F) reduced peritoneal B1 cells, defined as B220^{int}CD5^{int}. In panels D and F, graphs depict mean +/- SD, and p values were determined using a two-tailed, unpaired t test.

Suppl. Figure 2



Supplementary Figure 2. Role of Foxo1 in TGFβ/Smad signaling in B cells.

(A) B cell proliferation in the presence or absence of TGFβ was measured. The MTS conversion assay was used to quantitate metabolically active cells 66hr after stimulation as indicated. (B) Effects of Smad4 and Foxo3a on the *Ccng2* and *Rbl2* promoters. A20 cells were cotransfected with the indicated reporter constructs and Foxo3a or/and Smad4 expression vectors, and analyzed for luciferase activity. The bars indicate the average luciferase activity normalized to protein concentration. Luciferase activity of the reporter gene in the absence of exogenous transactivator was designated as one. Rbl2- refers to the promoter in reverse orientation. The data shown are from the average of three independent experiments. Error bars indicate SD of the mean. Similar data were obtained using Foxo1 instead of Foxo3a. (C) Asssessment of endogenous Smads binding to the promoters of *Ccng2* and *Rbl2* in resting B cells, or in B cells treated with TGFβ. Chromatin immunoprecipitation (ChIP) assay was performed using primary B cells treated with or without 5ng/ml of TGFβ for 30 min before crosslinking of chromatin proteins to DNA by formaldehyde. Chromatin was sheared and immunoprecipitated by specific Abs (to Foxo1, Foxo3a, δEF1, Smad2/3-p and Smad4), or nonimmune IgG control. PCR in the exponential phase were detected by Southern blotting for *Ccng2* promoter amplicons, with the carbonic anhydrase (CA) promoter as a negative control.

Supplementary References

1 **Chen, J., Yusuf, I., Andersen, H. M. and Fruman, D. A.,** FOXO transcription factors cooperate with delta EF1 to activate growth suppressive genes in B lymphocytes. *J Immunol* 2006. **176**: 2711-2721.

2 **Yusuf, I., Kharas, M. G., Chen, J., Peralta, R. Q., Maruniak, A., Sareen, P., Yang, V. W., Kaestner, K. H. and Fruman, D. A.,** KLF4 is a FOXO target gene that suppresses B cell proliferation. *Int Immunol* 2008. **20**: 671-681.

3 **Dengler, H. S., Baracho, G. V., Omori, S. A., Bruckner, S., Arden, K. C., Castrillon, D. H., DePinho, R. A. and Rickert, R. C.,** Distinct functions for the transcription factor Foxo1 at various stages of B cell differentiation. *Nat Immunol* 2008. **9**: 1388-1398.