SUPPLEMENTAL MATERIAL

de Valle et al., http://www.jem.org/cgi/content/full/jem.20151182/DC1

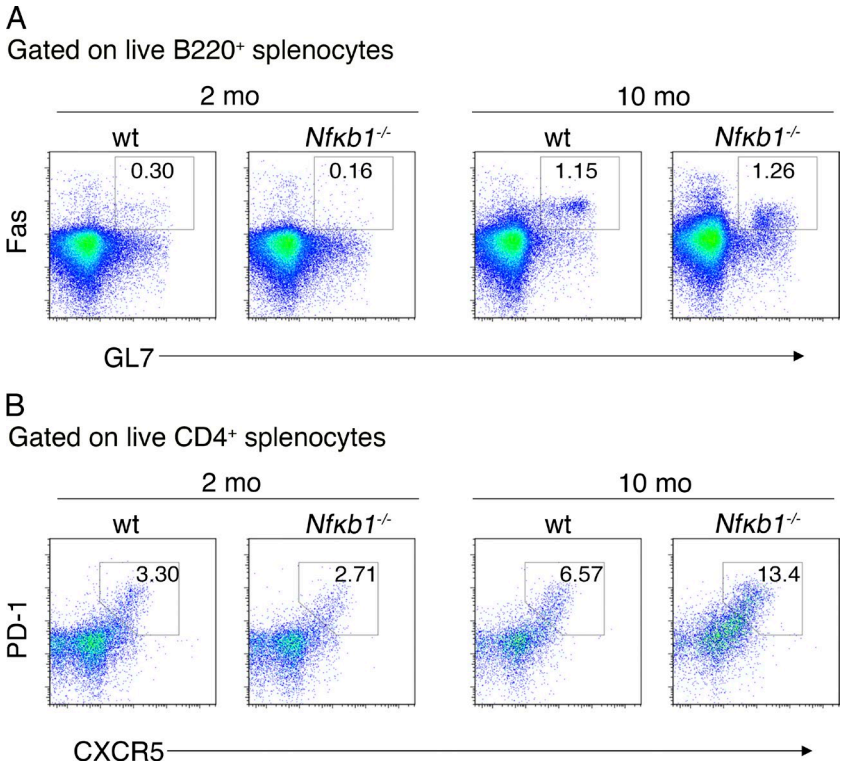

Figure S1. **Flow cytometry gating strategies for Fig. 1.** Flow cytometric analysis of GC B and T_{FH} cells in the spleens of WT and *Nfκb1*$^{-/-}$ mice at 2 and 10 mo. (A) Dot plots correspond to Fig. 1 E and show FAS versus GL7 expression on live B220^{+} splenocytes. The numbers indicate the proportion of FAS^{+}GL7^{+} GC B cells. (B) Dot plots correspond to Fig. 1 G and show PD-1 versus CXCR5 expression on live CD4^{+} splenocytes. The numbers indicate the proportion of PD-1^{+}CXCR5^{+} T_{FH} cells. Data are derived from two independent experiments (n = 3–5 mice/group).

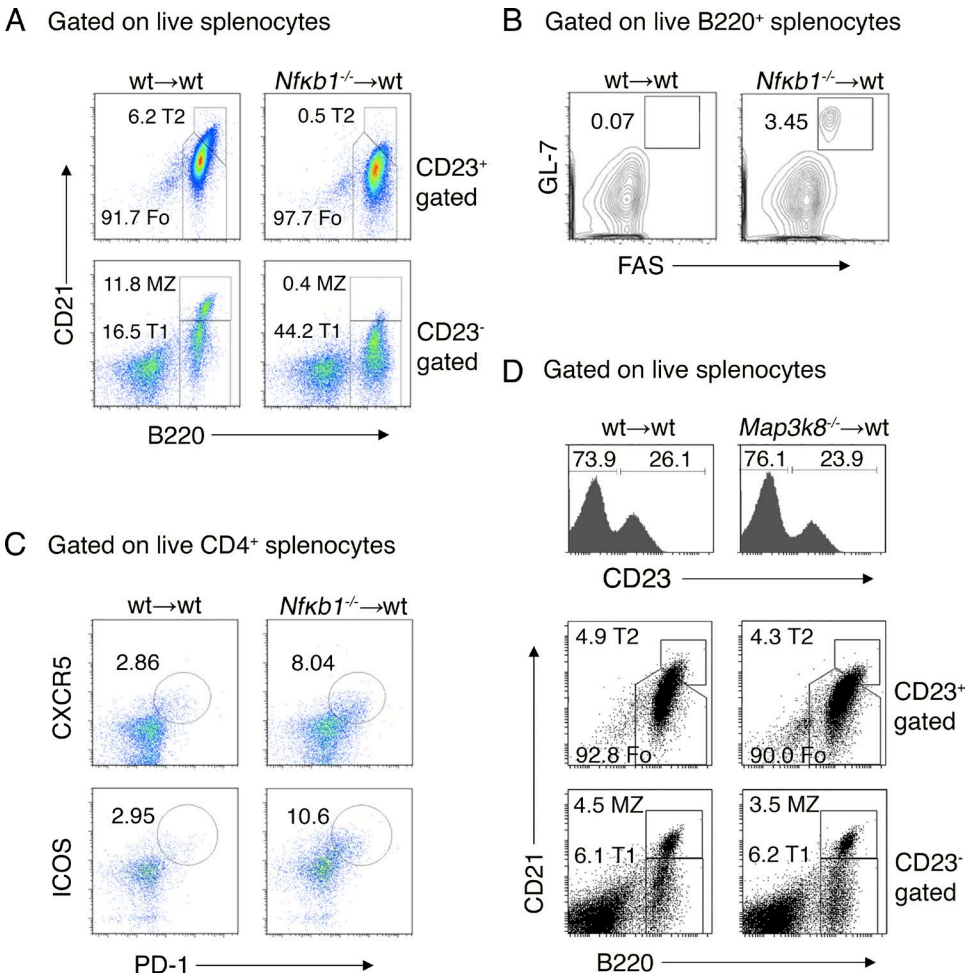

Figure S2. **Flow cytometry gating strategies for Fig. 3.** BM chimera mice were established as described in Fig. 2 and examined 7–10 mo after transplantation. (A) CD21 and B220 expression gated on splenic $CD23^+$ or $CD23^-$ populations as indicated. Gated regions show the proportion of T1 ($CD23^-B220^+CD21^{lo}$), T2 ($CD23^+B220^+CD21^{hi}$), MZ ($CD23^-B220^+CD21^{hi}$), and Fo ($CD23^+B220^+CD21^{lo}$) B cells enumerated in Fig. 3 B. (B) Gating strategy for Fig. 3 C showing FAS versus GL7 expression on live $B220^+$ splenocytes. The numbers indicate the proportion of GC B cells ($B220^+FAS^+GL7^+$). (C) Gating strategy for Fig. 3 D showing CXCR5 or ICOS versus PD-1 expression on $CD4^+$ splenocytes. Numbers indicate the proportion of T_{FH} cells ($CD4^+CXCR5^+ICOS^+PD\text{-}1^+$). Data in A–C were derived from at least three independent experiments (n = 6 mice/group). (D) Histograms show CD23 expression on total splenocytes, and dot plots show CD21 and B220 expression on gated $CD23^+$ or $CD23^-$ populations. The numbers represent the proportion of T1, T2, MZ, and Fo B cells shown in Fig. 3 I. Data were derived from two independent experiments (n = 4–5 mice/group).

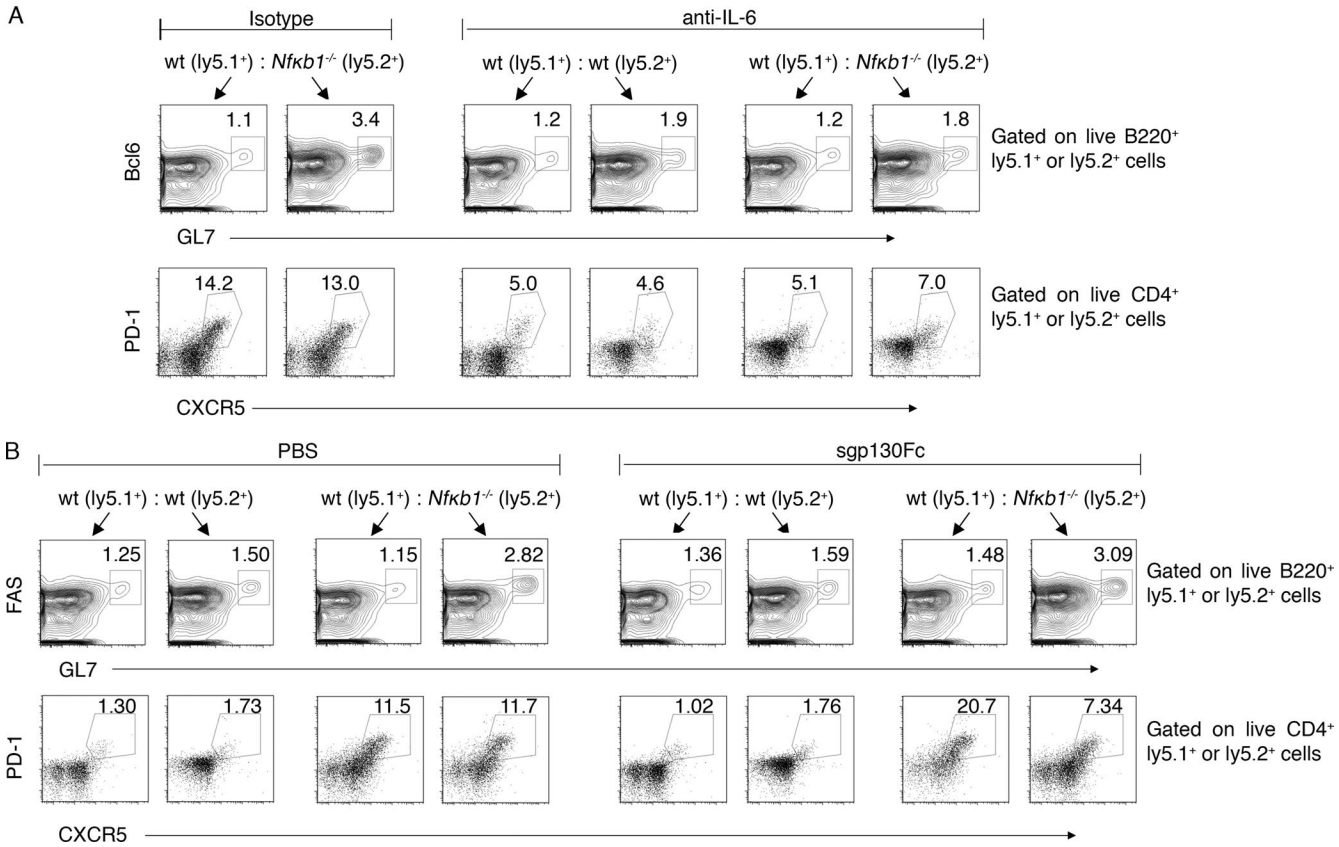

Figure S3. **Flow cytometry gating strategies for Fig. 7.** (A) As described in Fig. 7 (C and D), mBM-reconstituted mice of the indicated groups at 8 wk after transplantation were injected twice weekly for 6 wk with anti–IL-6 Ab or an Ig isotype–matched control Ab. Dot plots show gating strategies for the identification of GC B ($B220^+GL7^+Bcl6^+$) and T_{FH} ($CD4^+CXCR5^+PD\text{-}1^+$) cells from WT and *Nfκb1*$^{-/-}$ compartments defined on the basis of ly5.1 or ly5.2 expression (as indicated). (B) As described in Fig. 7 (E and F), mBM-reconstituted mice were injected twice weekly for 6 wk with sgp130Fc or PBS 8 wk after transplantation. Dot plots show gating of WT or *Nfκb1*$^{-/-}$ splenic GC B ($B220^+GL\text{-}7^+FAS^+$) and T_{FH} cells ($CD4^+CXCR5^+PD\text{-}1^+$). All data are representative of two individual experiments (n = 4–8 mice per group).

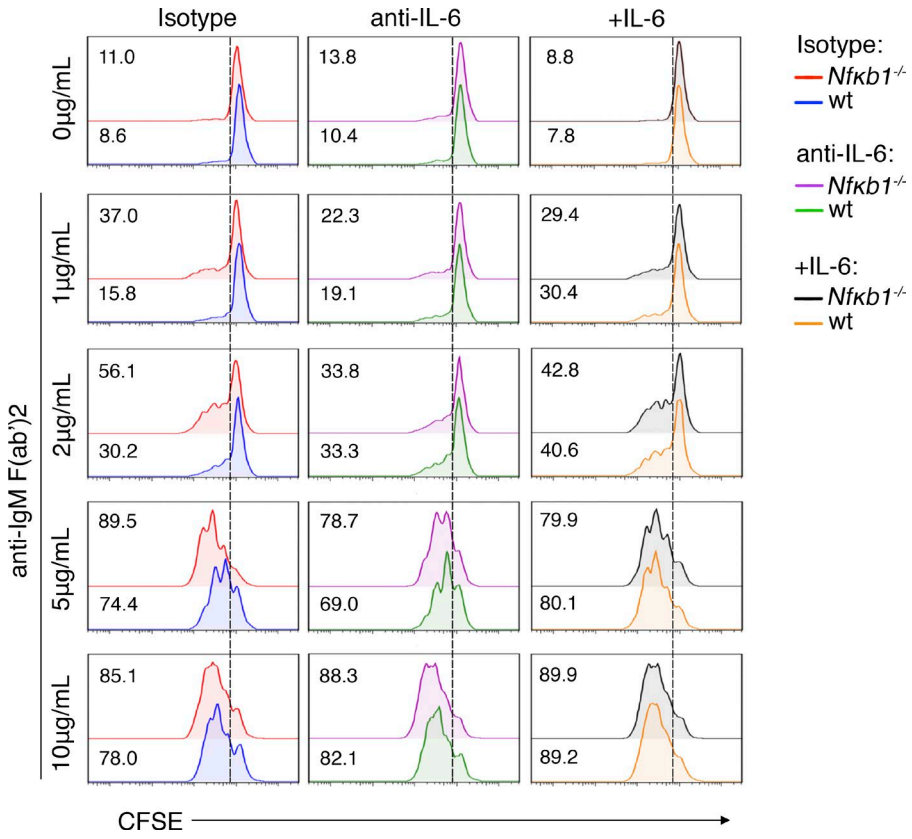

Figure S4. **Flow cytometry gating strategy for Fig. 9.** WT and *Nfκb1*$^{-/-}$ Fo B cells were labeled with CFSE and co-cultured as described in Fig. 9 (B and C). Histograms show CFSE expression profiles at 72 h, and numbers indicate the percentage of divided (CFSE^{-}) cells. Data are representative of two independent experiments (n = 5 mice/genotype).

Table S1. **Primers used for qPCR of ChIP DNA**

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
Il-6 region 1	TGGTAAATACAGAGCATTTGGGTG	TTGGGATAAAGTTGAGACAGGCT
Il-6 region 2	AGCCATTGCCCCCAGGAT	GCACATATGTAGCAGAGGACTGT
Il-6 region 3	CCTCTTCCCTGGGGTCTCA	TCAGAAGTCTCAACTAACCTGGAC
Il-6 region 4	GGGGTTTCCAACTTCAGTCCA	AGTTGGTCCAATGACTAGCCC
Tnfα (Yan et al., 2012)	AACCCTCTGCCCCCGCGATG	TCCTCGCTGAGGGAGCTTCTGC

REFERENCE

Yan, Q., R.J. Carmody, Z. Qu, Q. Ruan, J. Jager, S.E. Mullican, M.A. Lazar, and Y.H. Chen. 2012. Nuclear factor-κB binding motifs specify Toll-like receptor-induced gene repression through an inducible repressosome. *Proc. Natl. Acad. Sci. USA*. 109:14140–14145. http://dx.doi.org/10.1073/pnas.1119842109