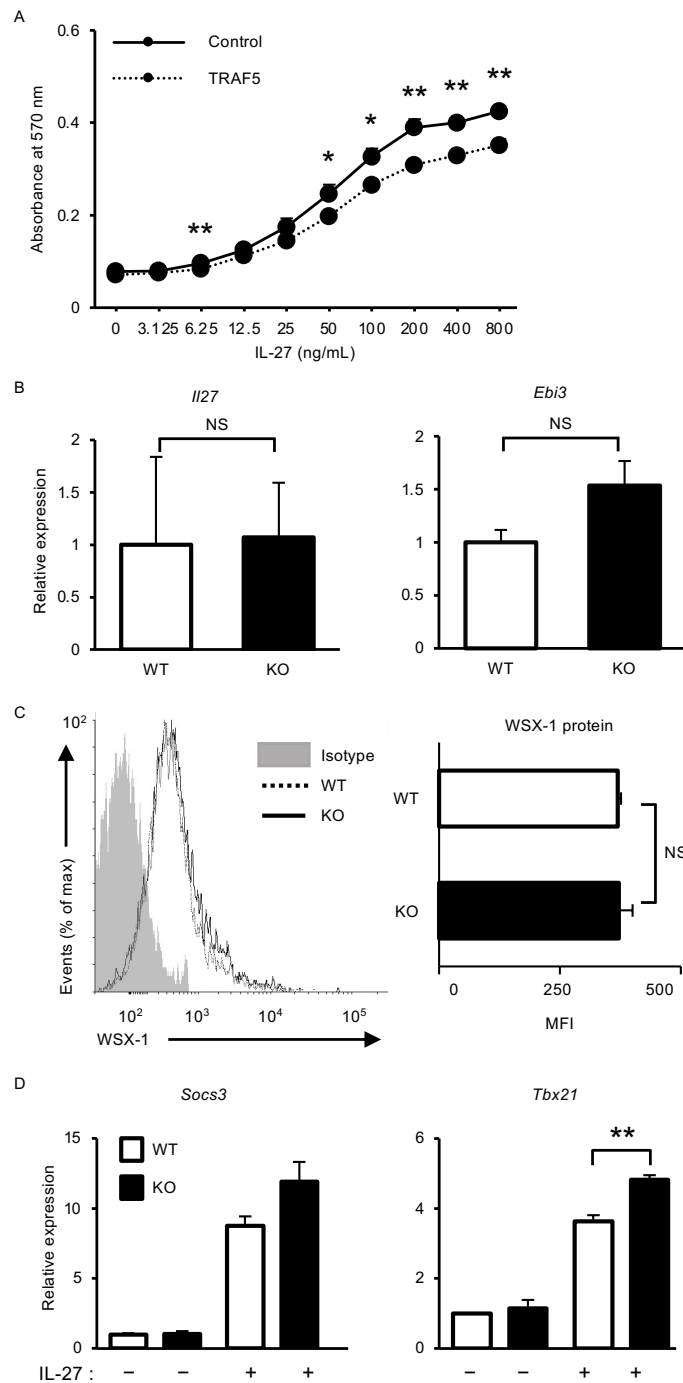
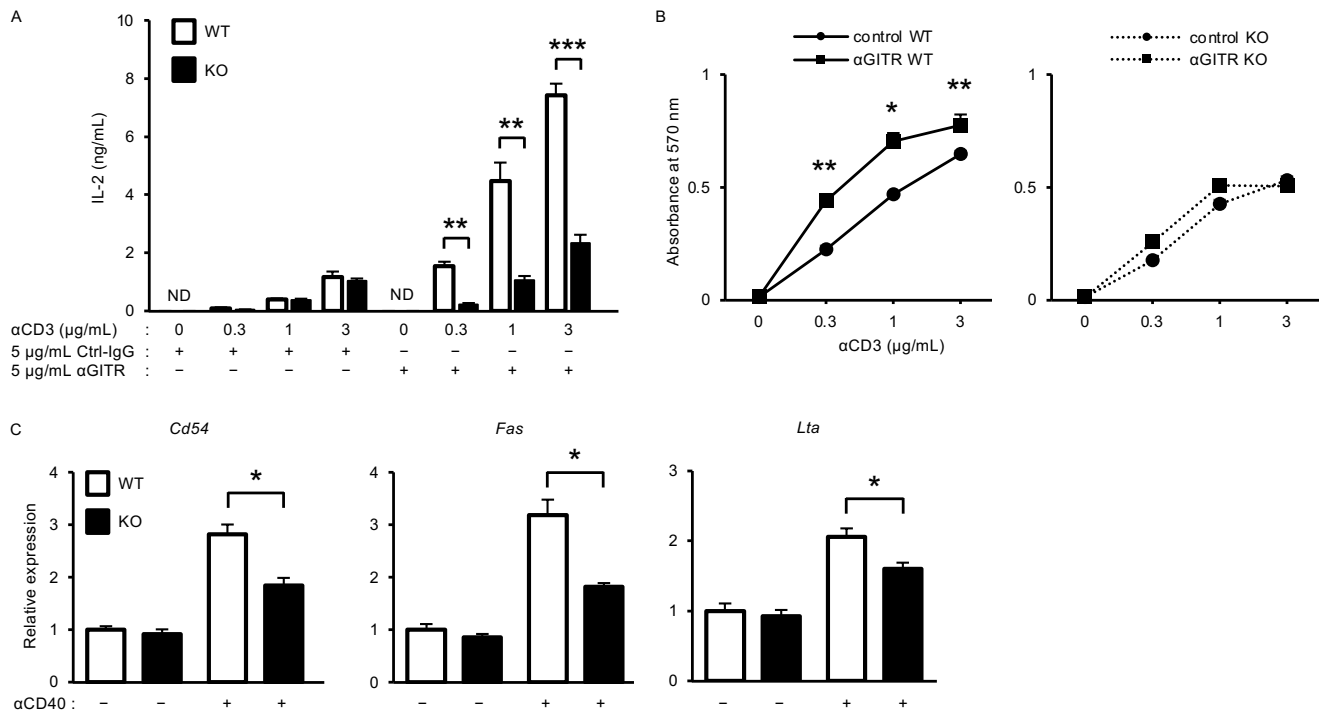


SUPPLEMENTAL FIGURE 1.



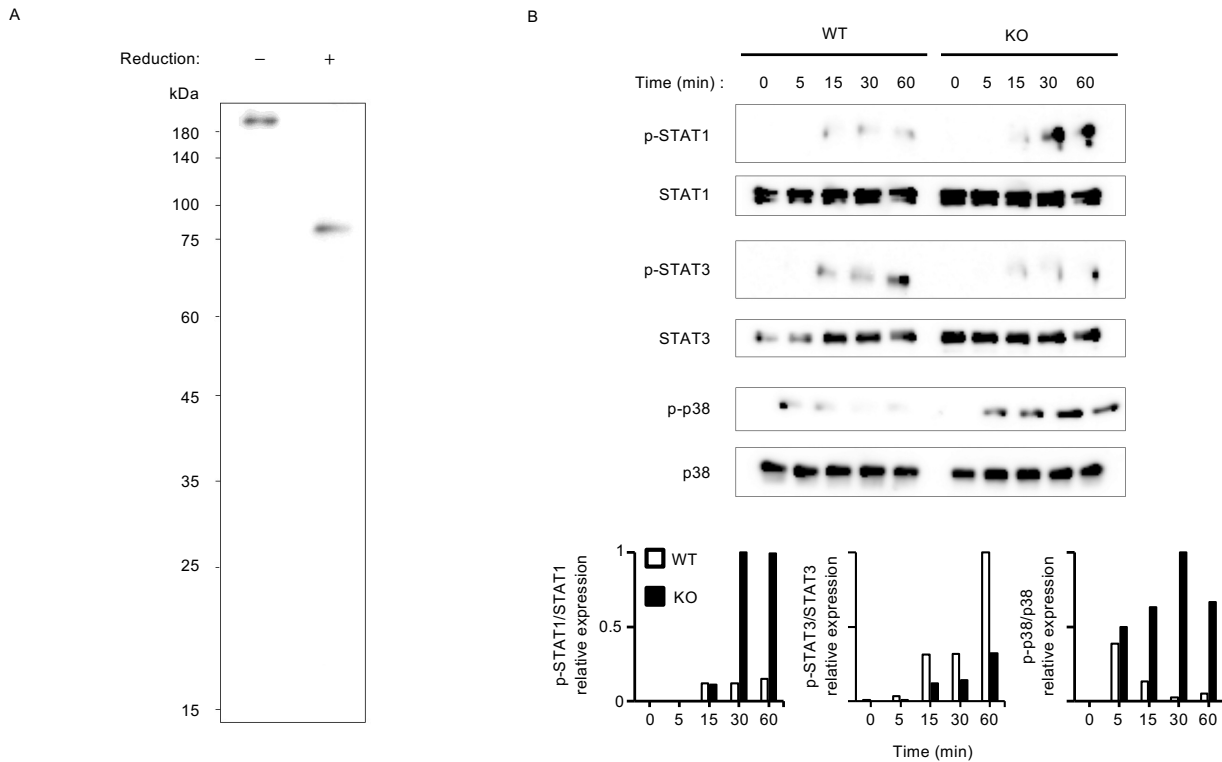
SUPPLYEMENTAL FIGURE 1. (A) Cell proliferative responses of BAF-gp130 cell line stably transduced with retroviral vector expressing GFP alone (control vector) or GFP and TARF5, cultured for 3 d with graded doses of IL-27, followed by addition of MTT. (B) Quantitative RT-PCR analysis of *Il27* and *Ebi3* mRNA in splenocytes from *Traf5*^{+/+} (WT) and *Traf5*^{-/-} (KO) naïve mice. (C) Representative flow cytometry histograms showing expression of WSX-1 protein on purified splenic B cells from *Traf5*^{+/+} and *Traf5*^{-/-} mice (left). Numbers indicate mean fluorescence intensity (MFI) of WSX-1 staining (right). (D) Quantitative RT-PCR analysis of *Socs3* and *Tbx21* mRNA in *Traf5*^{+/+} and *Traf5*^{-/-} B cells unstimulated (-) or stimulated for 2 h with 100 ng/mL IL-27 (+). Data are presented relative to the expression of *Ppib* mRNA. Data are mean \pm SEM (n = 3) and from one experiment representative of two independent experiments with similar results. NS, not significant, **p* < 0.05, ***p* < 0.01 (Student's *t*-test).

SUPPLEMENTAL FIGURE 2.



SUPPLEMENTAL FIGURE 2. (A, B) TRAF5 promotes IL-2 production and cell proliferation mediated by TCR and GITR in CD4⁺ T cells. Naïve CD4⁺ T cells from *Traf5*^{+/+} and *Traf5*^{-/-} mice were cultured with indicated concentrations of plate-bound anti-CD3 mAb in the presence of soluble anti-GITR mAb (5 μg/mL) or control IgG (5 μg/mL) for 3 days. (A) Primary IL-2 from activated naïve CD4⁺ T cells was measured by ELISA. (B) Cell proliferative responses of naïve CD4⁺ T cells was measured by MTT assay. (C) TRAF5 promotes CD40 signaling in B cells. Quantitative RT-PCR analysis of *Icam1*, *Fas*, and *Lta* mRNA in *Traf5*^{+/+} and *Traf5*^{-/-} B cells unstimulated (-) or stimulated for 2 h with 10 μg/mL anti-CD40 mAb (+). Data are presented relative to the expression of *Ppib* mRNA. Data are mean ± SEM (n = 3) and from one experiment representative of at least two independent experiments with similar results. ND, not detected, **p* < 0.05, ***p* < 0.01, ****p* < 0.001 (Student's *t*-test).

SUPPLEMENTAL FIGURE 3.



SUPPLEMENTAL FIGURE 3. IL-27-dependent phosphorylations of STAT1, STAT3, and p38 MAPK in CD4⁺ T cells. **(A)** Immunoblot analysis of IL-27-Fc with anti-PA-tag antibody. **(B)** Immunoblot analysis of STAT1 phosphorylated at Tyr701 (p-STAT1), total STAT1, STAT3 phosphorylated at Tyr705 (p-STAT3), total STAT3, p38 phosphorylated at Thr180/Tyr182 (p-p38), and total p38 in *Traf5*^{+/+} and *Traf5*^{-/-} CD4⁺ T cells. Ratios of phosphorylated STAT1 to total STAT1, phosphorylated STAT3 to total STAT3, and phosphorylated p38 to total p38 in *Traf5*^{+/+} and *Traf5*^{-/-} CD4⁺ T cells stimulated for indicated times with IL-27-Fc absorbed on Dynabeads protein G, presented relative to the highest ratio, set as 1.