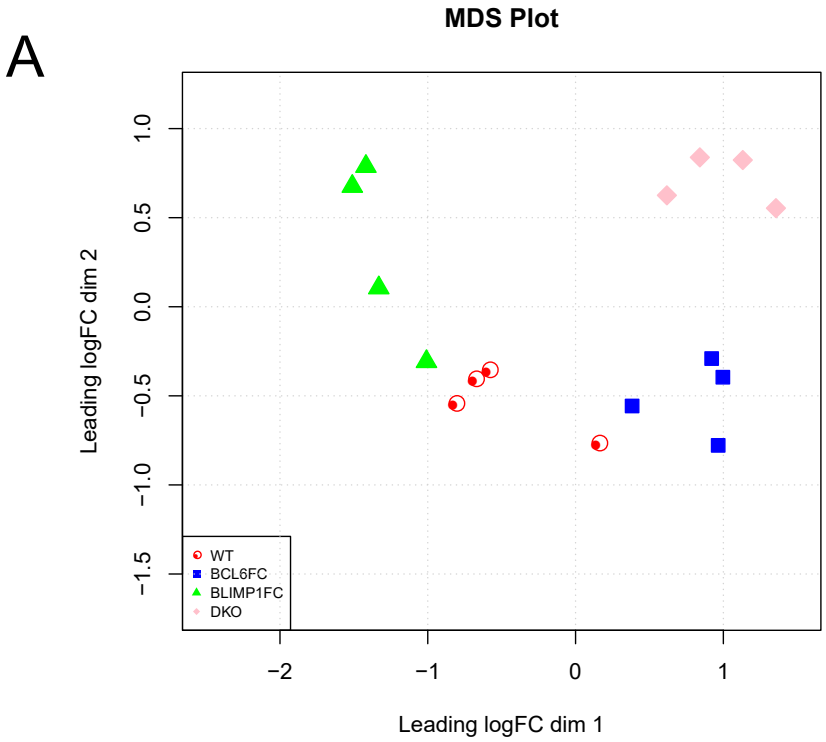
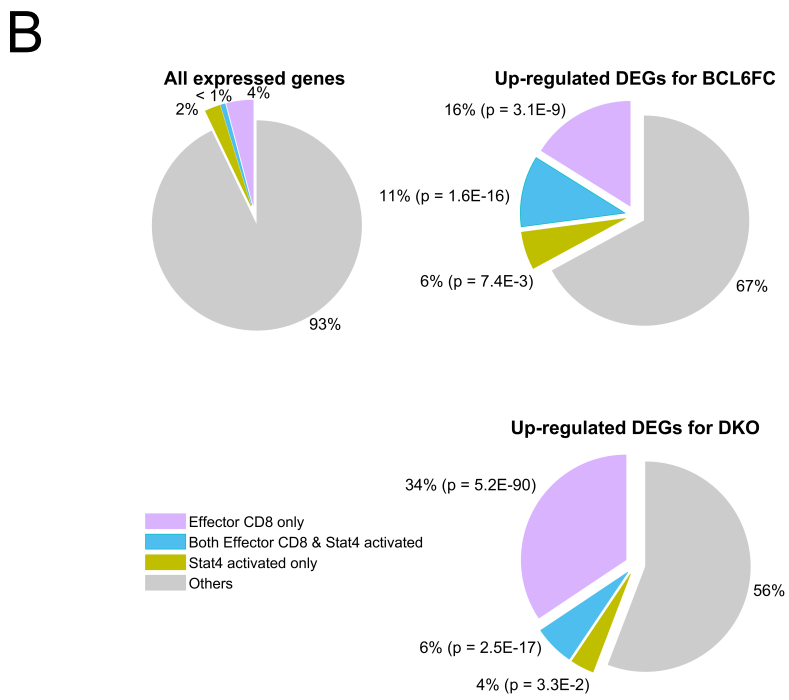
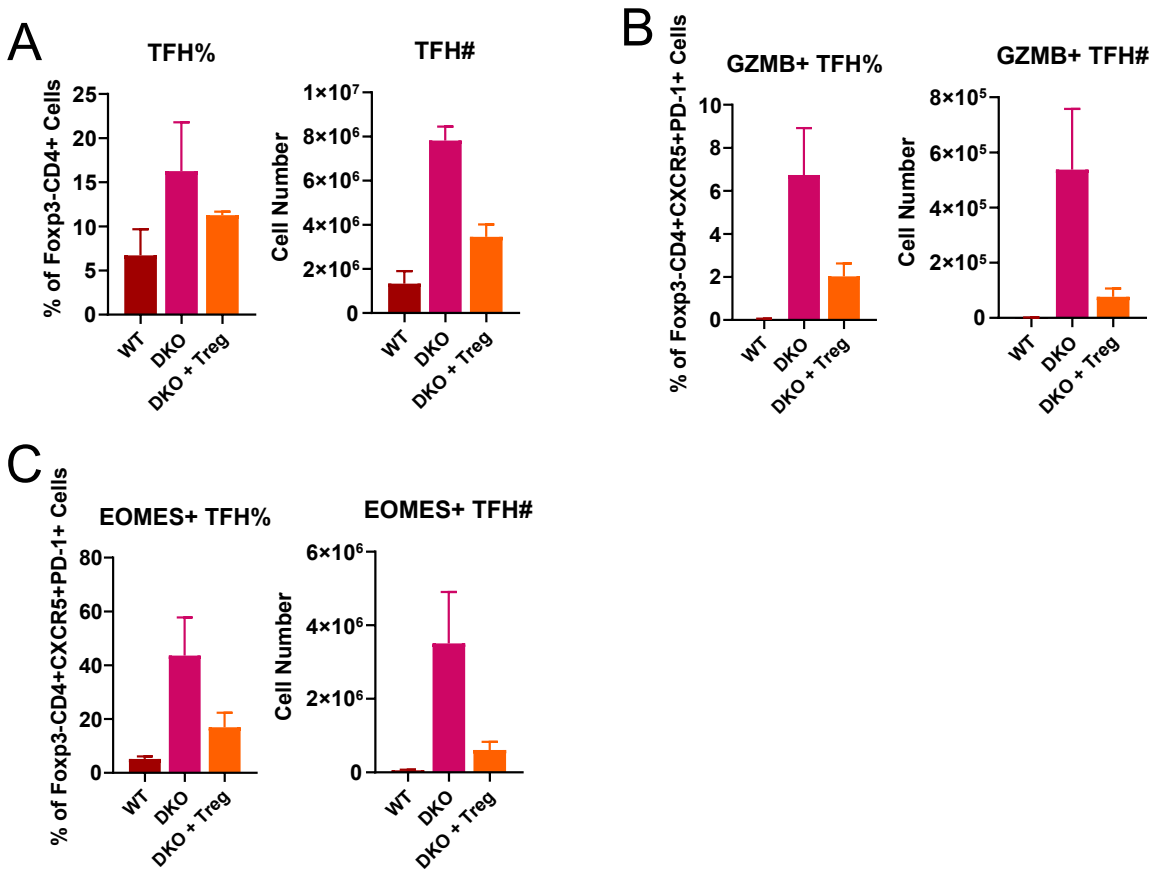
Supplementary Figure 1. RNAseq bioinformatic analysis and comparison with CD8 effector and Stat4 activated gene datasets. A. Principal component type analysis of gene expression data from TFH cells from WT, Bcl6FC, Blimp1FC and DKO mice. MDS = multidimensional scaling. **B.** Enrichment of Stat4 and effector CD8 T cell genes in Bcl6FC and DKO TFH, related to Figure 2C. Effector CD8 T cell DEGs, Stat4 activated DEGs and genes common to both types of DEGs are shown as fractions of all expressed genes and fractions of up-regulated genes in Bcl6FC and DKO TFH cells, with p values indicated for the enrichment.





Supplementary Figure 2: Suppression of cytotoxic-like TFH cells by WT Tregs. WT and DKO mice were immunized with sheep red blood cells (SRBC) i.p. and analyzed for development of Granzyme B- and Eomes-expressing TFH cells similar to Figure 4. One group of DKO mice were injected i.v. with CD25+ WT Tregs (500,000 per mouse) the day before immunization. TFH cells were analyzed 7 days after immunization. N = 3. Experiment was performed twice with similar results. **A.** Mean ± SEM TFH cell percentage and number. **B.** Mean ± SEM Granzyme B+ TFH cell percentage and number. **C.** Mean ± SEM Eomes+ TFH cell percentage and number.



Supplementary Figure 3: Alteration of IgG isotype by loss of TFR cells, Treg-specific Blimp1 correlating with increased cytotoxic-like TFH cells. WT, Blimp1FC and DKO mice were immunized with SRBC i.p.. 7 days after immunization, serum was analyzed for SRBC-specific (A) IgG1 and (B) IgG2b levels by ELISA. A ratio of the ODs for the 2 types of IgG are shown in (C). Graphs shown mean of data. Error bars show SEM. P values were calculated by t test where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$. N = 12 mice. Data shown is combined from 3 experiments. ANOVA with Tukey post hoc analysis was used for statistical significance.