

Supplemental Figure Legends

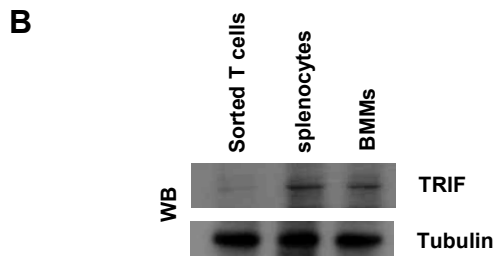
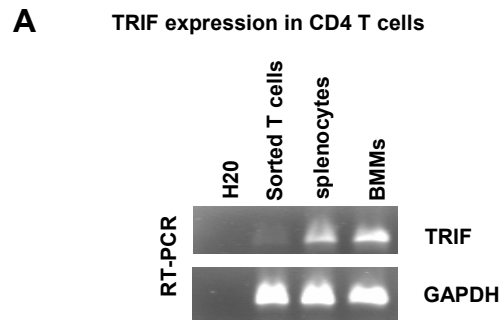
Supplemental Figure 1. TRIF expression in CD4 T cells. **(A)** RT-PCR analysis of TRIF mRNA expression in highly purified CD4 T cells. Total RNA was isolated with Trizol reagent from double-sorted CD4 T cells and was reverse-transcribed with SuperScript III reverse transcriptase. PCR was performed using primers for murine TRIF and GAPDH. Samples from total splenocytes and BMMs were included as positive controls. **(B)** Western blot analysis of TRIF protein in CD4 T cells. Double-sorted CD4 T cells, splenocytes and BMMs were lysed in lysis buffer (1% TX-100, 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1.0 mM EDTA, 1.0 mM Na₃VO₄, and a protease inhibitor mixture). Protein samples were separated on 10% SDS-PAGE, transferred to nitrocellulose membranes, and immunoblotted with an anti-TRIF antibody. Immunoblot with an antibody against Tubulin protein was used as loading control.

Supplemental Figure 2. **(A)** Co-culture of CD4 T cells with BMDCs. BMDCs from wt mice, TRIF deficient or IFNAR deficient mice were stimulated with LPS (100ng/ml) for 24 hours, then were cultured with wt naïve CD4 T cells in the presence of anti-CD3 (1μg/ml) for 72 hours. IL-17 production by T cells was measured by ELISA. **(B)** Similar to (A) except TRIF deficient CD4 T cells were used.

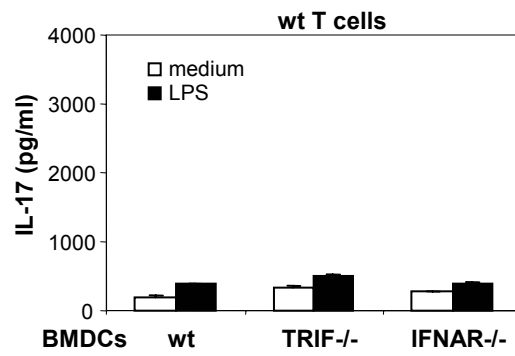
Supplemental Figure 3. Cytokine production from TRIF deficient BMMs and BMDCs. Wt and TRIF deficient BMMs or BMDCs were stimulated with 100ng/ml of LPS for 24 hours. The concentration of a cytokine indicated in culture supernatants was measure by ELISA.

Supplemental Figure 4. Effect of type I IFN on LPS-induced cytokine production in IFNAR deficient BMMs and BMDCs. Wt and IFNAR deficient BMMs or BMDCs were stimulated with 100ng/ml of LPS for 24 hours. The concentration of a cytokine indicated in culture supernatants was measure by ELISA.

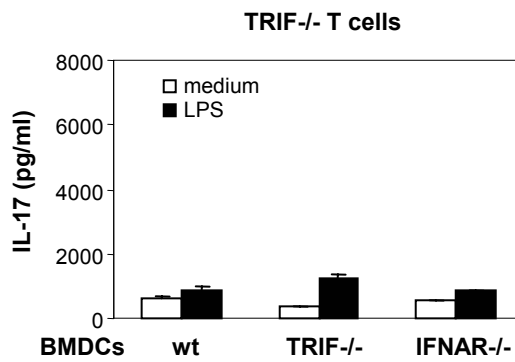
Supplemental Figure 5. **(A)** Wt CD4 T cells were cultures in Th17 condition (anti-CD3/anti-CD28, IL-6 and TGFβ) in the presence of CM from IFNβ-stimulated wt macrophages with anti-IL-27 antibody or control IgG. After 72 hours, Cells were restimulated with anti-CD3 antibody and were stained for surface CD4 and intracellular IL-17. Plots were gated on CD4+ T cells, and numbers indicate percentage of IL17+CD4+ cells of total CD4 cells. **(B)** IFNβ-mediated inhibitory effects on IL-17 production from TRIF deficient T cells. Purified TRIF deficient CD4 T cells were culture under Th17 differentiation condition in the presence of CM from IFNβ-stimulated wt macrophages with anti-IL-27 antibody. After 72 hours, IL-17 production by CD4 T cells was determined by ELISA.

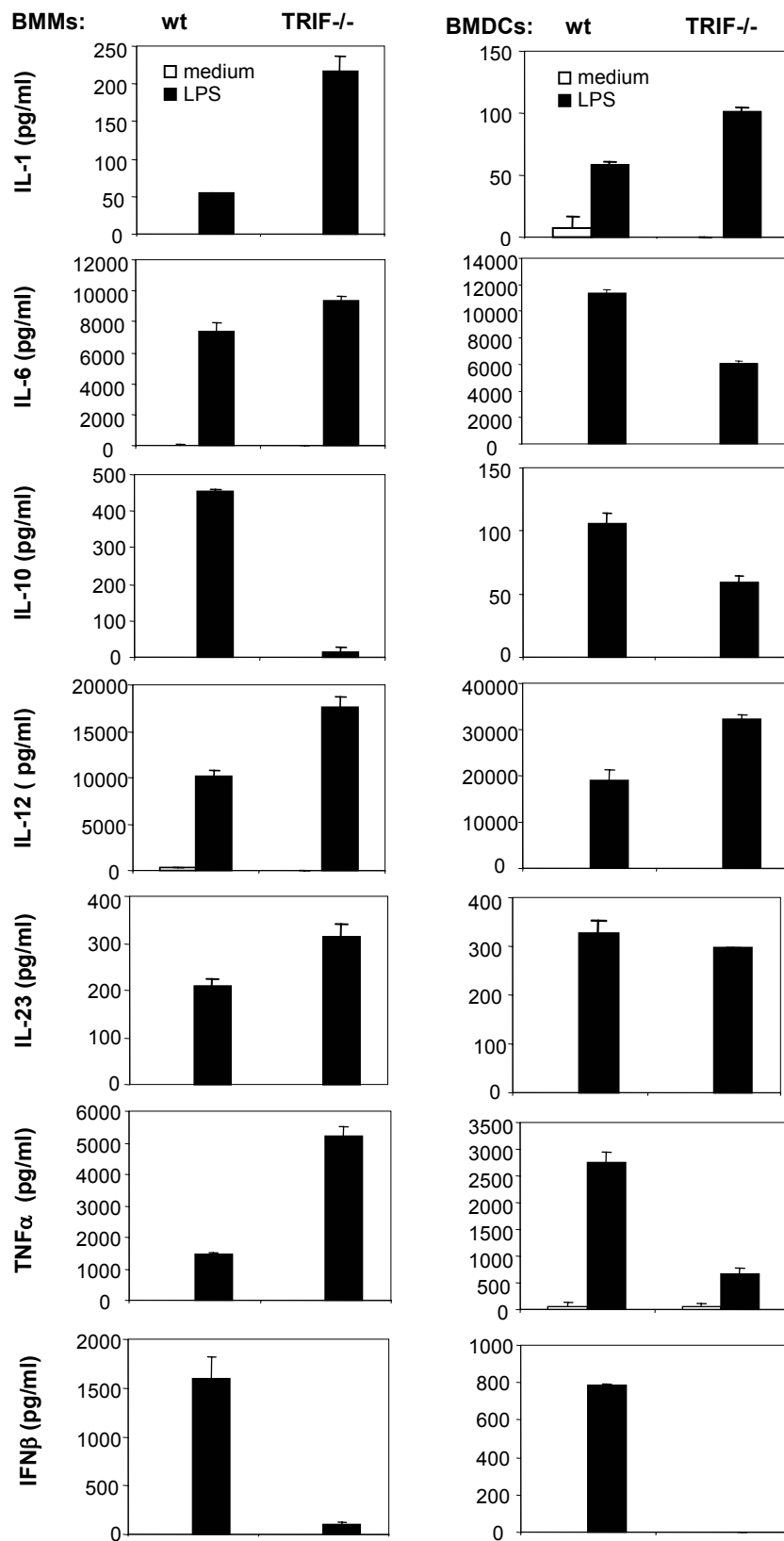


A

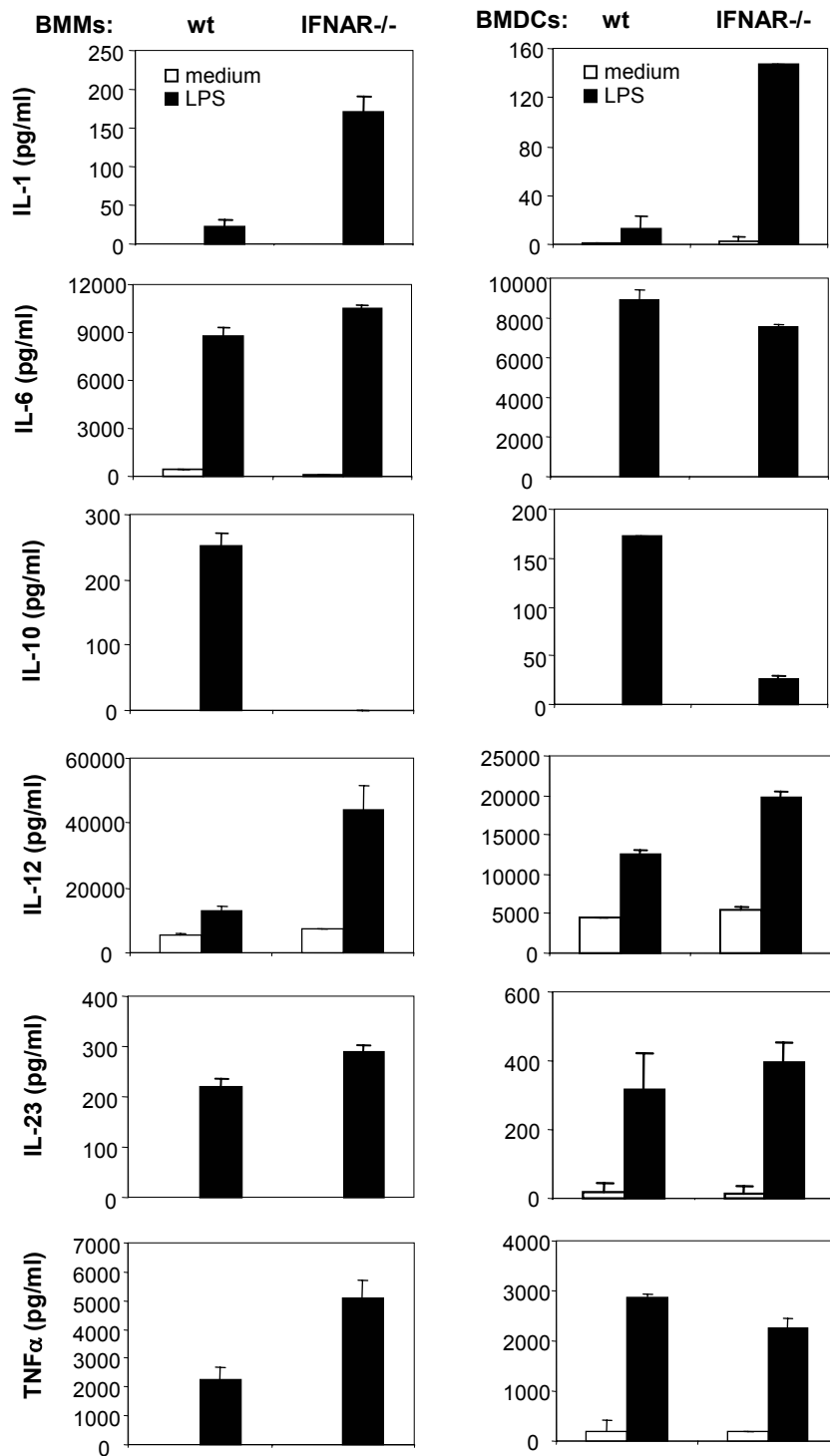


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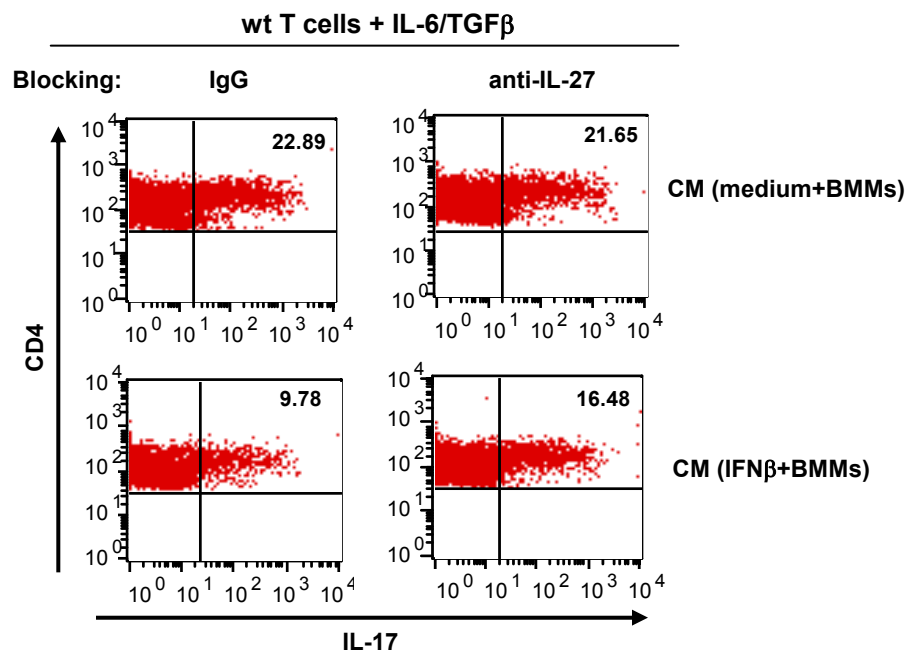


Supplemental Figure 3



Supplemental Figure 4

A



B

