Supplemental material JCB

Bohgaki et al., http://www.jcb.org/cgi/content/full/jcb.201103053/DC1

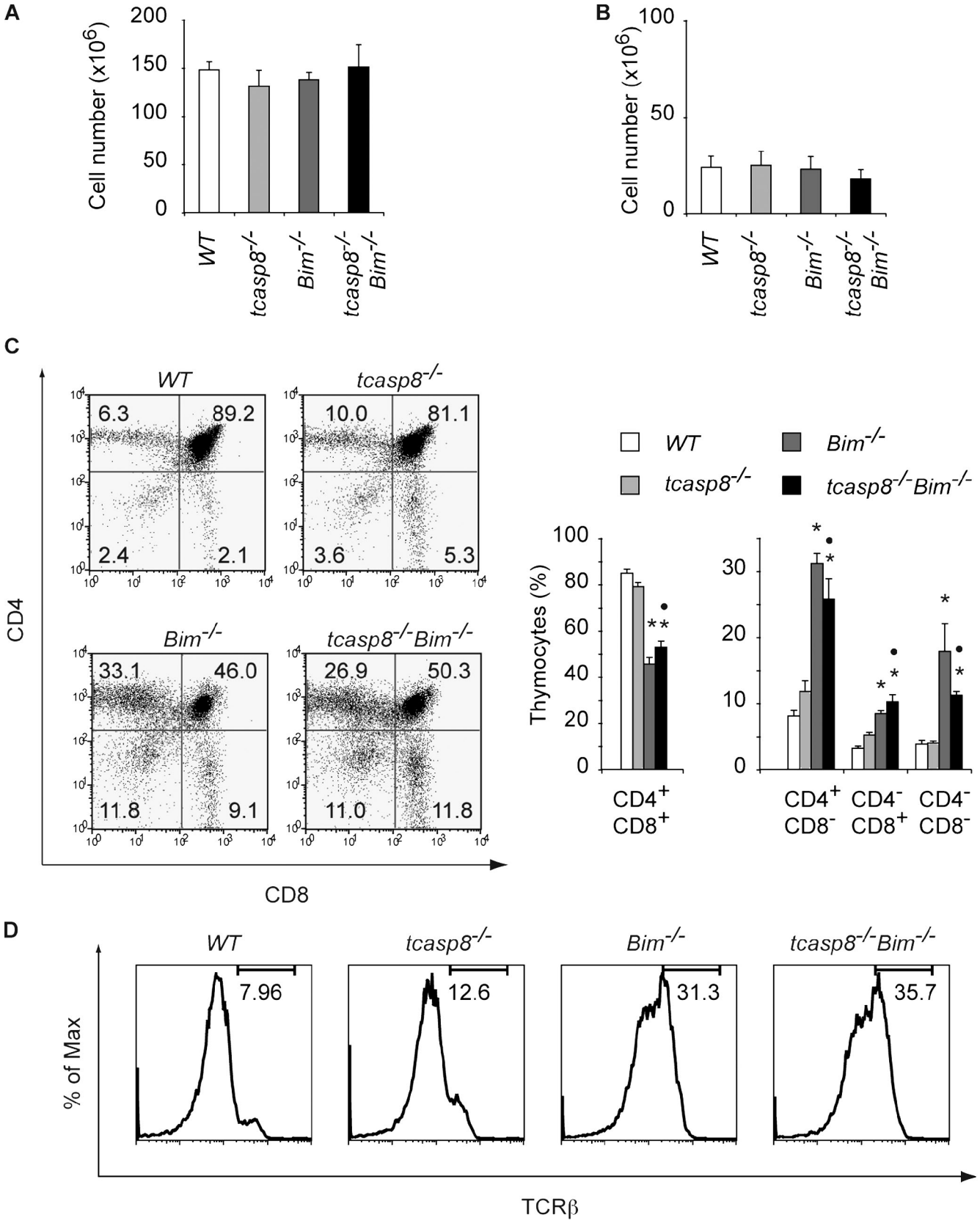

Figure S1. **Thymic T cell populations in *tcasp8*$^{-/-}$*Bim*$^{-/-}$ and control mice.** (A and B) Total thymocyte numbers of young (A) and old (B) mice of the indicated genotypes are shown. Data represent the mean ± SEM for five mice of each genotype. (C) Representative FACS analyses of thymocytes from old mice of the indicated genotypes are shown (left). Histograms show the mean percentages of each thymocyte subpopulation from five mice for each genotype (right). Data represent the mean ± SEM (error bars). (D) Representative analyses of the expression of TCR-β on thymocytes from old mice of the indicated genotypes are shown. *, $P < 0.05$ compared with WT mice; ●, $P < 0.05$ compared with *tcasp8*$^{-/-}$ mice.

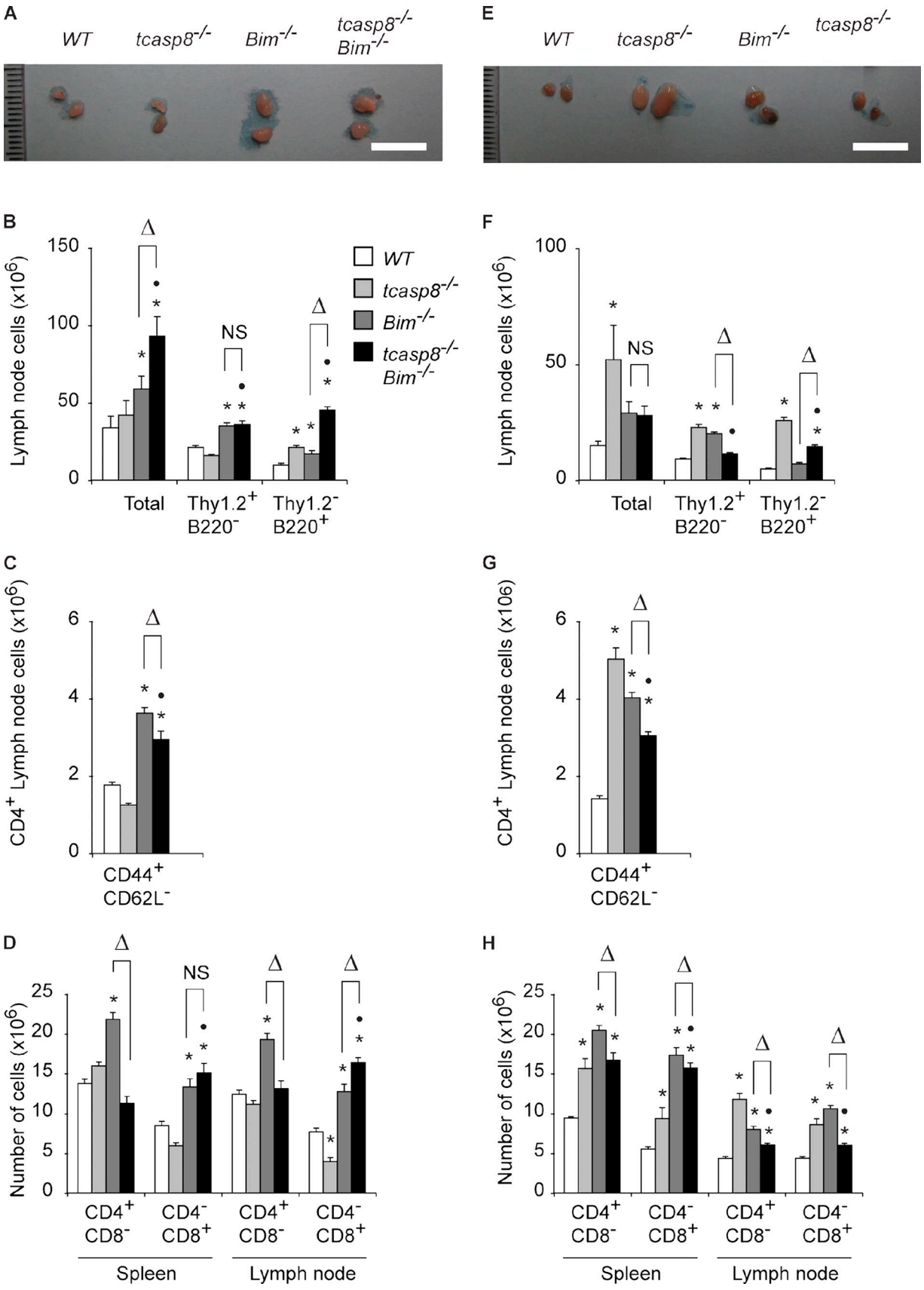

Figure S2. **Effect of caspase-8 loss in T cells on lymphadenopathy caused by loss of Bim.** Representative photographs of LNs from young (A) and old (E) *tcasp8*$^{-/-}$*Bim*$^{-/-}$, *tcasp8*$^{-/-}$, *Bim*$^{-/-}$, and WT mice. Numbers of total, Thy1.2^{+}B220^{-} T cells, and Thy1.2^{-}B220^{+} B cells in LNs of young (B) and old (F) mice of the indicated genotypes are indicated. The numbers of effector memory T cells (CD4^{+}CD44^{+}CD62L^{-}) in LNs from young (C) and old (G) mice of the indicated genotypes are shown. Numbers of CD4^{+} and CD8^{+} T cells in spleens and LNs of young (D) and old (H) mice of the indicated genotypes are shown. Data represent the mean ± SEM (error bars) of 4–7 mice for each genotype and age; statistical analysis was performed by using the ANOVA test. *, $P < 0.05$ compared with WT mice; ●, $P < 0.05$ compared with *tcasp8*$^{-/-}$ mice; Δ, $P < 0.05$ compared with *Bim*$^{-/-}$ mice.

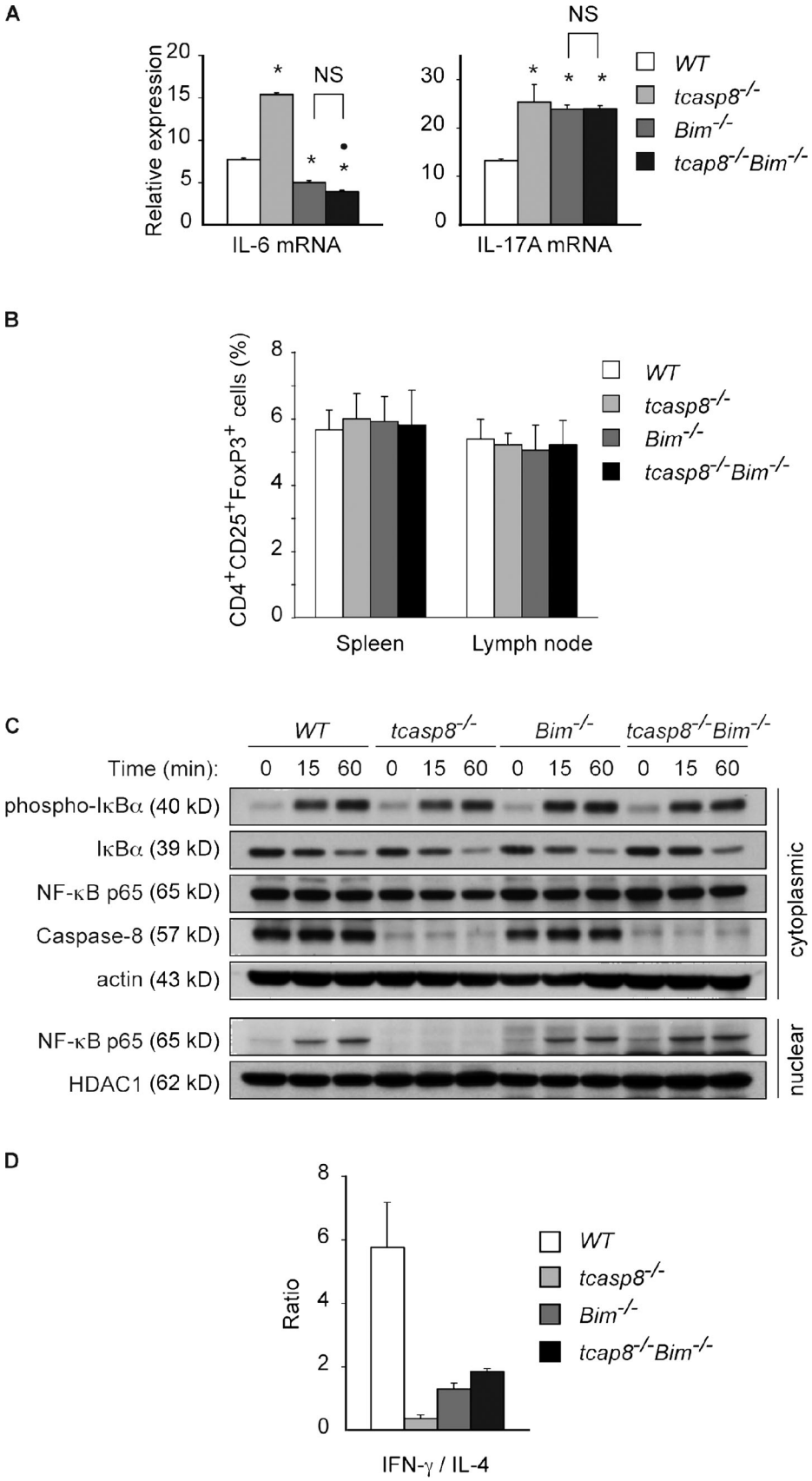

Figure S3. **Cytokine production levels and the ratio of Treg in periphery in *tcasp8*$^{-/-}$*Bim*$^{-/-}$ mice.** (A) Gene expression levels of *IL-6*, *IL-17*, and *TNF* in purified T cells from *tcasp8*$^{-/-}$*Bim*$^{-/-}$, *tcasp8*$^{-/-}$, *Bim*$^{-/-}$, and WT mice. (B) Effect of caspase-8 inactivation in *Bim*$^{-/-}$ T cells on the ratio of Treg. The percentage of CD4^{+} gated cells is indicated. Histograms represent the mean ± SEM (error bars; n = 3–5). (C) Representative immunoblots of the levels of phosphorylated and total IκBα, and total NF-κB p65 after anti-CD3/anti-CD28 antibody T cell stimulation. (D) The ratio of intracellular expression of IFN-γ/IL-4 was determined by FACS analysis. Naive CD4^{+} T cells were cultured under Th1- or Th2-specific conditions and restimulated with anti-CD3/anti-CD28 for 6 h. *, $P < 0.05$ compared with WT mice; ●, $P < 0.05$ compared with *tcasp8*$^{-/-}$ mice. Data are derived from three independent experiments of young mice. Histograms represent the mean ± SEM (error bars).

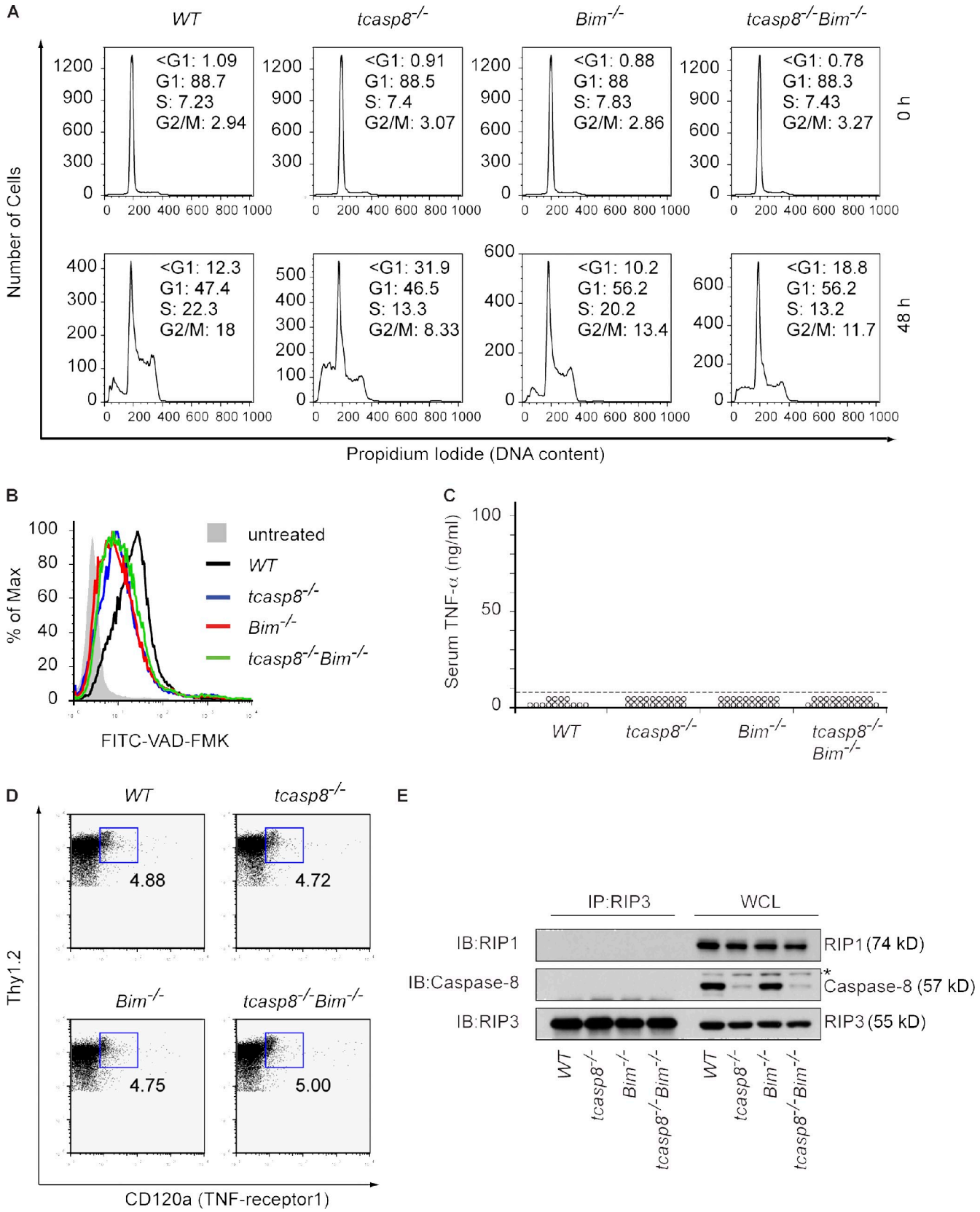

Figure S4. **Cell cycle analysis, caspase activities, and necroptosis complex in peripheral T cells treated with anti-CD3 and anti-CD28 antibodies.** (A) Representative cell cycle progression of *tcasp8*$^{-/-}$*Bim*$^{-/-}$, *tcasp8*$^{-/-}$, *Bim*$^{-/-}$, and WT peripheral T cells stimulated with anti-CD3/anti-CD28 antibodies. (B) Representative levels of in vivo caspase activities in peripheral T cells from *tcasp8*$^{-/-}$*Bim*$^{-/-}$, *tcasp8*$^{-/-}$, *Bim*$^{-/-}$, and WT mice. T cells were stimulated with anti-CD3/anti-CD28 antibodies for 24 h, and their caspase activity levels were examined by FITC-VAD-FMK staining. (C) Expression levels of serum TNF in *tcasp8*$^{-/-}$*Bim*$^{-/-}$, *tcasp8*$^{-/-}$, *Bim*$^{-/-}$, and WT mice (n = 14–22). Mice were >6 mo of age. The dotted line represents the limit of sensitivity of the ELISA kit. (D) Representative expression levels of surface CD120a (TNF-receptor 1) in peripheral T cells from WT and mutant mice. (E) Representative immunoblot analysis of RIPK1 and RIPK3 interaction after anti-CD3/anti-CD28 antibody stimulation of T cells. RIPK3 immunoprecipitates from 24-h-stimulated T cells from *tcasp8*$^{-/-}$*Bim*$^{-/-}$, *tcasp8*$^{-/-}$, *Bim*$^{-/-}$, and WT mice were immunoblotted with anti RIPK1, anti–caspase-8, and anti- RIPK3. The asterisk indicates a nonspecific band.

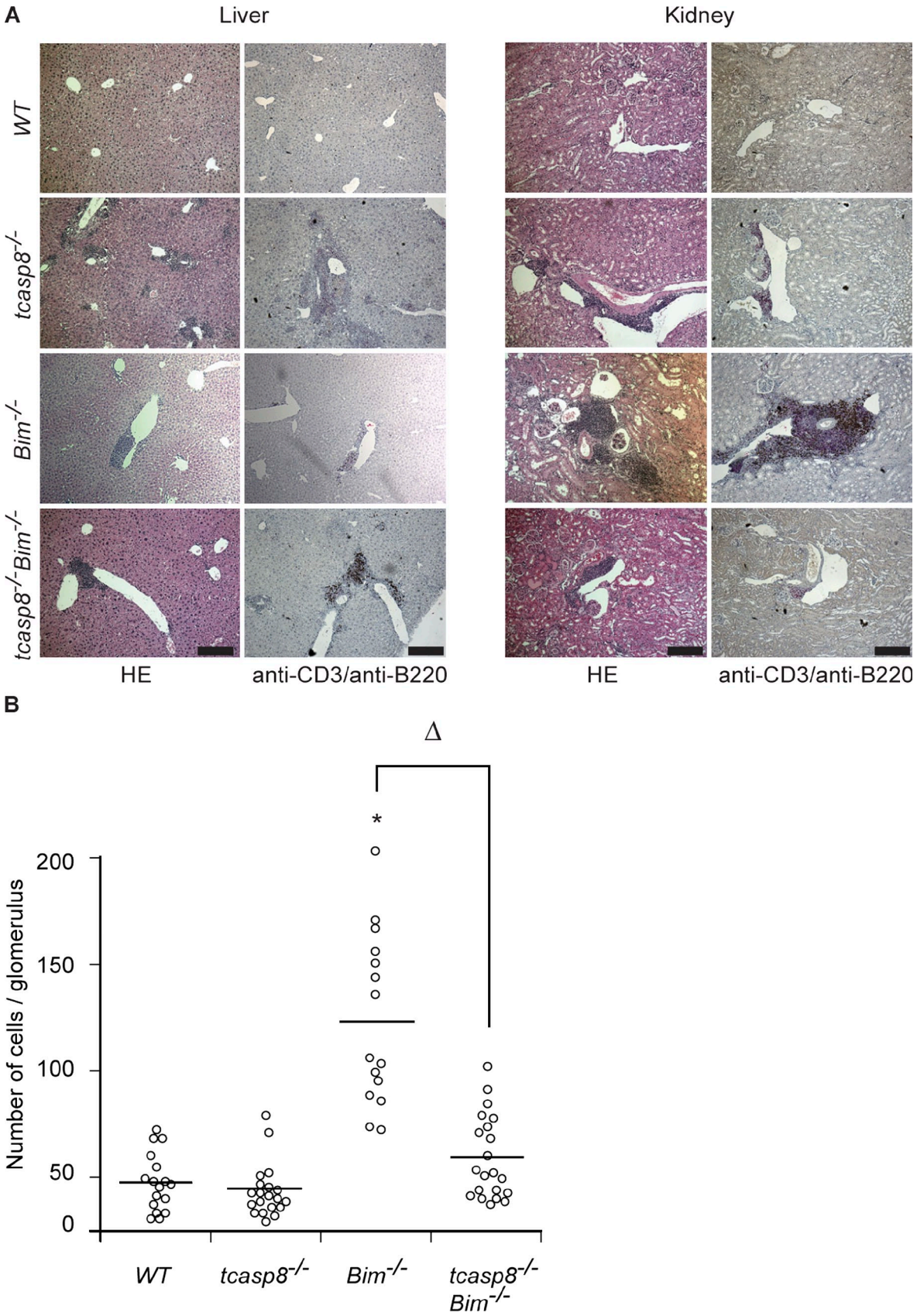

Figure S5. **Loss of caspase-8 in T cells suppresses lymphocyte infiltration and autoimmune kidney disease of Bim-deficient mice.** (A) Liver and kidney sections from 13-mo-old *tcasp8*$^{-/-}$*Bim*$^{-/-}$, *tcasp8*$^{-/-}$, *Bim*$^{-/-}$, and WT mice were stained with HE or anti-CD3 antibodies and anti-B220 antibodies. Bars, 100 µm. (B) Numbers of glomerular cells are shown. Numbers of cells in glomeruli were determined from three mice for each genotype. *, $P < 0.05$ compared with WT mice; Δ, $P < 0.05$ compared with *Bim*$^{-/-}$ mice.