Supp Figure S1

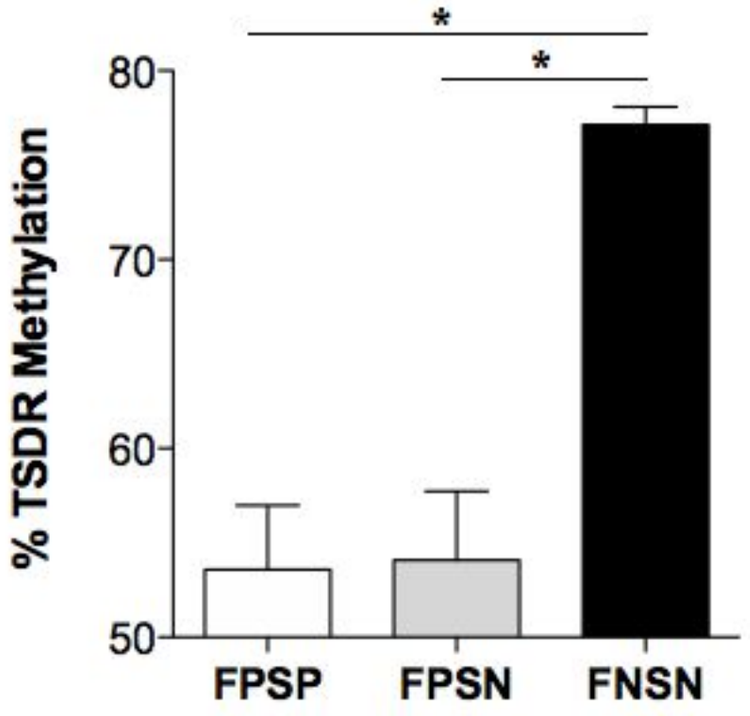


Figure S1. FPSP and FPSN clones have equally demethylated TSDR regions. Genomic DNA was extracted from female-derived primary FPSP, FPSN and FNSN clones and analyzed for TSDR methylation. Shown is the mean + SEM of % methylation of 10 cytosine guanine dinucleotides (CpGs) of the human Treg specific demethylation region (TSDR). n= 2-4 clones per group. Statistical analysis was done with the one-way ANOVA followed by Tukey's post-test.

Supp Figure S2

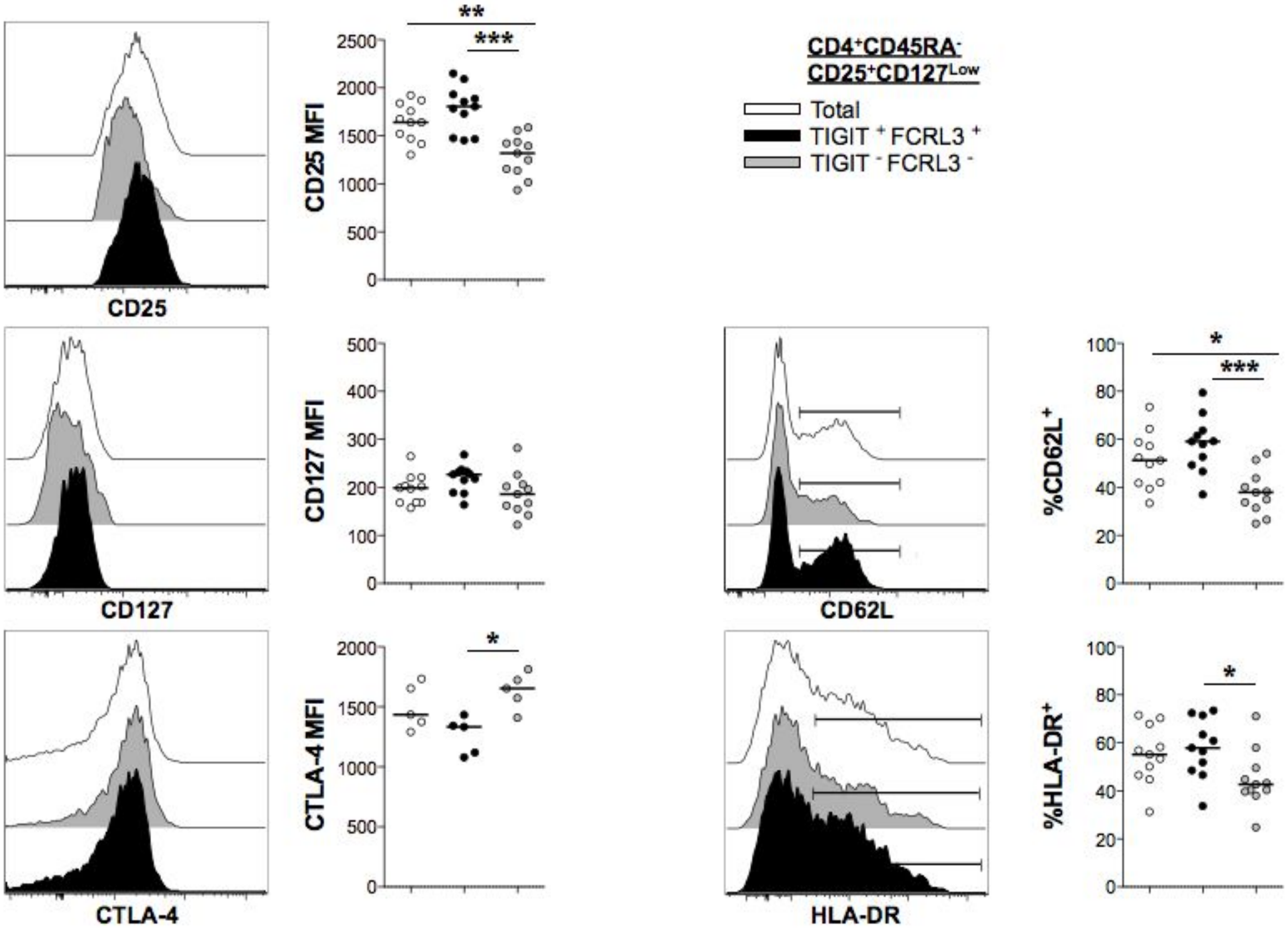


Figure S2. Analysis of the expression of Treg-associated molecules on TIGIT$^+$FCRL3$^+$ vs. TIGIT$^-$FCRL3$^-$ Treg cells. PBMCs from healthy subjects were analyzed *ex vivo* by flow cytometry. Shown are representative FACS plots as well as the combined analysis of marker expression on Treg subsets of 11 healthy donors. Statistical analysis was done with the one-way ANOVA followed by Tukey's post-test

Supp Figure S3

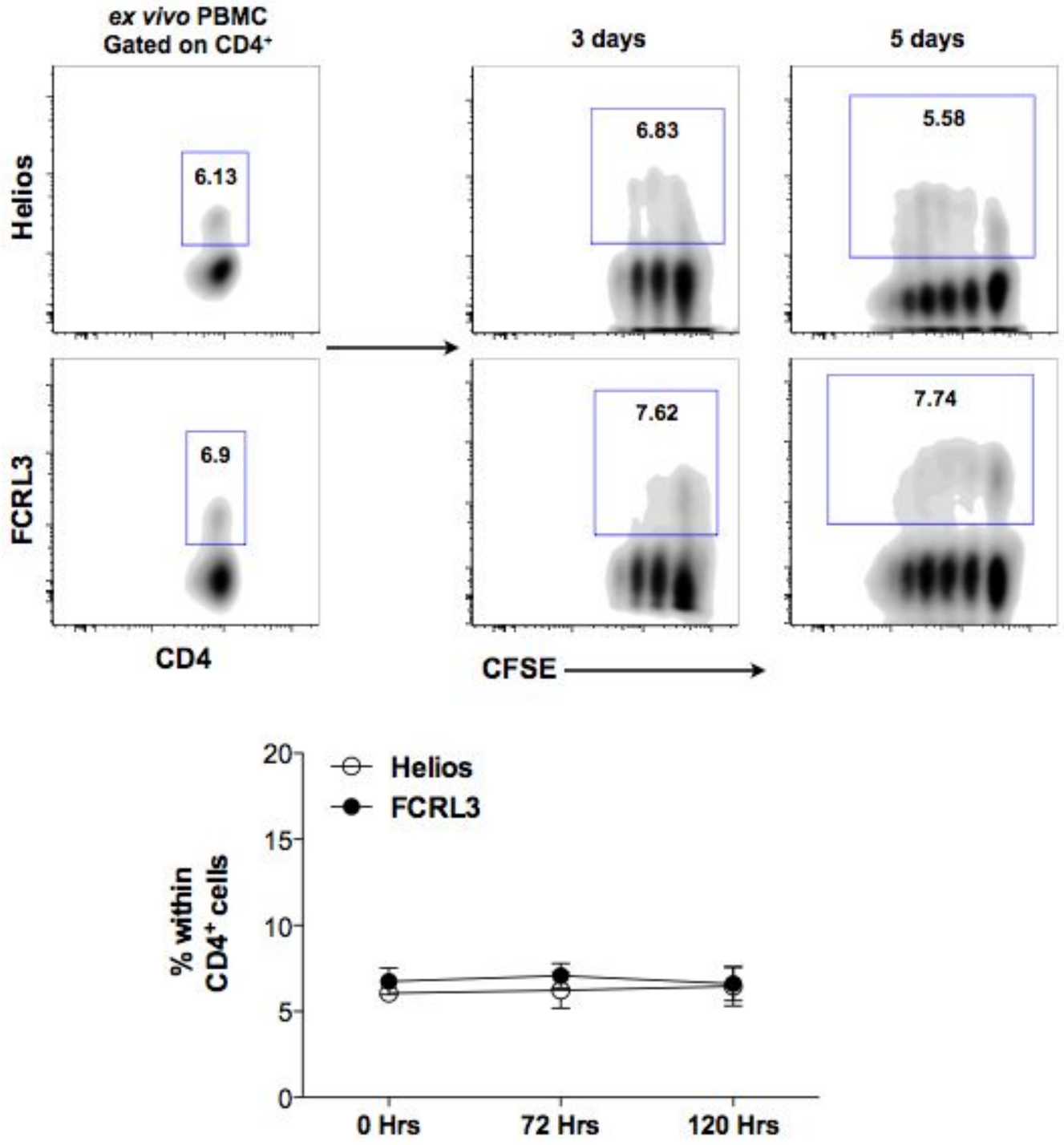
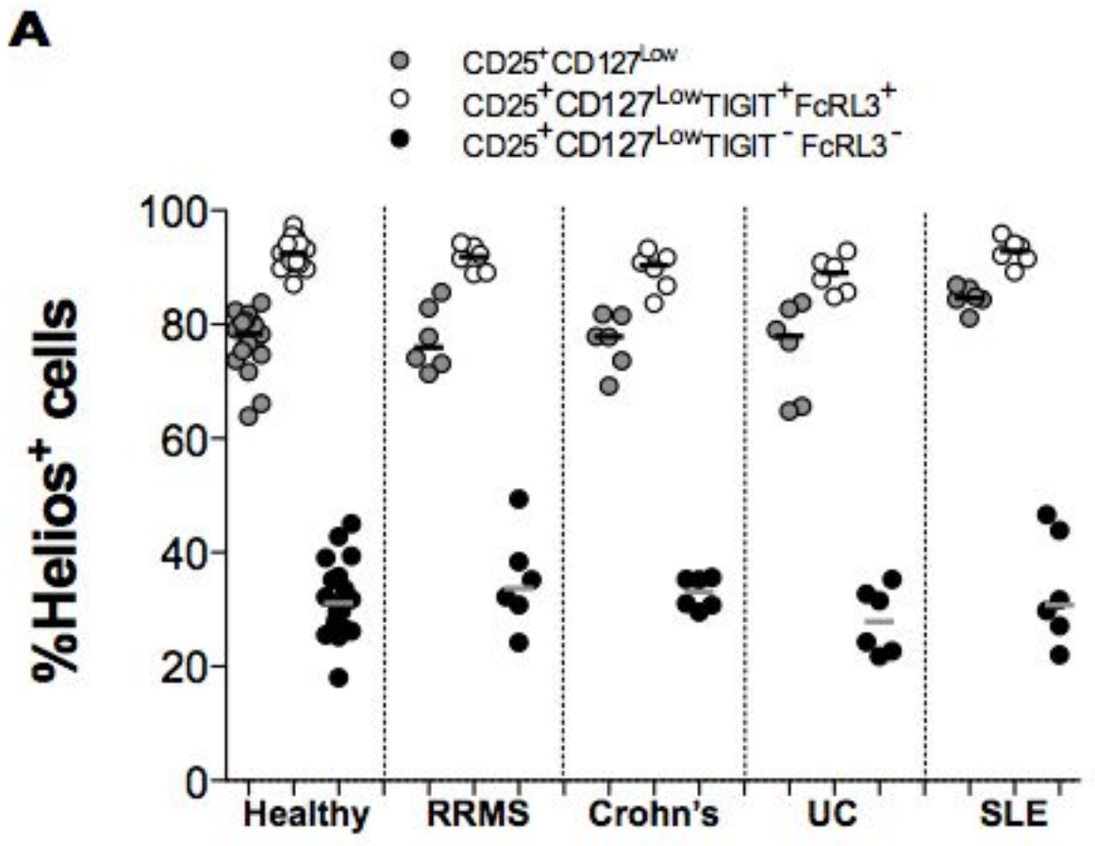


Figure S3. Helios and FCRL3 are not induced on Tconv upon *in vitro* activation of PBMC. Whole PBMC from 3 healthy individuals were labeled with CFSE and stimulated with anti-CD3/anti-CD28-coated beads at a ratio of 2 beads: 1 cell for 5 days. Shown is the expression of Helios and FCRL3 on $CD4^+$ cells at day 0, 3 and 5.

Supp Figure S4



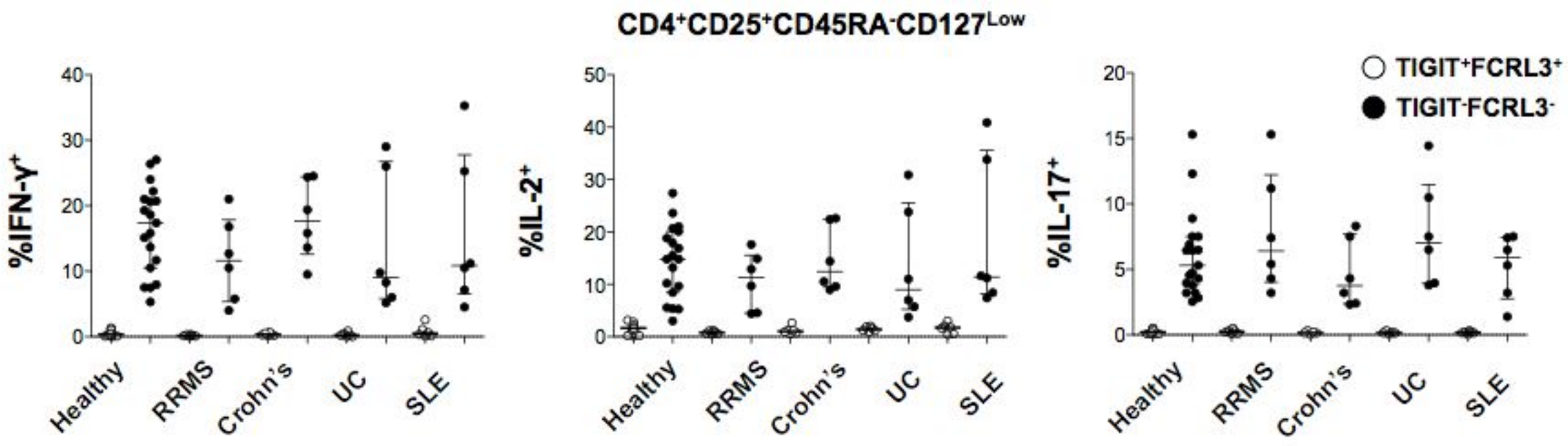


Figure S4. Phenotype and cytokine production in TIGIT$^+$FCRL3$^+$ vs. TIGIT$^-$FCRL3$^-$ Treg cells of healthy and autoimmune donors. PBMCs from untreated patients with relapsing-remitting multiple sclerosis (n=6), Crohn's disease (n=6), ulcerative colitis (n=6), and treated systemic lupus erythematosus (n=6; SLEDAI-2k >6) and their age and sex-matched healthy controls (n=19) were analyzed by flow cytometry directly *ex vivo* **(A)** or after incubation with PMA (25 ng/ml), ionomycin (1 µg/ml) and GolgiStop for 4hrs **(B)**. Shown is **A)** the percentage of Helios-expressing cells in TIGIT/FCRL3-identified Treg subsets, and **B)** the frequency of cytokine-producing cells in the indicated subsets.