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Supplementary Materials for

The adaptor protein TRAF3 inhibits interleukin-6 receptor signaling in B cells to limit plasma cell development

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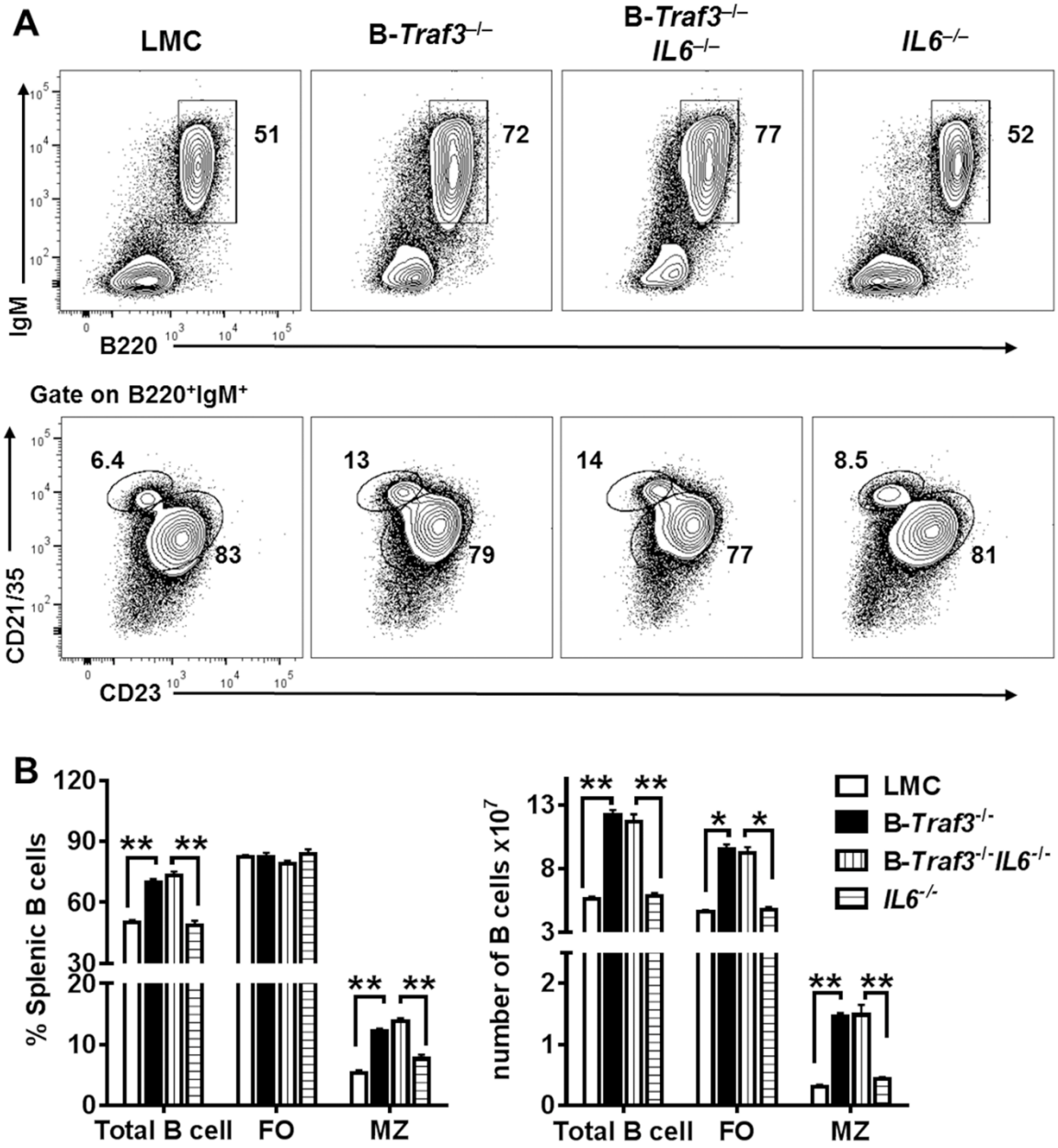

Fig. S1. The increase in the number of B cells caused by TRAF3 deficiency is independent of IL-6. (**A**) Representative plots from the flow cytometric analysis of total B cells ($B220^{+}IgM^{+}$), marginal zone (MZ) B cells ($B220^{+}IgM^{+}CD21/35^{hi}CD23^{low}$), and follicular (FO) B cells ($B220^{+}IgM^{+}CD21/35^{int}CD23^{+}$) from the indicated strains of mice. Outlined areas and numbers indicate the percentages of the different populations. (**B**) The percentages (left) and numbers (right) of total B cells, FO B cells, and MZ B cells from the indicated mice. Data are means ± SEM of at least three mice per group. $^{**}P < 0.01$, $^{*}P < 0.05$ by one-way ANOVA.

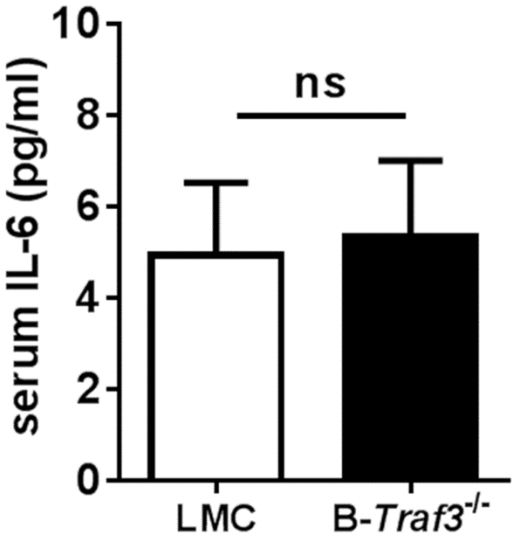

Fig. S2. Loss of TRAF3 in B cells does not alter the amounts of serum IL-6 in mice. ELISA was performed to determine the basal serum concentrations of IL-6 in LMC and B-*Traf3*$^{-/-}$ mice. Data are means ± SEM of five mice per group. ns, not statistically significant.

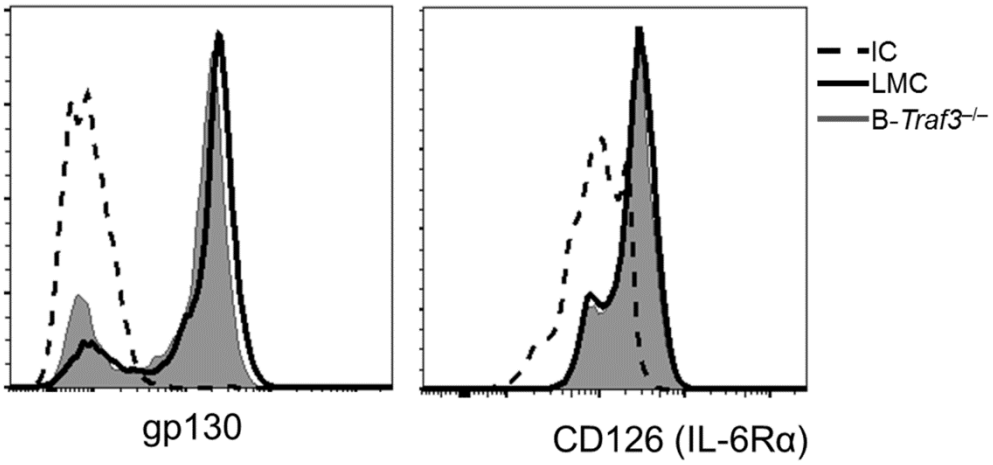

Fig. S3. Loss of TRAF3 does not affect IL-6R abundance on B cells. Splenic B cells isolated from LMC or B-*Traf3*$^{-/-}$ mice were incubated with antibodies specific for gp130 (left) or CD126 (right) and then were analyzed by flow cytometry. Three mice of each genotype were analyzed per experiment. Representative flow cytometry plots from one of two experiments are shown. IC, isotype control antibody.

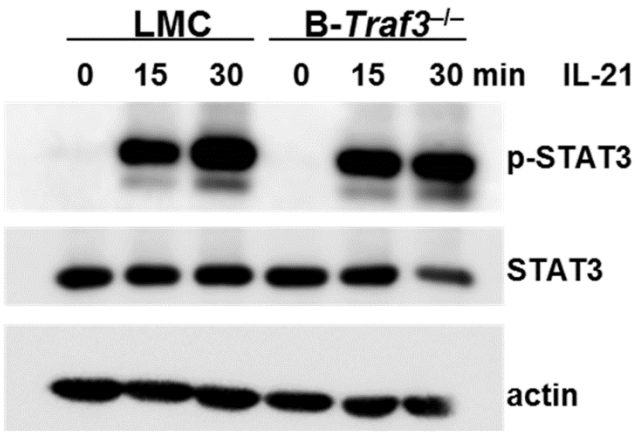

Fig. S4. TRAF3 is not required for the IL-21–dependent activation of STAT3. Splenic B cells isolated from the indicated mice were left untreated or were treated with IL-21 (50 ng/ml) for the indicated times. Whole-cell lysates were then analyzed by Western blotting with antibodies specific for the indicated proteins. Western blots are from a single experiment and are representative of three independent experiments.

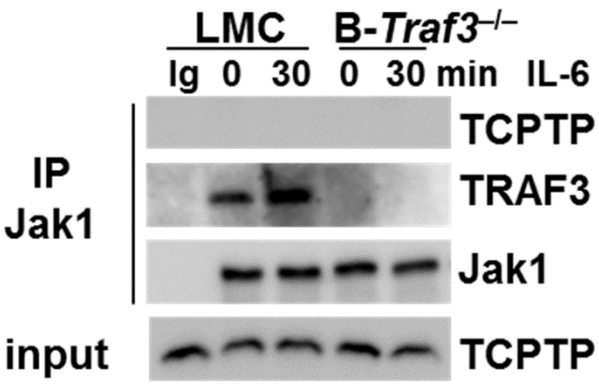

Fig. S5. TCPTP does not associate with Jak1 in B cells in response to IL-6. Splenic B cells from the indicated mice were left untreated or were treated with IL-6 for 30 min. Cell lysates were then subjected to immunoprecipitation (IP) with an antibody against Jak1, and the precipitates were analyzed by Western blotting with antibodies specific for the indicated proteins. Whole-cell lysates (input) were also analyzed to detect TCPTP. Blots are from a single experiment and are representative of two independent experiments.

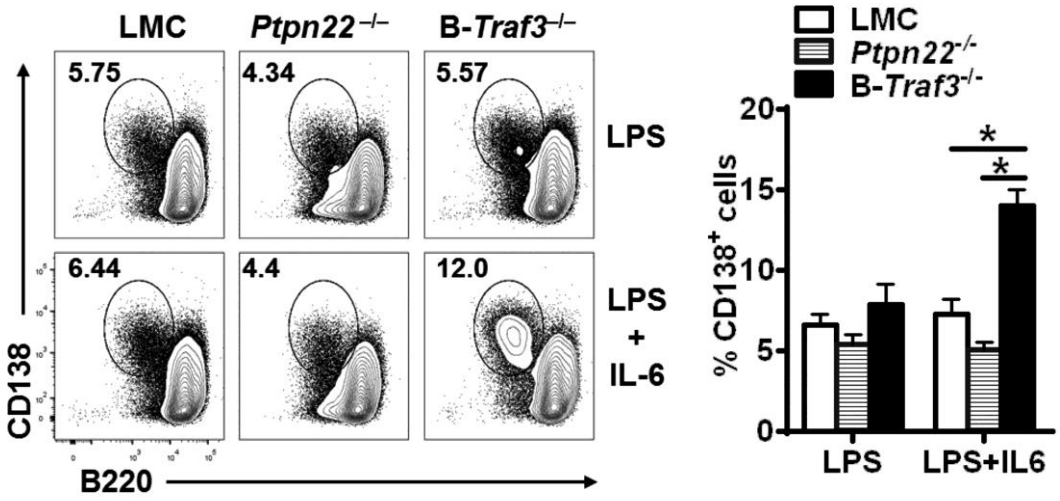

Fig. S6. PTPN22-deficient B cells do not show enhanced PC differentiation in vitro in response to LPS and IL-6. Splenic B cells from the indicated mice were stimulated with LPS in the presence or absence of IL-6 for 3 days. $CD138^{+}B220^{low}$ PC populations were then analyzed by flow cytometry. Dead cells were excluded from the analysis. Left: Flow cytometry plots from one representative experiment. The outlined areas and numbers indicate the percentages of $CD138^{+}B220^{low}$ PCs. Right: Quantification of the percentages of $CD138^{+}B220^{low}$ PCs from the indicated mice. Data are means ± SEM from three independent experiments.