Supplemental Figures

Figure S1

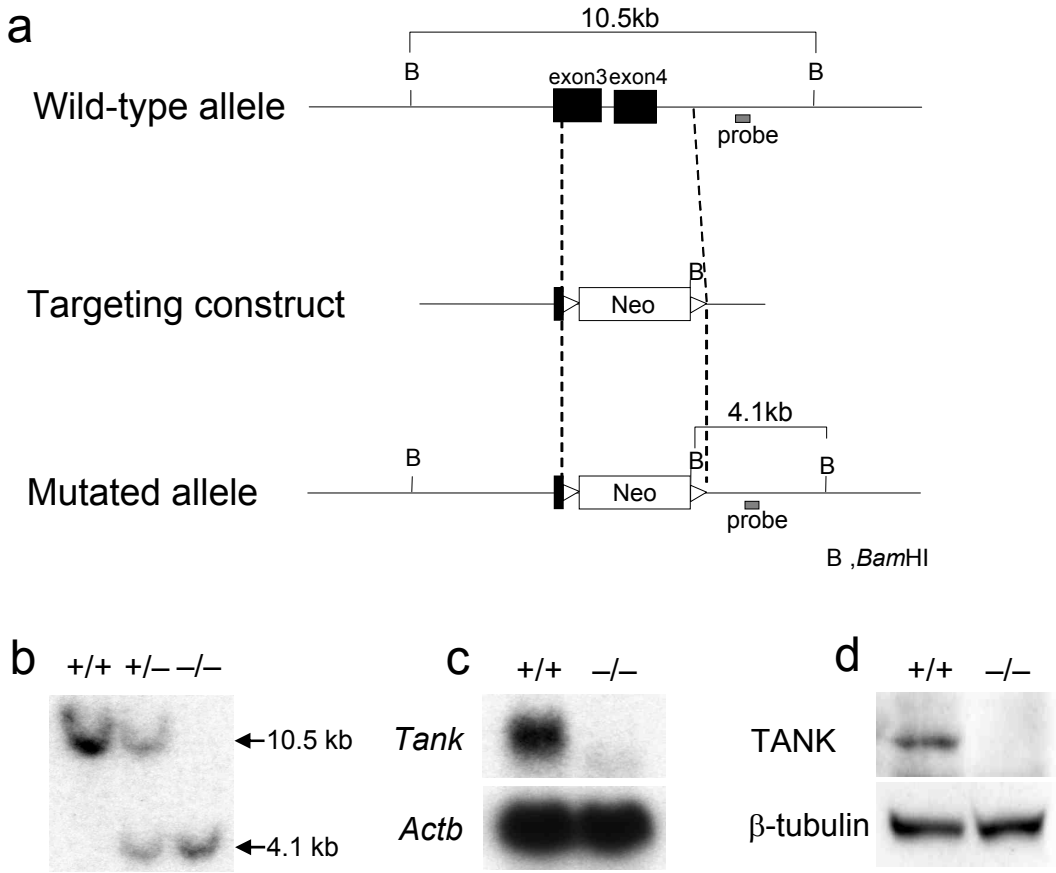

Figure S1 Targeted disruption of *Tank*

(a) Structure of the mouse *Tank* gene, the targeting vector, and the predicted disrupted gene. Closed boxes denote the coding exon. B, *Bam*H1

(b) Southern blot analysis of offspring from the heterozygote intercrosses. Genomic DNA was extracted from mouse tails, digested with *Bam*HI, separated by electrophoresis, and hybridized with the radiolabeled probe indicated in (a).

(c) Nothern blot analysis of the expression of TANK mRNA. Total RNA from wild-type and *Tank*$^{-/-}$ peritoneal macrophages were extracted and subjected to the Northern blot analysis for the expression of TANK mRNA. The same membrane was rehybridized with an *Actb* probe.

(d) Immunoblot analysis for the expression of TANK protein in wild-type and *Tank*$^{-/-}$ macrophages. Cell lysates from peritoneal macrophages were immunoblotted with antibody to TANK. Probing for β-tublin was used to ensure equal loading.

Figure S2

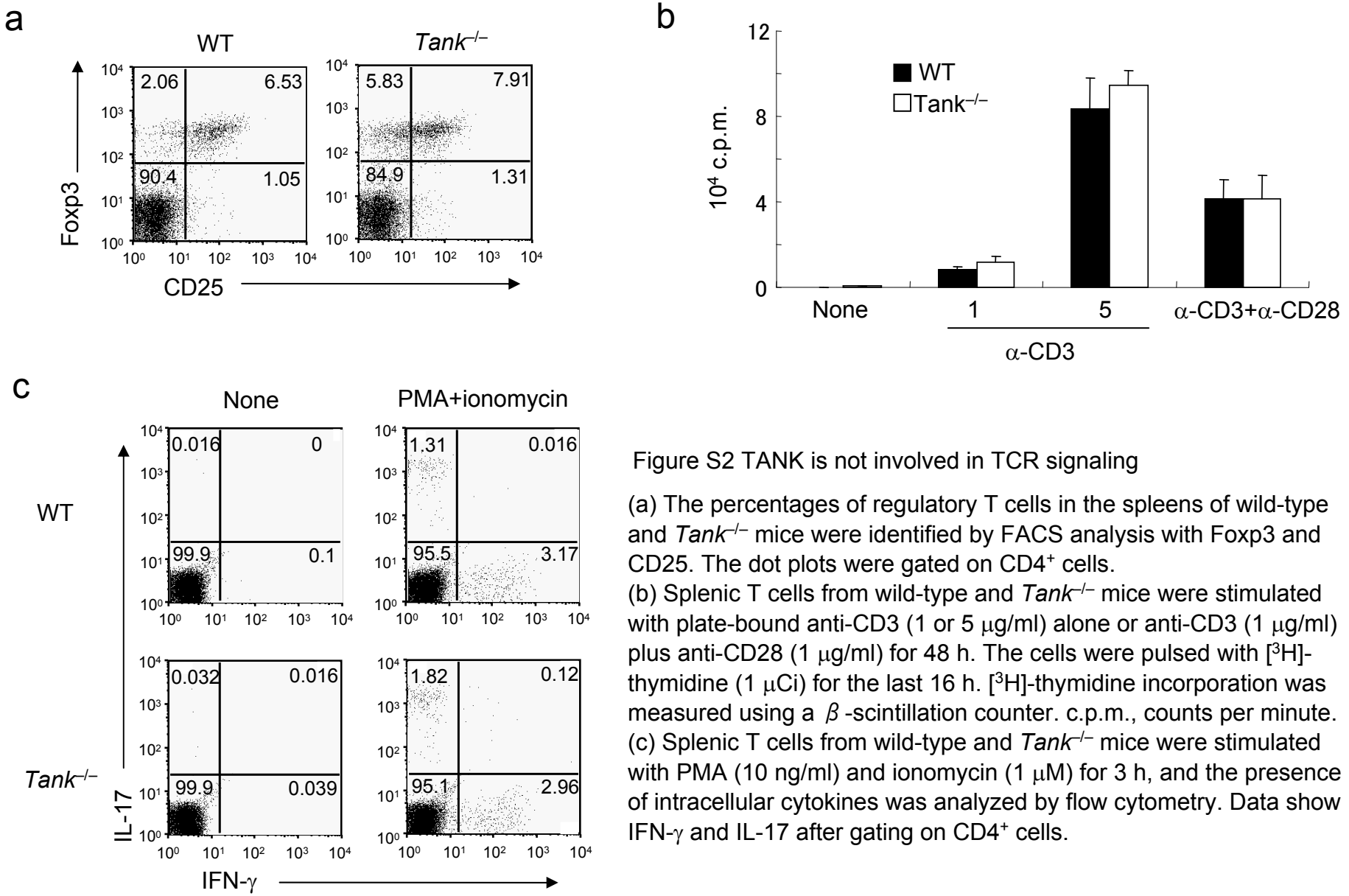

Figure S2 TANK is not involved in TCR signaling

(a) The percentages of regulatory T cells in the spleens of wild-type and *Tank*$^{-/-}$ mice were identified by FACS analysis with Foxp3 and CD25. The dot plots were gated on CD4$^+$ cells.
(b) Splenic T cells from wild-type and *Tank*$^{-/-}$ mice were stimulated with plate-bound anti-CD3 (1 or 5 μg/ml) alone or anti-CD3 (1 μg/ml) plus anti-CD28 (1 μg/ml) for 48 h. The cells were pulsed with [^{3}H]-thymidine (1 μCi) for the last 16 h. [^{3}H]-thymidine incorporation was measured using a β-scintillation counter. c.p.m., counts per minute.
(c) Splenic T cells from wild-type and *Tank*$^{-/-}$ mice were stimulated with PMA (10 ng/ml) and ionomycin (1 μM) for 3 h, and the presence of intracellular cytokines was analyzed by flow cytometry. Data show IFN-γ and IL-17 after gating on CD4$^+$ cells.

Figure S3

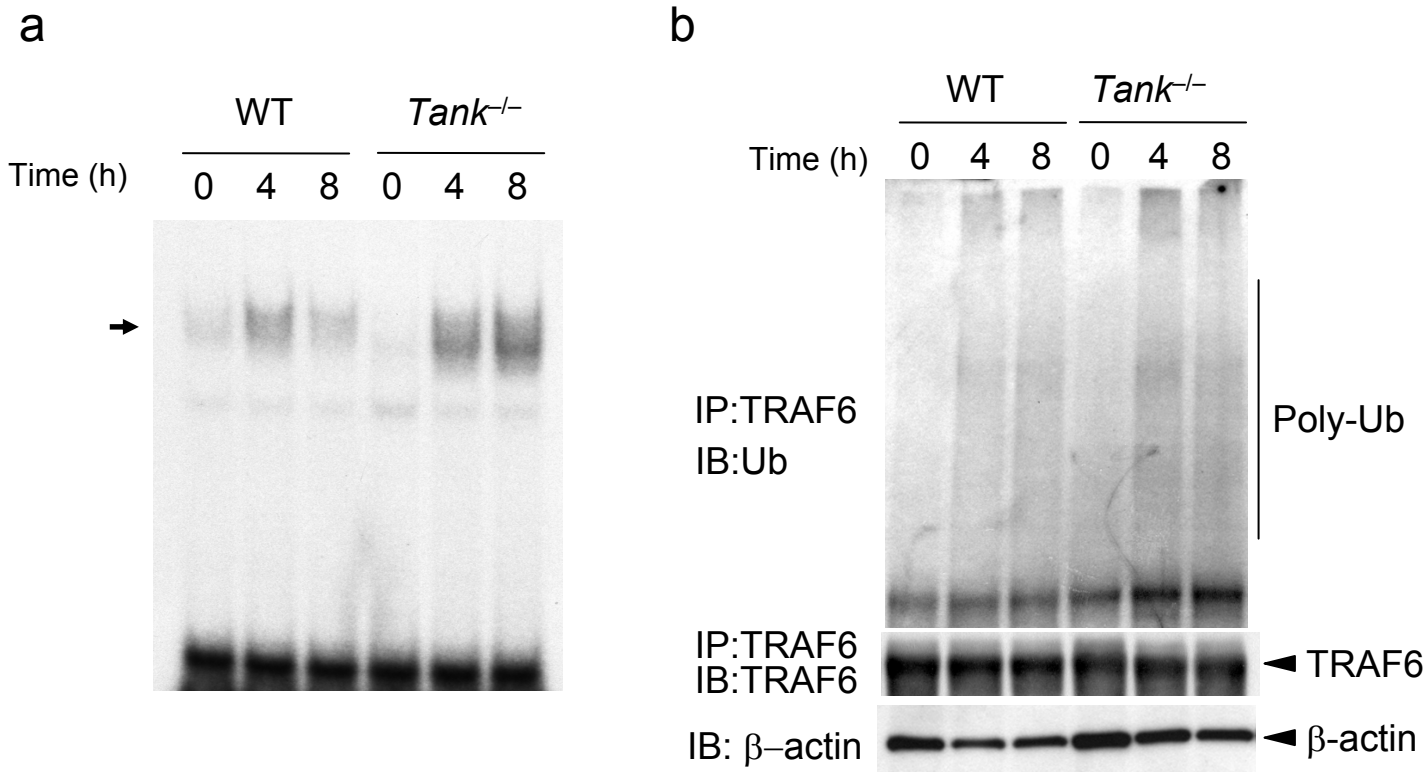

Figure S3 Enhanced NF-κB activation and TRAF6 ubiquitination after BCR stimulation in *Tank*$^{-/-}$ B cells

(a) Splenic B cells from wild-type and *Tank*$^{-/-}$ mice were stimulated with 10 μg/ml anti-IgM for the indicated periods. Nuclear extracts were prepared and the NF-κB DNA-binding activity was determined by EMSA. The arrow indicates the induced NF-κB complex.
(b) Cell lysates of splenic B cells treated with 10 μg/ml anti-IgM for the indicated periods were immunoprecipitated with anti-TRAF6, followed by immunoblot analysis with anti-Ub. Immunoblots of TRAF6 and β-actin are shown as a loading control.

Figure S4

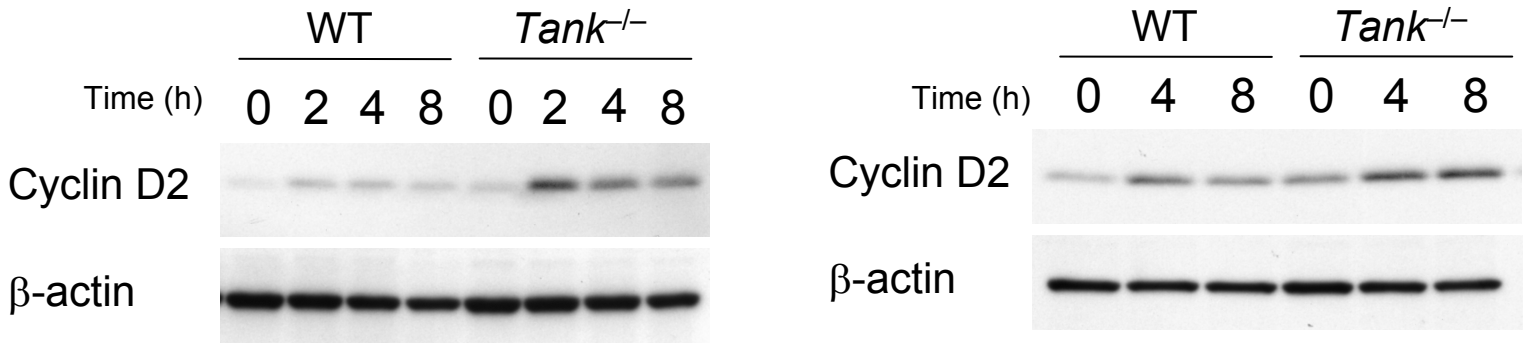

Figure S4 Enhanced expression of cyclin D2 after CD40 and BCR stimulation in *Tank*$^{-/-}$ B cells

Splenic B cells from wild-type and *Tank*$^{-/-}$ mice were stimulated with 5 μg/ml anti-CD40 (left) or 10 μg/ml anti-IgM (right) for the indicated periods. Cell lysates were prepared and the expression of cyclin D2 was determined by immunoblot analysis. Immunoblots for β-actin are shown as a loading control.