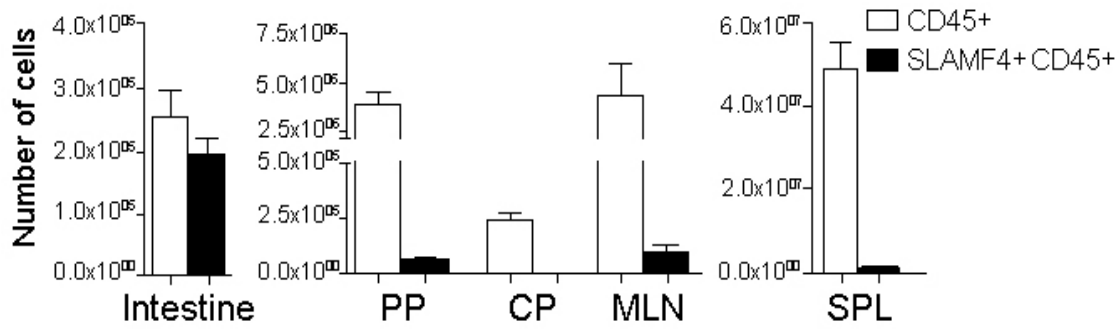
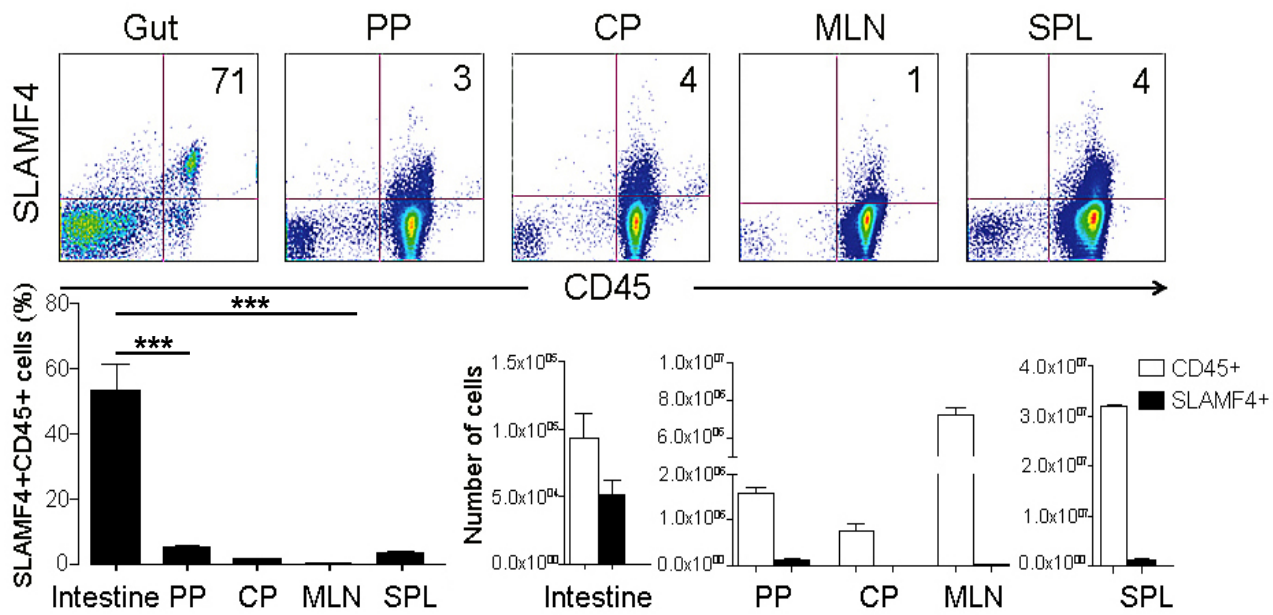
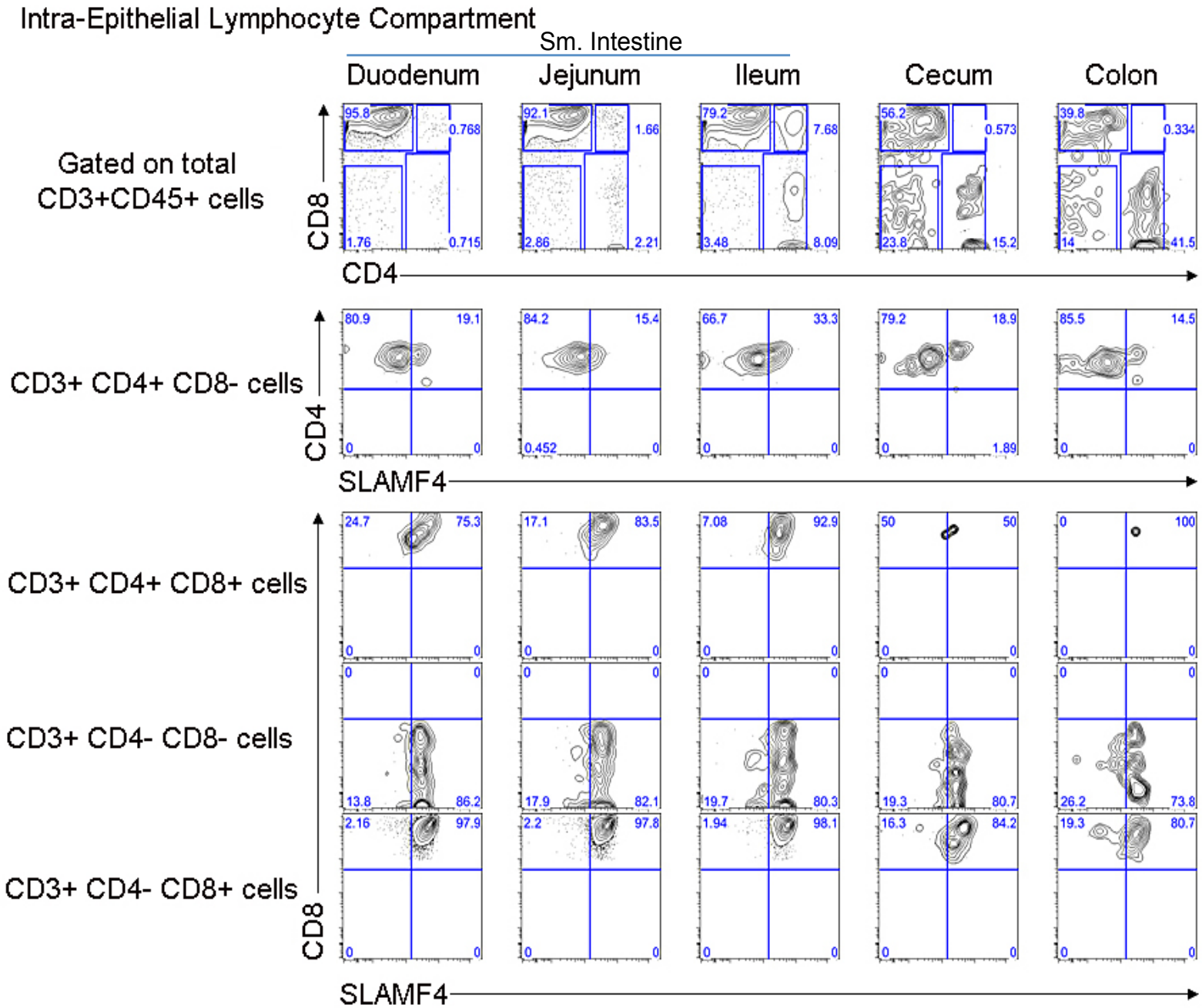
a. Mouse strain: C57BL/6



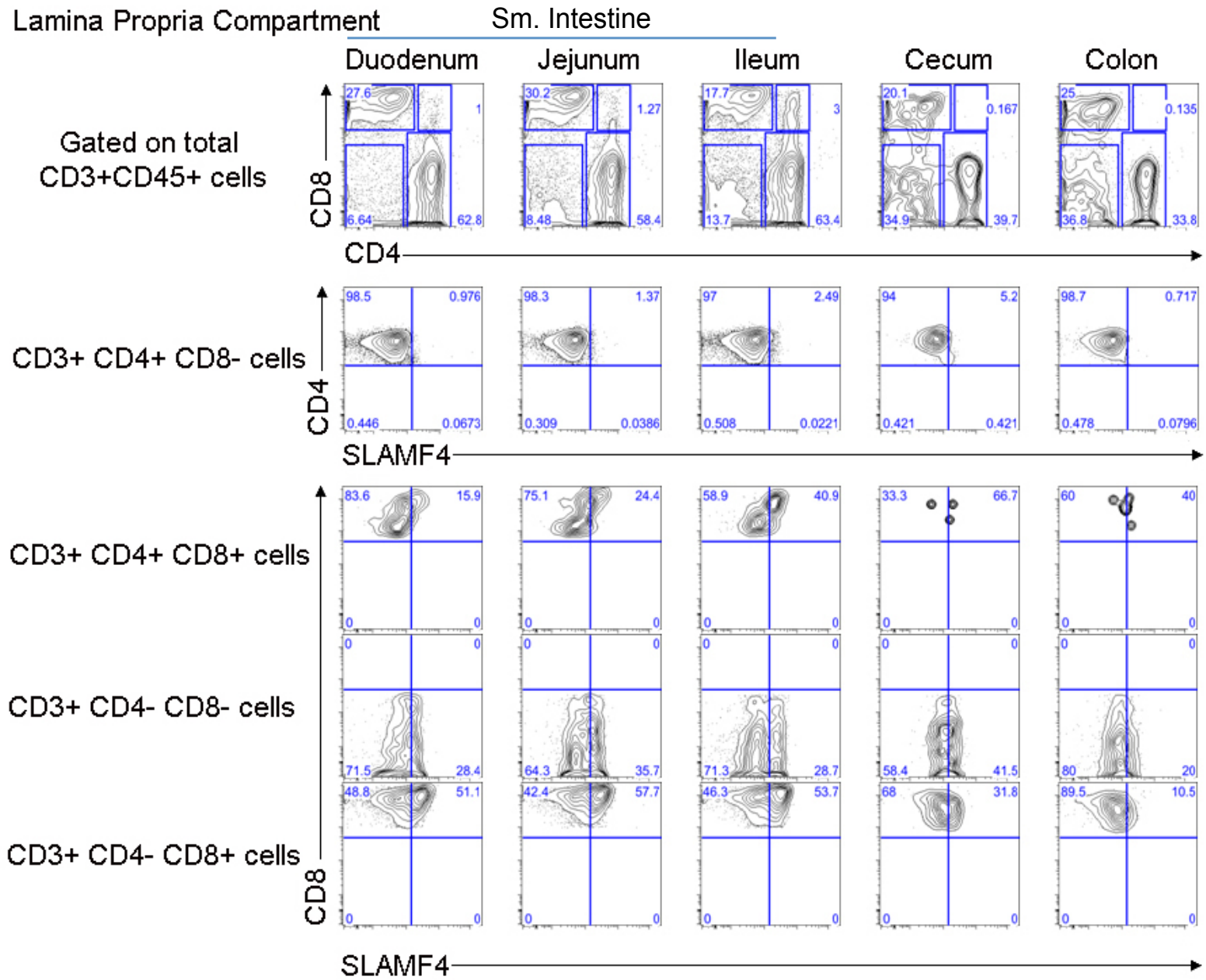
b. Mouse strain: BALB/c



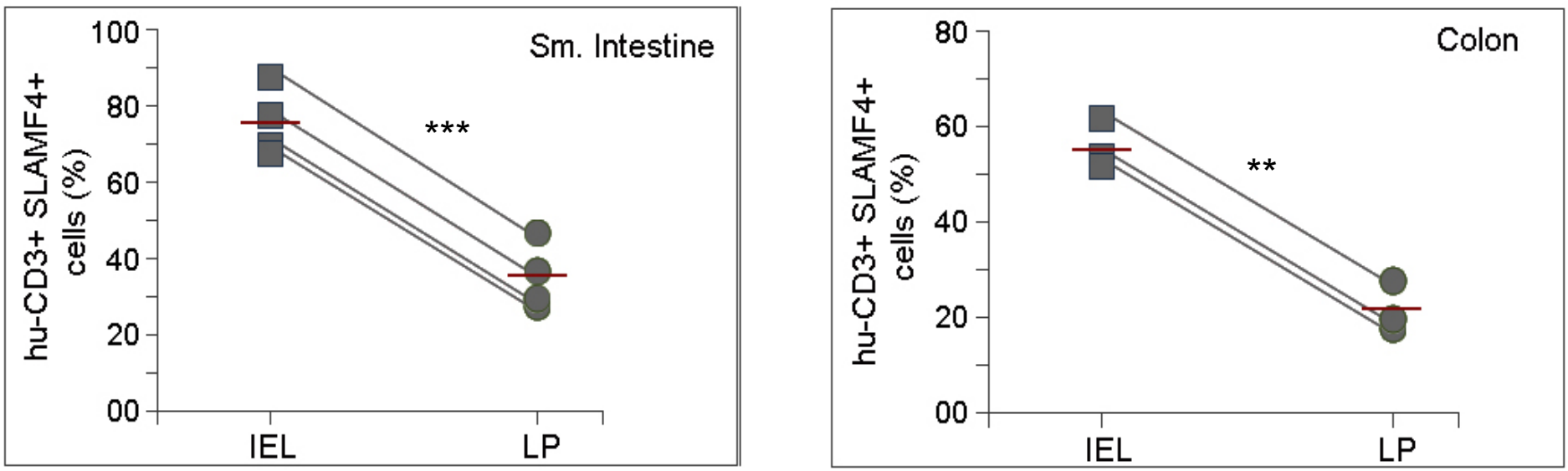
Supplemental Figure 1. SLAMF4 is a marker of CD45+ immune cells of the intestinal mucosa. (a) Most hematopoietic derived-cells in the gut mucosa, but not the Peyer's patch (PP), cecal patch (CP), mesenteric lymph nodes (MLN), or spleen (SPL) of C57BL-6 mice express SLAMF4 (*n*=8-20). Data are shown as means of total numbers of SLAMF4+CD45+ cells ± s.e.m. Error bars represent s.e.m. **(b)** CD45+ cells in the gut mucosa, but not in other tissues of BALB/c mice express SLAMF4. Numbers indicate the % CD45+ cells that are SLAMF4+. *Below*, data are shown as means of % of CD45+ and total numbers of SLAMF4+CD45+ cells ± s.e.m. Error bars represent s.e.m. Data are from 3 experiments using 2 mice per experiment.



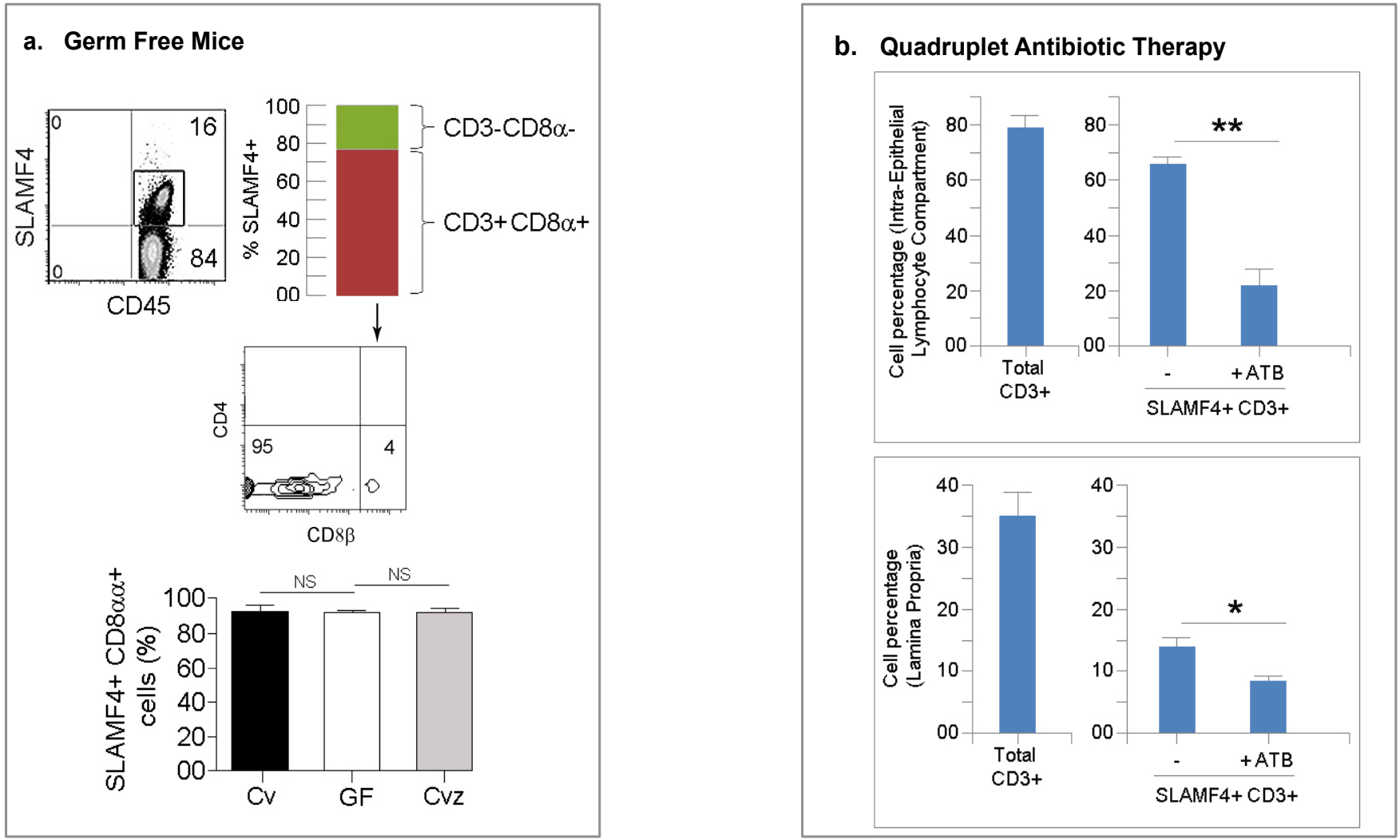
Supplemental Figure 2. SLAMF4 expression on T lymphocytes in the IEL compartment. SLAMF4 is a expressed by different T lymphocyte subsets in the IEL compartment of the duodenum, jejunum, and Ileum (which constitute the small intestine) as well as cecum, and colon. Dot plots are representative of *n*=4. Numbers indicate the % CD3+CD45+ T cell subsets (*upper panel*) and % SLAMF4-expressing CD3+CD45+ T cell subsets (*lower panels*).



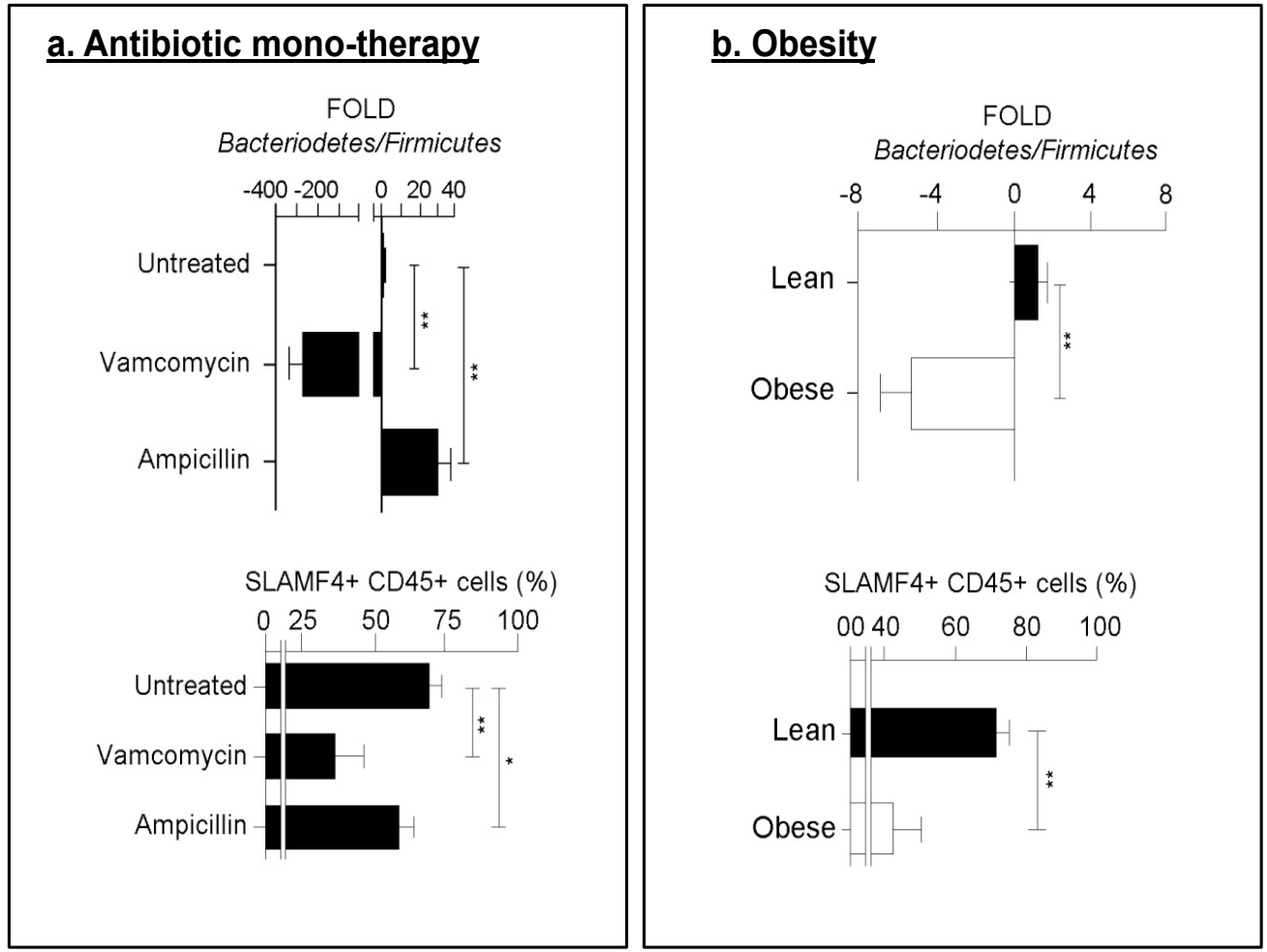
Supplemental Figure 3. SLAMF4 expression on T lymphocytes in the lamina propria. SLAMF4 is a expressed by different T lymphocyte subsets in the lamina propria of the duodenum, jejunum, and Ileum (which constitute the small intestine) as well as cecum, and colon. Dot plots are representative of *n*=4. Numbers indicate the % CD3+CD45+cell subsets (*upper panel*) and % SLAMF4-expressing CD3+CD45+ T cell subsets (*lower panels*).



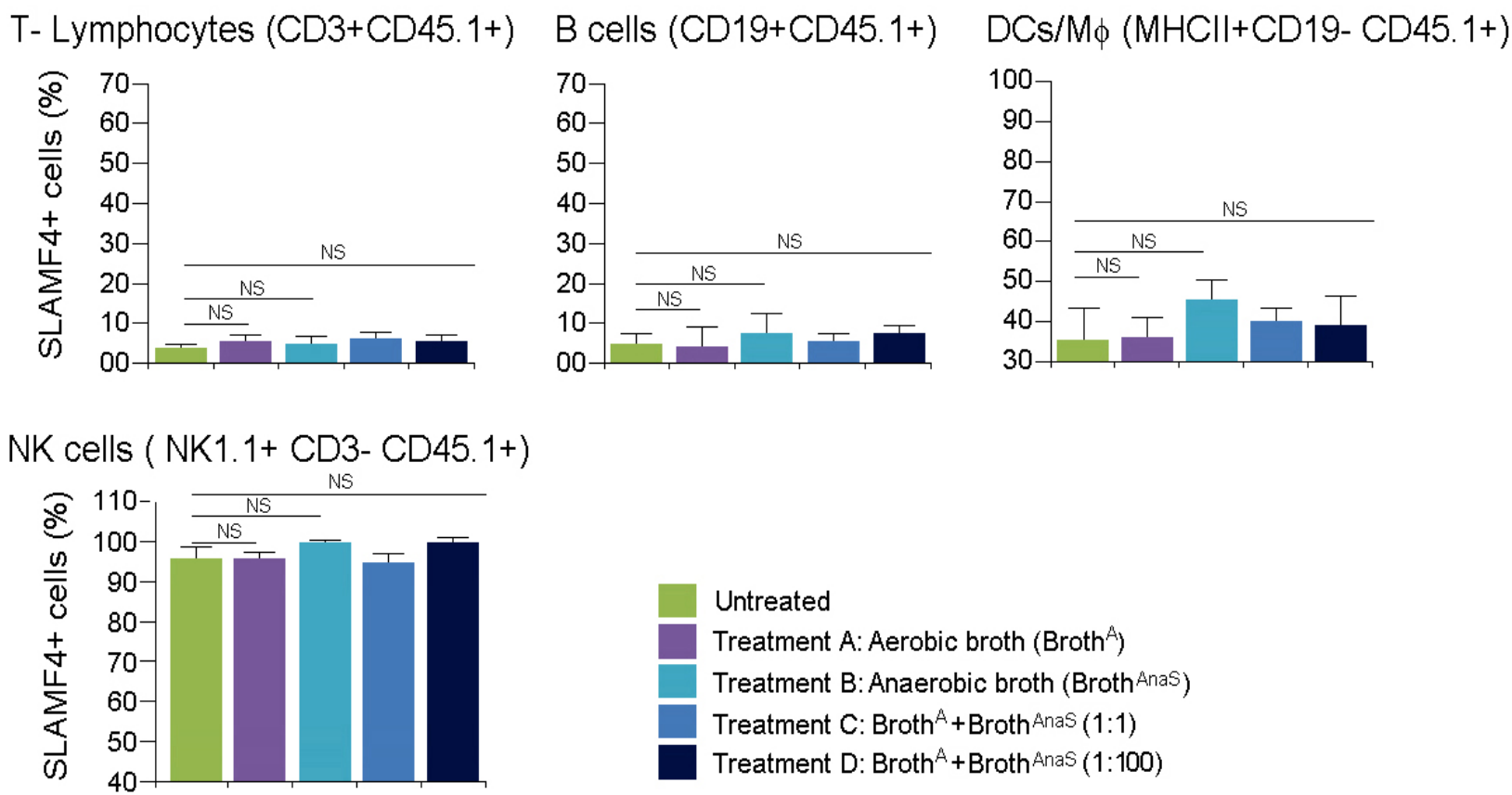
Supplemental Figure 4. SLAMF4 expression by total CD3+ cells in human intestinal mucosa. Human small intestine and colon samples were obtained, IEL and LP fractions prepared, and stained for flow cytometry with anti-human SLAMF4, anti-CD45, and anti-CD3 antibodies. Data indicate SLAMF4 expression as % of CD45+ CD3+ cells and relationship between IEL and LP for each tissue sample. (*n*=4 small intestine, *n*=3 colon, ***P* < 0.005, ****P* < 0.0005 using student's two-tailed t test.



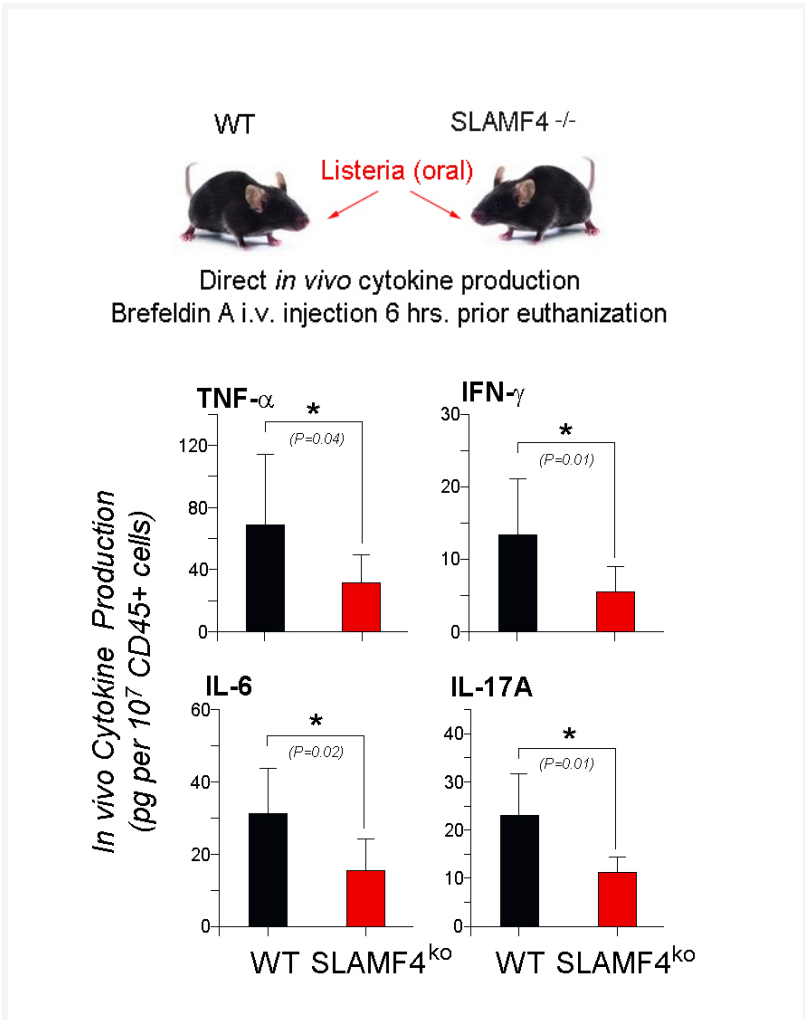
Supplemental Figure 5. (a) Natural CD8 T cells (CD3+CD8α+CD8β-CD4-) express SLAMF4 in germ-free (GF) BALB/c mice. In GF mice, SLAMF4 expression on natural CD8αα T cells is similar to their counterparts in conventional (Cv) and conventionalized (CvZ) mice. Data are shown as means of % natural CD8αα T cells that are SLAMF4+ ± SD. Error bars represent SD. Data were generated from 3 experiments using 2 to 3 mice per experiment. **(b)** Mice were left untreated (-) or treated with a combination of 4 antibiotics (ATB; ampicillin, metronidazole, neomycin, and vancomycin). Bar graphs show % intestinal CD3+ CD45+ cells that remain SLAMF4+ after ATB treatment. Data are from *n*=4. A 2-tailed Student's *t* test distribution with paired groups was evaluated for statistical significance. $P > .05$ was considered not significant (NS), *$P < .05$ was considered significant, and **$P < .005$.

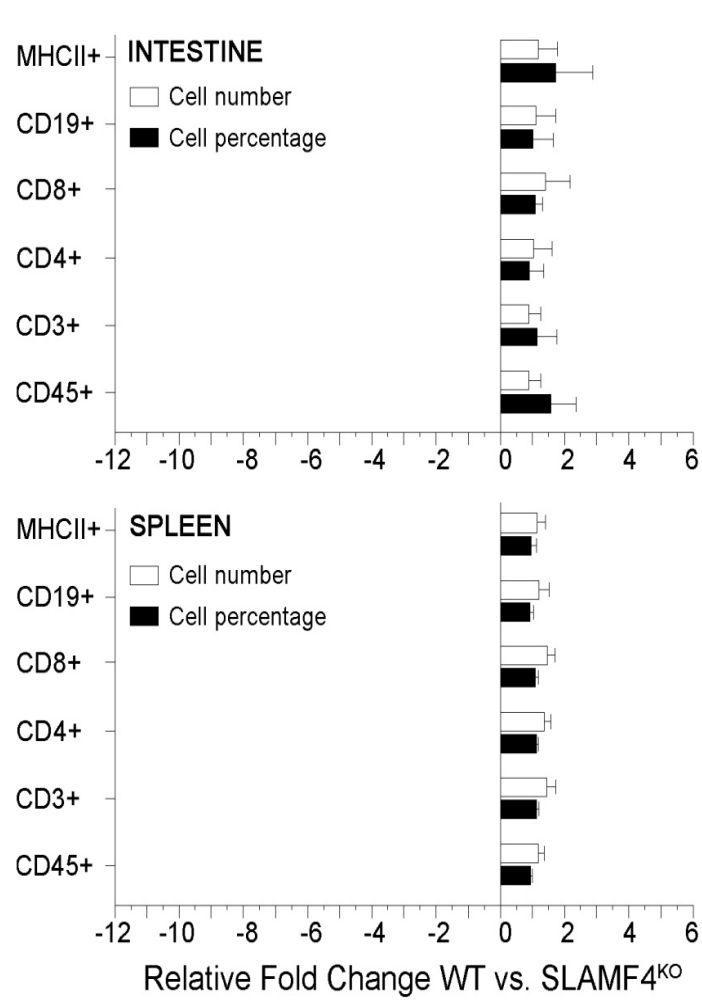


Supplemental Figure 6. Subtle changes of the gut microbiota affect SLAMF4 expression in the intestine. (a) B6 mice were left untreated or treated with ampicillin (1 mg/mL) or vancomycin (0.5 mg/mL) in the drinking water. Four weeks later, fecal DNA was made from intestinal contents and analyzed for the proportions of *Bacteroidetes* and *Firmicutes* by qPCR. *Below*, gut mucosal cell preparations were made, stained for SLAMF4 and CD45, and analyzed by flow cytometry. Data are shown as means of % CD45+ intestinal cells that remain SLAMF4+. **(b)** Bar graph shows shifts in bacterial populations (fold *Bacteroidetes*/*Firmicutes*) in the gut of obese (DIO) mice compared to lean (control) animals. *Below*, pooled IELs and LPs were analyzed by flow cytometry. Data are shown as means of % gut CD45+ cells that are SLAMF4+. Data are from n=5 (a) and n=9 (b) per animal group. Data are shown as means ± s.e.m. Error bars represent s.e.m. A 2-tailed Student's *t* test distribution with paired groups was evaluated for statistical significance. **P* < 0.05 was considered significant, ***P* < 0.005.



Supplemental Figure 7. Stimulation of splenocytes with control bacterial broths. BHI broth (Aerobic, Broth^{A}) and OxyRase^{TM} AnaSelect supplemented-BHI broth (anaerobic, Broth^{AnaS}) were used to stimulate CD45.1 B6 splenocytes. Eighteen hours later, immune cell types such as T lymphocytes (CD3+), NK cells (NKp46+), B cells (CD19+), and other APCs (CD19-MHCII+) were examined for the expression of SLAMF4 by flow cytometry. For this, propidium iodide (PI) plot of total CD45+ cells was used to select PI^{-} events which represent viable CD45 cells. Overall cell viability was >75%. Data are shown as means of % of each cell type expressing SLAMF4 ± SD. Error bars represent SD. Data were generated from *n*=6. A 2-tailed Student's *t* test distribution with paired groups was evaluated for statistical significance. P > 0.05 was considered not significant (NS).





Supplemental Figure 8. Characterization of SLAMF4^{KO} mice. (a) Direct *in vivo* cytokine production in the gut mucosa following Lm infection was assessed as descibed in [53]. Briefly, Brefeldin-A (250 µg) was injected *i.v.* into mice 4.5 days post Lm-infection, and 6 hours later, mice were euthanized. Single CD45+ cell suspensions isolated from pooled IEL and LP cells were homogenized. The supernatants were used to assess cytokine production using BD multiplex bead-based immunoassays (BD Bioscience). Data are shown as means of pgs of produced cytokine per 10^7 gut CD45+ cells ± SD. Error bars represent SD. Data are from 2 experiment using 4 mice per group per experiment. **(b)** Immune cell composition was assessed by flow cytometry using age- and sex-matched groups of cohoused *SLAMF4^{ko}* mice and WT littermates. Splenocytes and gut mucosal cell preparations (IEL+LP) were made from each strain, stained for CD45, CD3, CD4, CD8, CD19, and MHC-II and analyzed by flow cytometry. Data are shown as means of % and total number of each cell type ± SD. Error bars represent SD. Data are from 3 experiments using 2 to 4 mice per group per experiment. A 2-tailed Student's *t* test distribution with paired groups (WT vs. SLAMF4^{ko}) was evaluated for statistical significance. *$P < 0.05$ was considered significant.