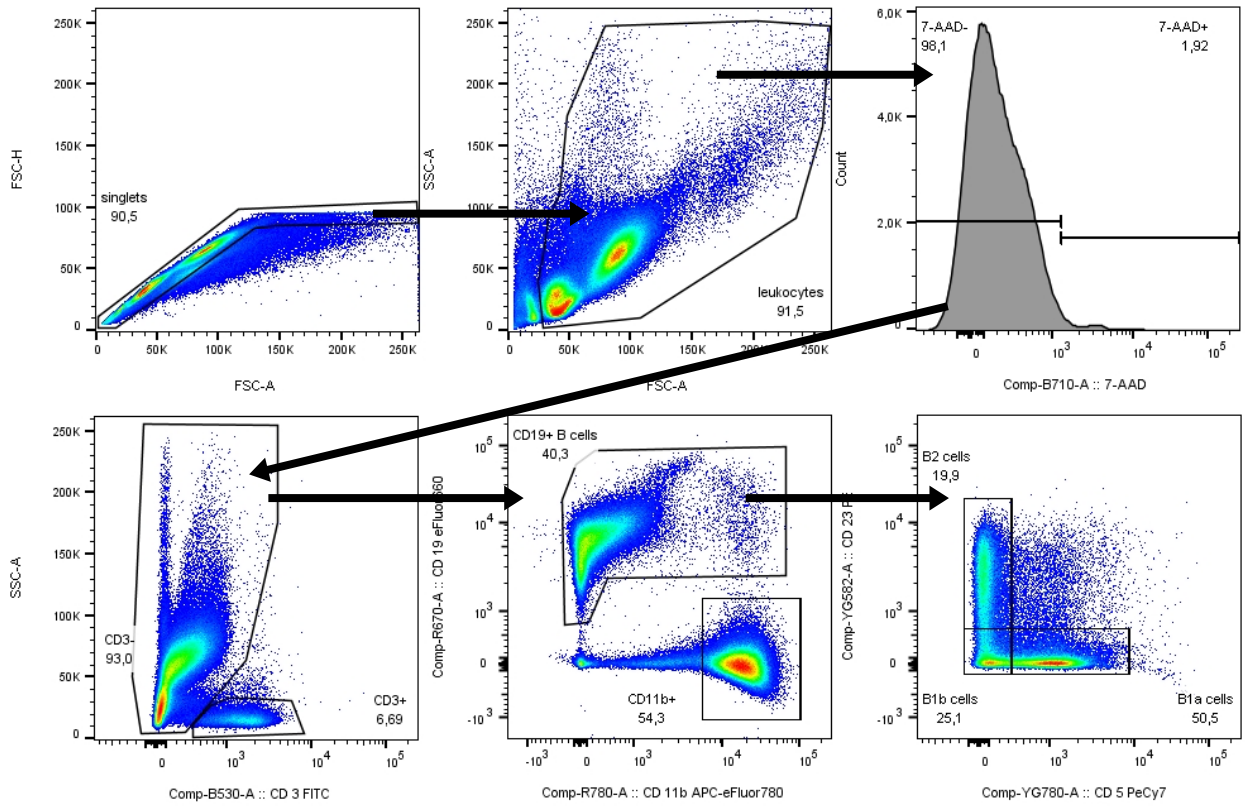
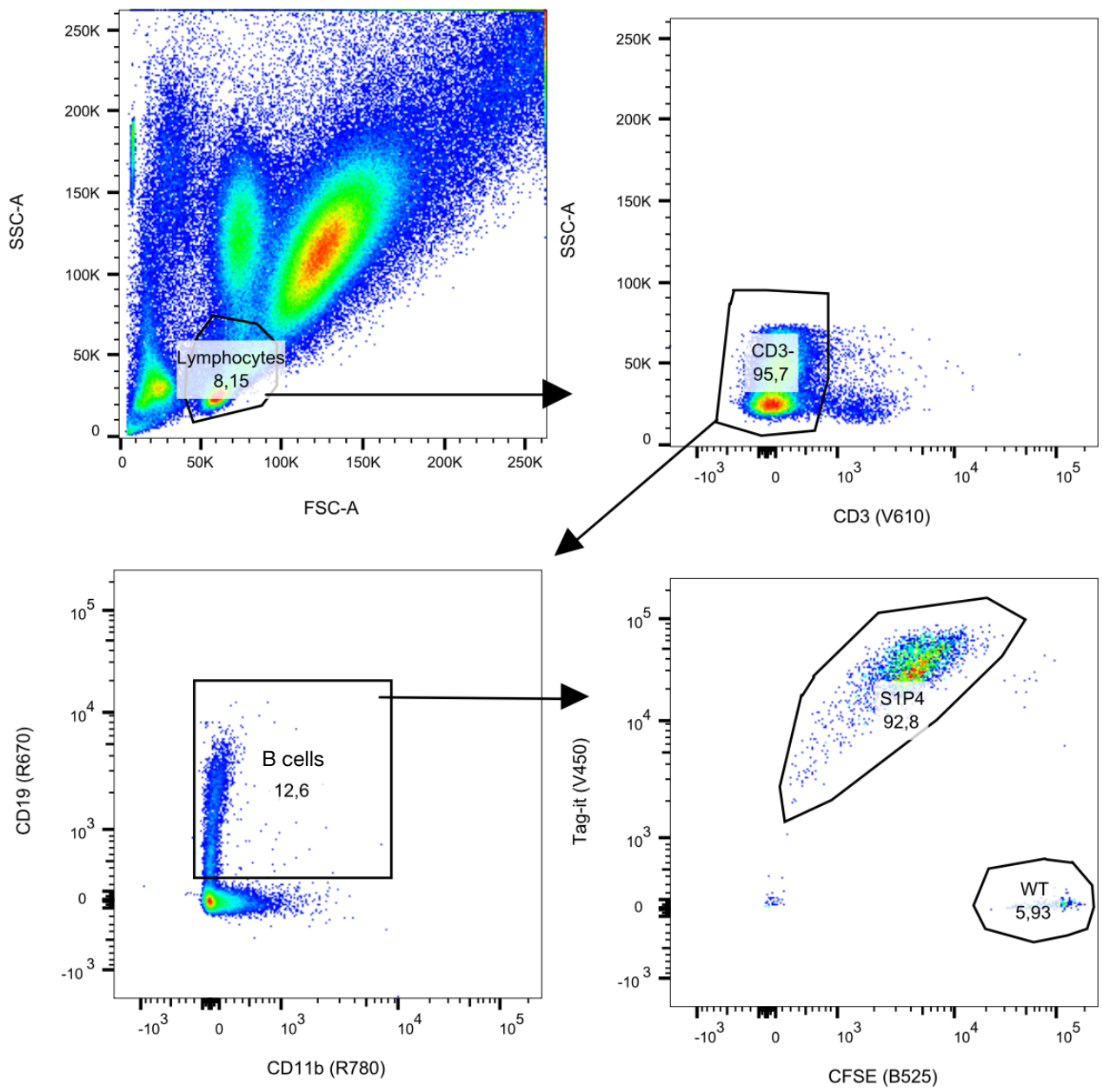
Supplemental material: Impact of $S1PR_4$-signalling on peritoneal B cell trafficking

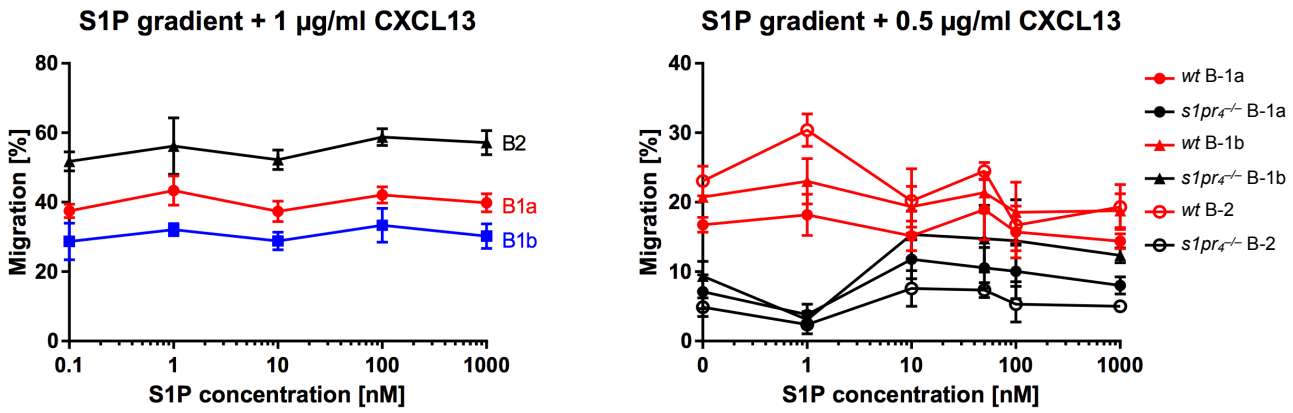
Content:



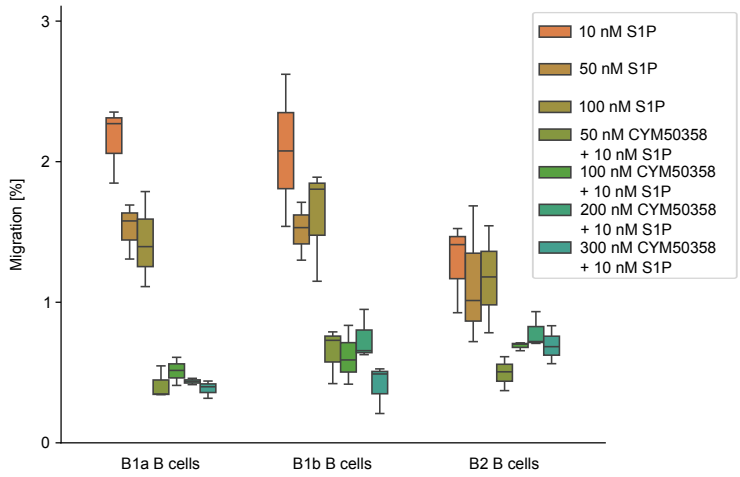
Supplemental Figure S1: Gating strategy of peritoneal B cell subpopulations. Populations were classified as follows: B cells CD3$^-$ CD19$^+$; B-1a cells CD3$^-$ CD19$^+$ CD5$^+$ CD23$^-$; B-1b cells CD3$^-$ CD19$^+$ CD5$^-$ CD23$^-$; B-2 cells CD3$^-$ CD19$^+$ CD5$^-$ CD23$^+$.



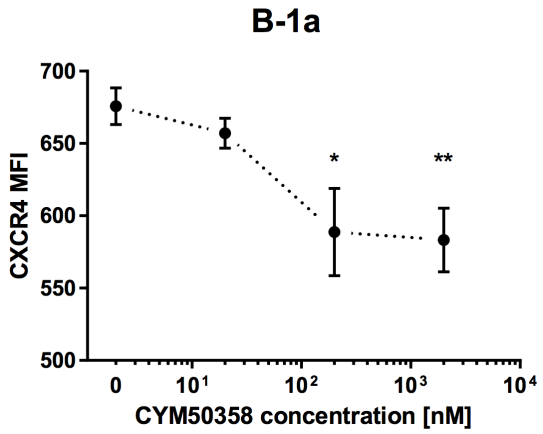
Supplemental Figure S2: Exemplary gating strategy of labeled peritoneal cells. Peritoneal cells were labeled with CFSE or Tag-it® and transferred to *scid* mice. Peritoneal lavage fluid of recipients was analyzed by flow cytometry. After identification of peritoneal subpopulations by the same antibodies as above, each subpopulation was gated for Tag-it® (V450) against CFSE (B525) to differentiate the genotypes.



Supplemental Figure S3: Migration of peritoneal B cells toward gradients of S1P combined with high CXCL13 concentrations. (A): At high concentrations of 1 µg/ml CXCL13 no effect of the bottom S1P concentrations can be detected in transwell migration assays (*n=6*). **(B)**: At a concentration of 500 ng/ml CXCL13, no clear concentration dependent peak can be observed across the S1P gradient (*n=6*) between wildtype (*wt*) and S1PR$_4$-deficient (*s1pr4*$^{-/-}$) cells.



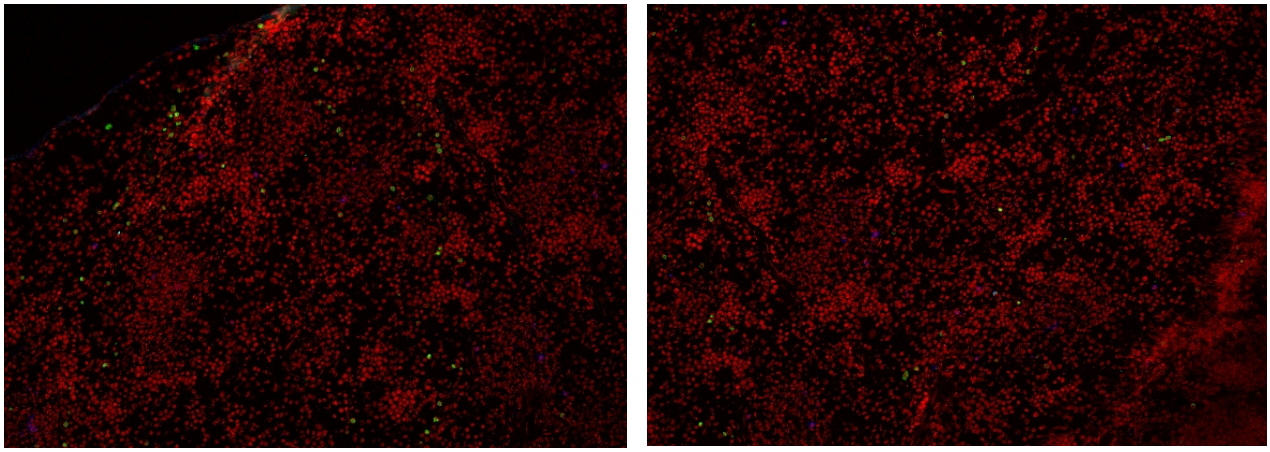
Supplemental Figure S4: Migration of peritoneal B cells towards S1P in presence of the S1PR$_4$-specific antagonist CYM50358. Transwell migration assay results of n = 3 biological replicates from wildtype mice toward various concentrations on S1P and CYM50358 in the lower chamber.



Supplemental Figure S5: Concentration dependent effect of S1PR$_4$-specific antagonist CYM50358 on CXCR4 expression. CXCR4 expression was quantified using flow cytometry of n = 5 biological replicates from wildtype mice after incubation with various concentrations of CYM50358 in the presence of S1P. Significance was calculated using paired Student's t-test.

wt - CFSE | *s1pr4*$^{-/-}$ - Tag-it®

wt - Tag-it® | *s1pr4*$^{-/-}$ - CFSE



Supplemental Figure S6: Analysis of splenic sections 48 hours after adoptive transfer. Peritoneal cells of *wt* and *s1pr4*$^{-/-}$ mice were labelled with CFSE (FITC channel) or Tag-it® (DAPI channel) and transferred into the peritoneal cavity of *scid* mice. Forty-eight hours after transfer, spleens of recipient mice were harvested and analyzed. Images show nuclei stained with Draq5 (red), transferred Tag-it® labelled cells (blue) and transferred CFSE-labelled cells (green). Only loose accumulations of cells can be seen. No compact follicular aggregates were found. Images representative for at least n = 3 animals per genotype.