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Supporting Information

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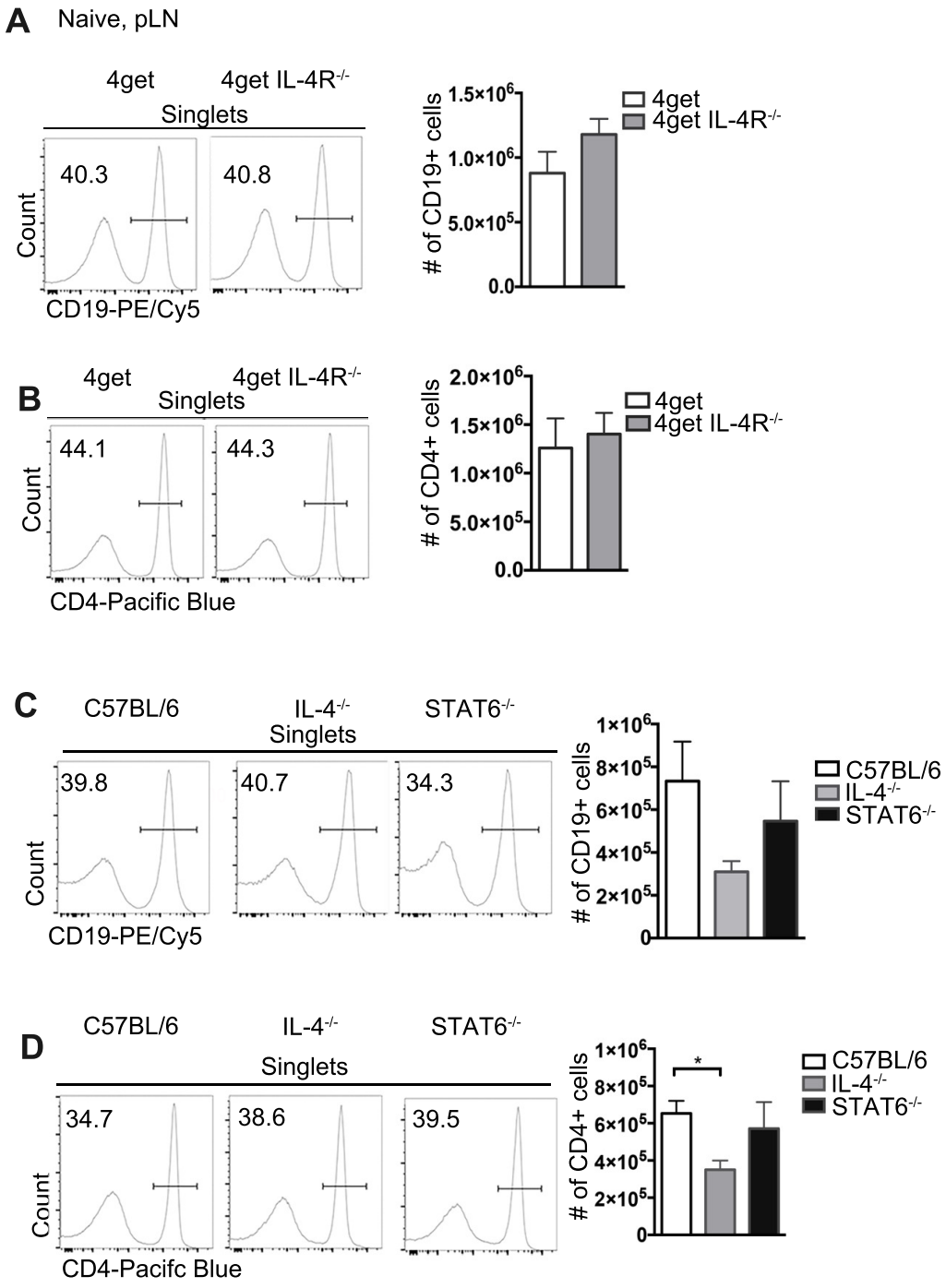
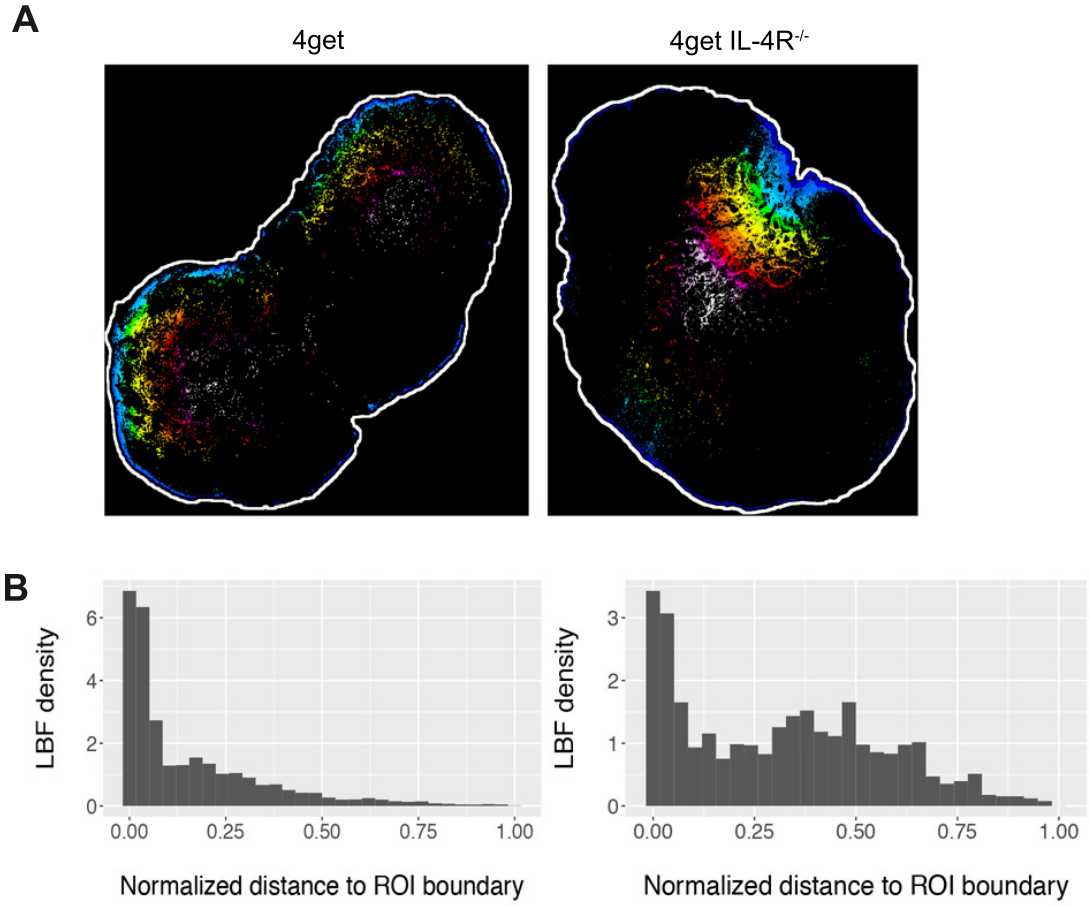
IL-4 promotes stromal cell expansion and is critical for development of a type-2, but not a type 1 immune response

Supplementary Figure 1



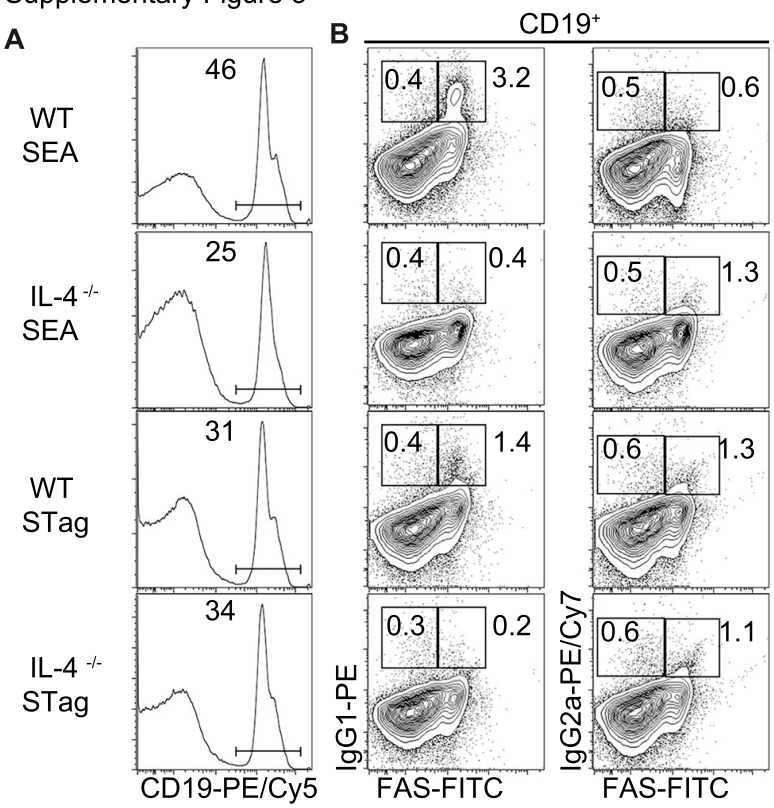
Supplementary Figure 1. Lack of IL-4Rα does not affect T and B cell numbers or high endothelial venules in naive peripheral lymph nodes. (A) Frequency and number of B cells by flow cytometry in WT (4get) and 4get IL-4RKO. (B) Frequency and number of T cells (C) CD19+ expression and B cell numbers in WT, IL-4KO and STAT6KO. (D) Frequency and number of CD4+ T cells in naive popliteal lymph node of WT, IL-4KO and STAT6KO. (E) Tile confocal imaging of naive pLN stained with B220 (red), CD4(green) and PNAd high endothelial venules (HEV)(gray). Scale bar: 200 µm. One representative image from three independent experiments is shown. FACS data depicts concatenated samples from >3 mice per group from four independent experiments. Bars show mean ±SEM from four independent experiments.Student t-test was used to determine statistical significance, *$p < 0.05$.

Supplementary Figure 2



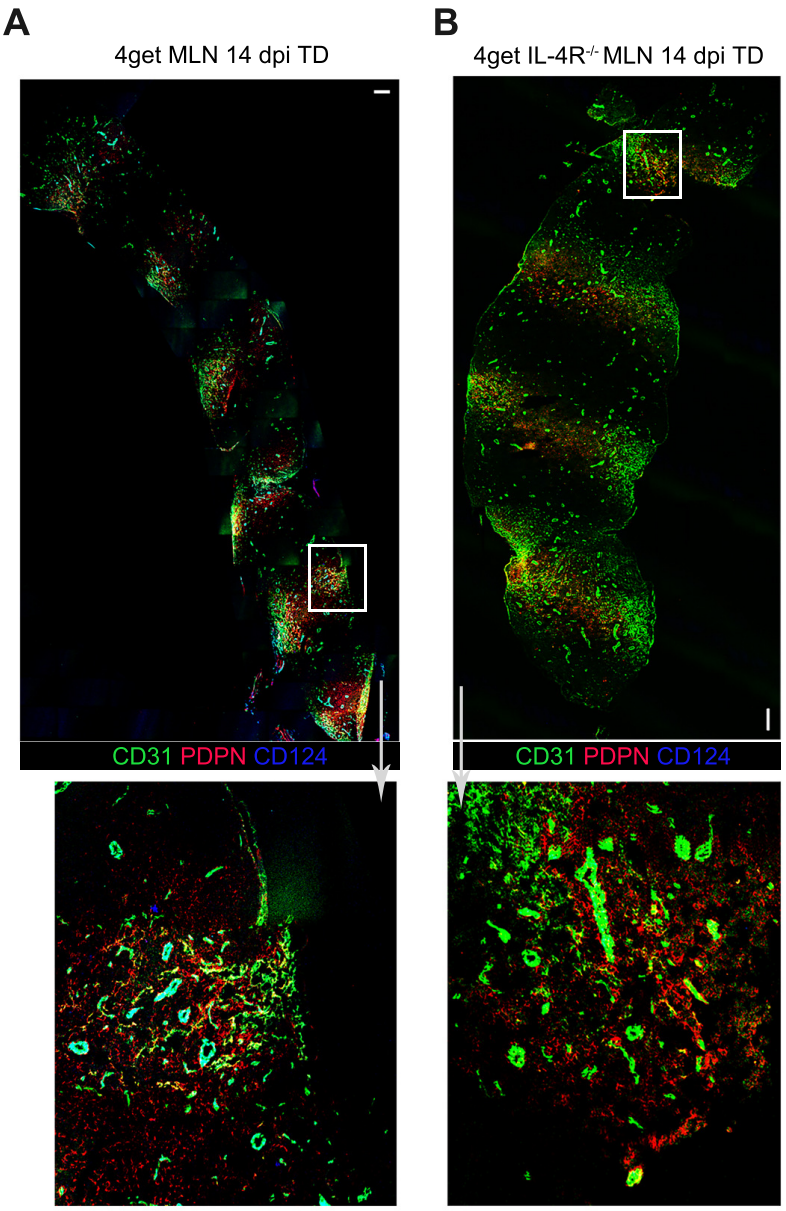
Supplementary Figure 2. Visualization of differences in spatial distribution of co-stained podoplanin/CD31 locally bright features (LBF). The LBF color encodes the distance to the Region of Interest (ROI) boundary (white). (A)The LBFs in the lymph node located close to the perimeter of the ROI (shown as blue, cyan and green) and the proportion of LBF which are away from the ROI boundary (encoded in yellow, red and magenta) is shown. (B) Quantification of the LBF densitiy to the normalized distance to the ROI boundary. Image is representative of >3 mice from two independent experiments.

Supplementary Figure 3



Supplementary Figure 3. In absence of IL-4, Type 2, but not Type 1 antigens, fail to induce a humoral response. C57BL/6 and IL-4KO of 6-8 weeks of age were immunized with 30µg of *S. mansoni* antigens or *T. gondii* antigens. 8 days post-immunization, draining popliteal lymph nodes were collected to obtain single cell suspensions and analyzed by flow cytometry.(A) Expression of CD19+ cells (B cells) gated from lymphocytes, (B) Isotype-switched IgG1 (FAS+IgG1+ GC B cells or Fas−IgG1+ memory B cells) and IgG2a (GC B cells or memory) from B cells.Plots represent concatenated samples from five independent experiments and >3 mice per group.

Supplementary Figure 4



Supplementary Figure 4: Mesenteric lymph node organization following immunization with alum-adjuvanted Tenatus/Diphteria. Mesenteric lymph nodes from 4get (control) and 4get IL-4Ralpha deficient mice were dissected, collected in OCT as described in the Material and Methods, and frozen. Tile confocal images from 14 days post immunization were obtained and imaged with a Leica TCS SP5 laser scanning confocal microscope (A) Frozen mesenteric lymph nodes from wild type (4get) mice were stained for CD31 (green), podoplanin (PDPN, red) and CD124 (blue). Scale bar: 200 µm.(B) Frozen MLN section from 4get IL-4R alpha stained with CD31(green),PDPN (red) and CD124 (blue) Scale bar: 200 µm. Images are representative of three experiments with three mice per group.