

Supporting Information

Kraus et al. 10.1073/pnas.0903786106

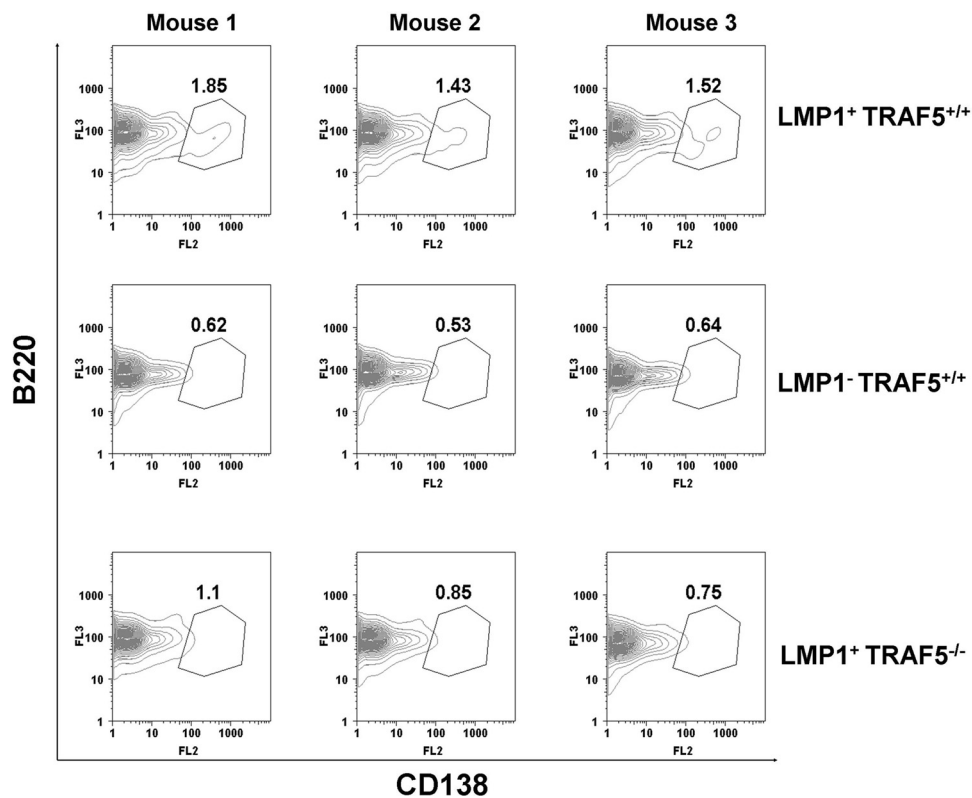


Fig. S1. CD138 expression on splenocytes from LMP1⁺TRAF5^{+/+} and LMP1⁺TRAF5^{-/-} mice. Spleens were collected from LMP1⁻TRAF5^{+/+}, LMP1⁺TRAF5^{+/+}, and LMP1⁺TRAF5^{-/-} mice of 3–4 months of age. The spleens were homogenized and stained with anti-B220 PerCp mAb, anti-CD19 FITC mAb, and anti-CD138 PE.

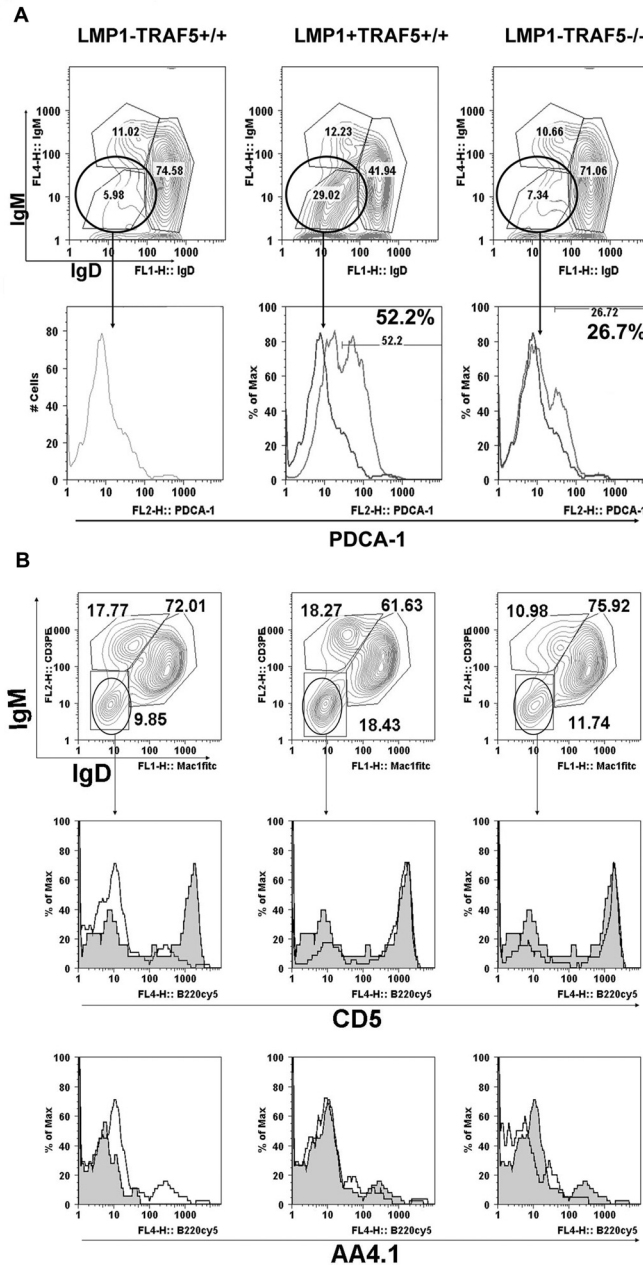


Fig. S2. B-cell and DC subsets in spleens from LMP1⁺TRAF5^{+/+} and LMP1⁺TRAF5^{-/-} mice. Spleens were collected from LMP1⁻TRAF5^{+/+}, LMP1⁺TRAF5^{+/+}, and LMP1⁺TRAF5^{-/-} mice of 3–4 months of age. The spleens were homogenized and stained with anti-B220 PerCp mAb, anti-IgD FITC mAb, anti-IgM APC mAb, and anti-PDCA-1 PE (A), anti-B220 PerCp mAb, anti-CD23 PE mAb and anti-IgM FITC mAb and anti-CD5 APC mAb or anti-B220 PerCp mAb, anti-CD23 PE mAb and anti-IgM FITC mAb and AA4.1 APC mAb (B).

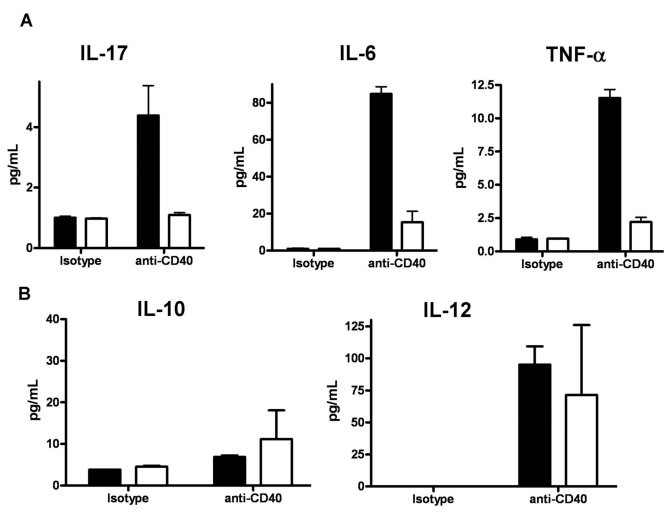


Fig. S3. The effect of TRAF5 deficiency on LMP1-mediated cytokine secretion by primary B lymphocytes. Supernatants of purified B cells from LMP1⁺TRAF5^{+/+} mice (black bars) or LMP1⁺TRAF5^{-/-} mice (white bars) stimulated with 5 μ g/mL anti-mCD40 (HM40.3), 5 μ g/mL isotype control, or 100 ng/mL CpG-B (2084) for 48 h were used in a multicytokine Luminex assay. IL-17, IL-6, and TNF- α (A). IL-10 and IL12 (B).

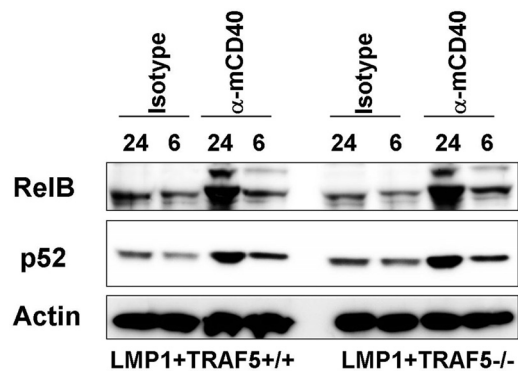


Fig. S4. NF- κ B2 activation in B lymphocytes from LMP1⁺TRAF5^{+/+} and LMP1⁺TRAF5^{-/-} mice. A total of 1×10^6 purified (>95% purity) splenic B cells from LMP1⁺TRAF5^{+/+} and LMP1⁺TRAF5^{-/-} mice of 8–12 weeks of age was stimulated with 5 μ g/mL anti-mCD40 (HM40.3), 5 μ g/mL isotype control for 6 or 24 h. RelB and p52 levels were measured by Western blot using RelB- and p52-specific Abs.

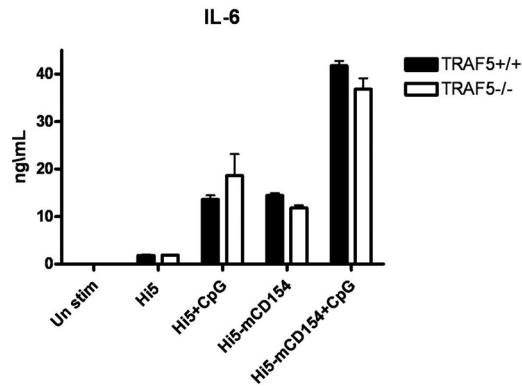
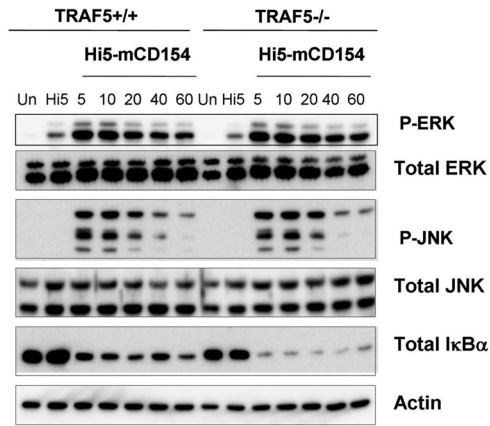


Fig. S5. Proximal CD40 signaling in B lymphocytes from TRAF5^{-/-} mice. A total of 2×10^6 purified B cells from TRAF5^{+/+} or TRAF5^{-/-} mice was stimulated at 37°C with 4×10^5 (5:1) Hi5 cells expressing membrane-bound CD154 or control Hi5 cells infected with wild-type baculovirus for 5, 10, 20, 40, and 60 min. Cells stimulated with Hi5 control were stimulated for 20 min. Activation of JNK, Erk, and IκBα degradation was detected by Western blot.