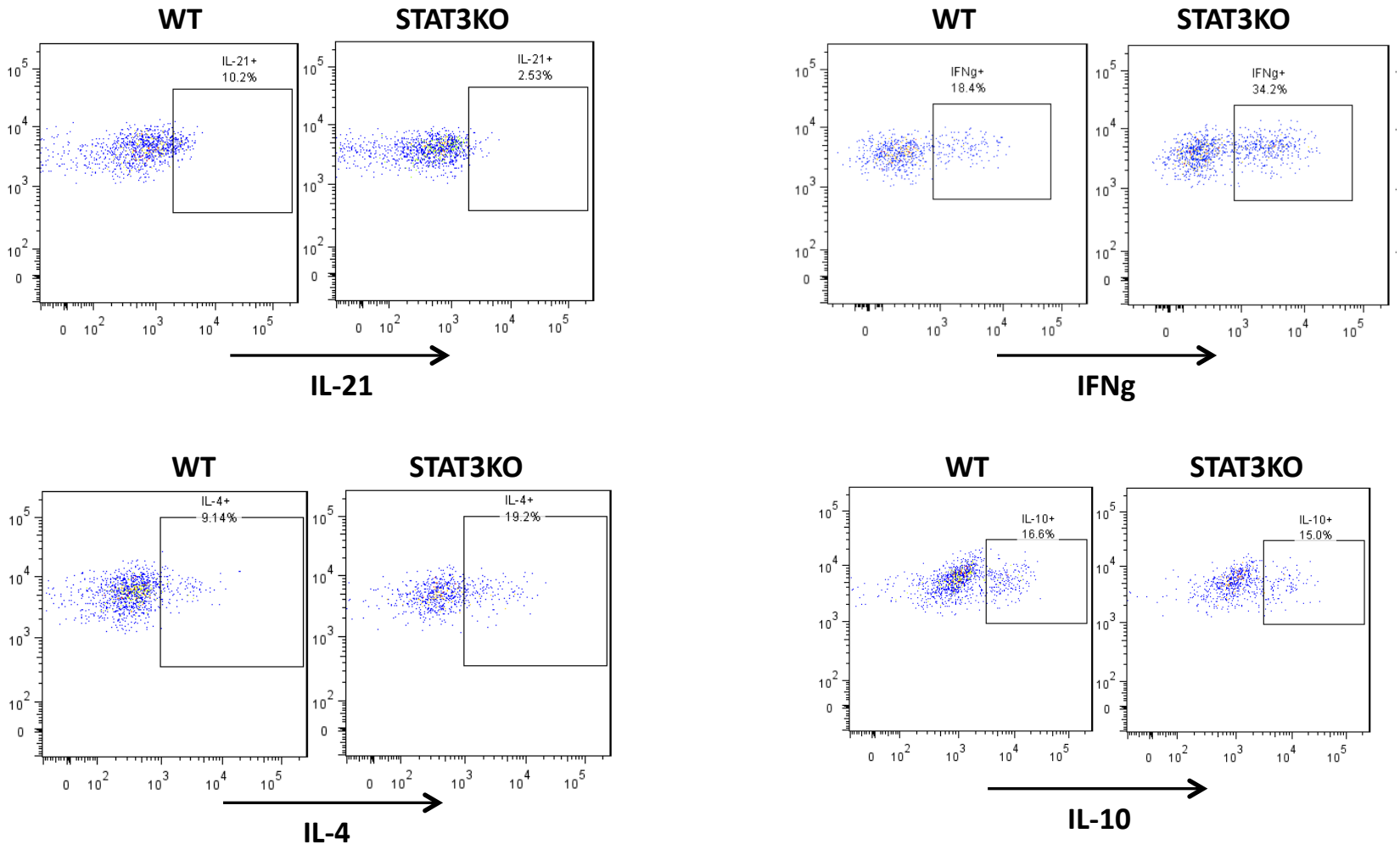
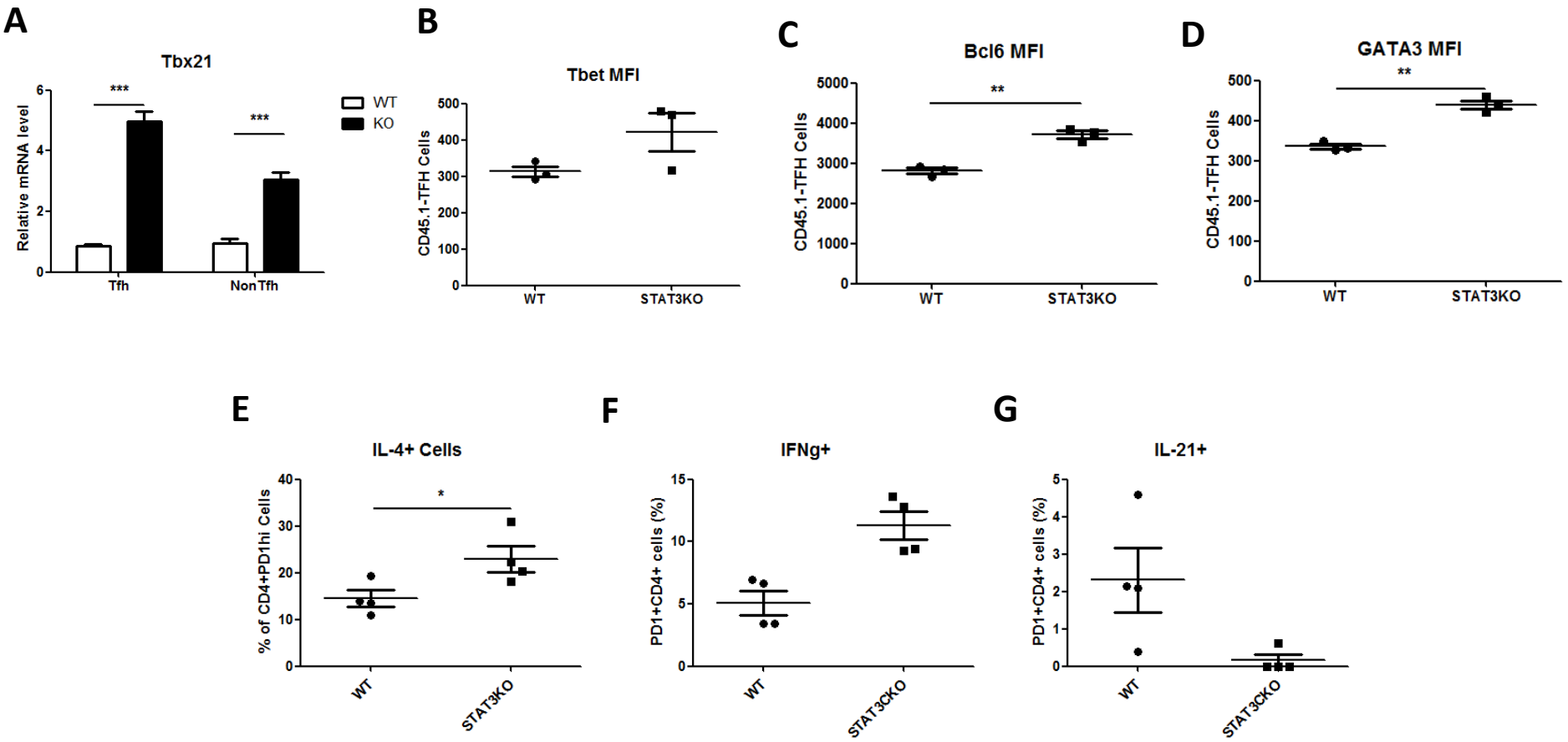
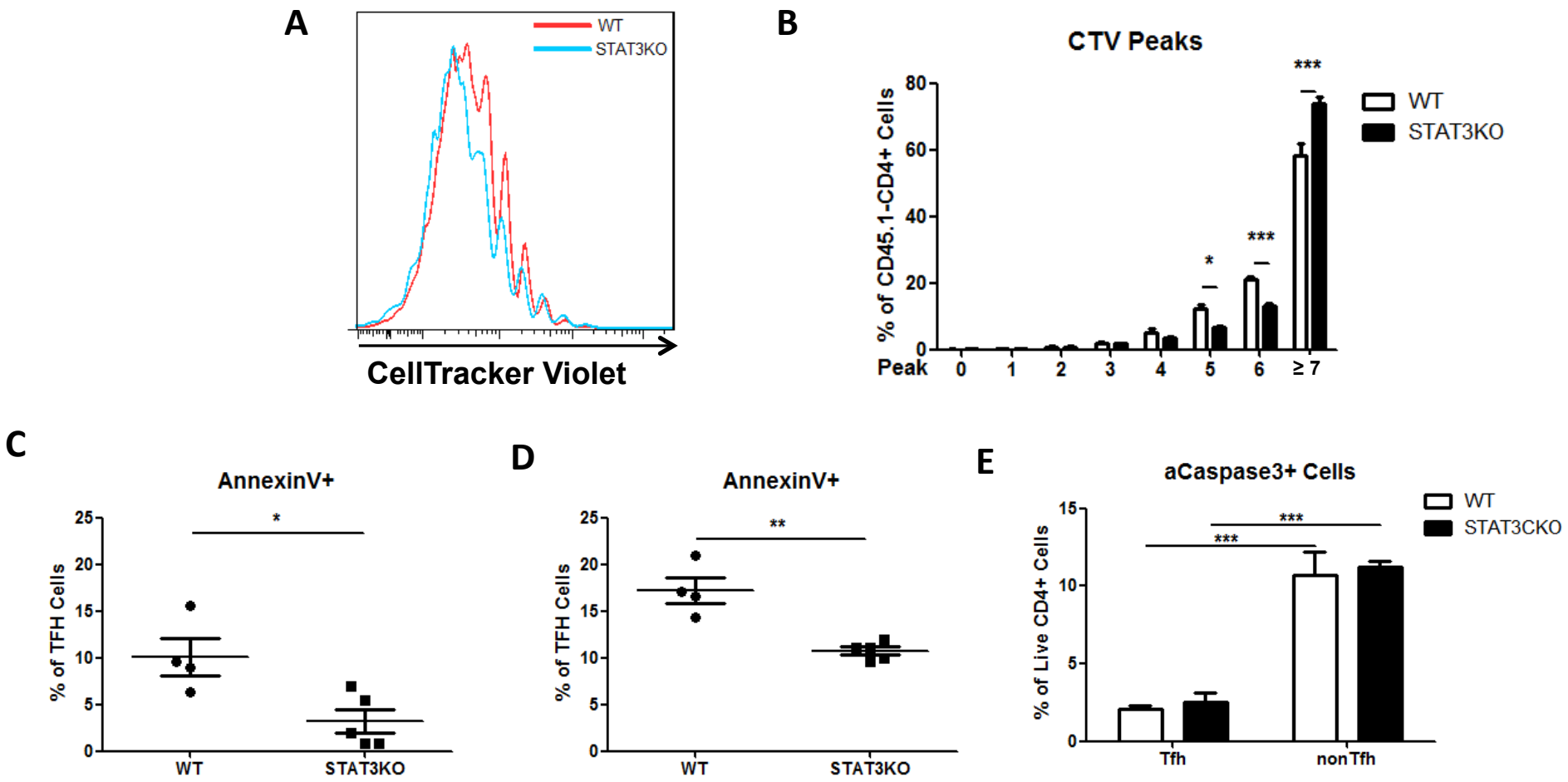


Supplemental Figure 1. Examples of cytokine staining and gates showing staining controls. Spleen cells were stimulated with PMA plus ionomycin and stained intracellularly for the indicated cytokines, 7 days after SRBC immunization as in Figure 6. Plots are gated on CD4+ cells. Gated regions show cytokine-positive cells. Isotype controls for each Ab were used to set gates; for isotype controls, 0-1% of the cells were in the gated region for all cytokines analyzed.



Supplementary Figure 2. Tfh cell intrinsic regulation of transcription factors and cytokines by Stat3. (A) T_{FH} cells in the Peyer's patches (PPs) of WT and STAT3KO mice were sorted by FACS and RNA isolated post-sort. Tbx21 (Tbet) mRNA levels were assayed by QPCR. (B-D) WT and STAT3KO OTII TCR transgenic CD45.2+ CD4+ T cells were transferred to CD45.1+ BoyJ mice, and the recipient BoyJ mice were immunized with OVA-Alum. T_{FH} cells were analyzed at 7 days after immunization. Graphs show Tbet, Bcl6 and Gata3 MFIs of CD45.1-TFH cells, where each symbol represents one mouse (n=3, mean ± SEM). (E-G) Cytokines in T_{FH} cell populations in the PPs of bone marrow chimeras were measured by intracellular cytokine staining (ICS) following PMA plus ionomycin staining in vitro.



Supplemental Figure 3. Increased proliferation and decreased death of Stat3-deficient CD4 T cells. A-B) WT and STAT3KO OTII TCR transgenic CD45.2+ CD4+ T cells were transferred to CD45.1+ BoyJ mice and immunized with OVA-Alum as in Figure 6. A) Histogram plot of Cell Tracker Violet (CTV)-labeled WT and STAT3KO OTII CD4+ T cells at 6 dpi with OVA-Alum. B) Graphs of average MFI of CTV-labeled WT and STAT3KO OTII CD4+ T cells. Each symbol represents one mouse (n=3, mean ± SEM). ***P* <0.01 (*t*-test). C) Annexin V+ percentage of T_{FH} cells assayed *ex vivo* from spleen 7 dpi with SRBC in WT and STAT3KO mice. Each symbol represents one mouse (n=4-5, mean ± SEM). **P* <0.05 (*t*-test). D) Annexin V + percentage of T_{FH} cells assayed *ex vivo* from PP (n=4-5, mean ± SEM). ***P* <0.01 (*t*-test). E) 7 dpi with SRBC, average percentage of activated Caspase3+ (aCaspase3+) cells in FACS-isolated spleen T_{FH} cells and non-T_{FH} cell populations, stimulated with anti-CD3 Ab overnight *in vitro* . (n=3, mean ± SEM). ****P* <0.001 (ANOVA).

Supplemental Figure 4. Defective Suppression of IL-4 by Bcl6 in Stat3-deficient T cells. WT and STAT3KO OTII TCR transgenic CD4+ T cells were cultured under Tfh conditions with APC and OVA peptide *in vitro* for 3 days. (A) Cells were stimulated with PMA plus ionomycin and analyzed for IFNg and IL-4 ICS and flow cytometry after just 3 days of culture. (B) Part of the primary culture was put into secondary culture under Th2 conditions with APC and OVA peptide *in vitro* for 5 days, before being stimulated with PMA plus ionomycin and analyzed for IFNg and IL-4 ICS and flow cytometry. Graphs show average percentages of IFNg+IL-4-, IFNg+IL-4+ and IFNg-IL-4+ cell CD4+ T cells (n=3, mean ± SEM). ****P* <0.001 (*ANOVA*).