Sustained signaling by canonical T helper cytokines throughout the reactive lymph node

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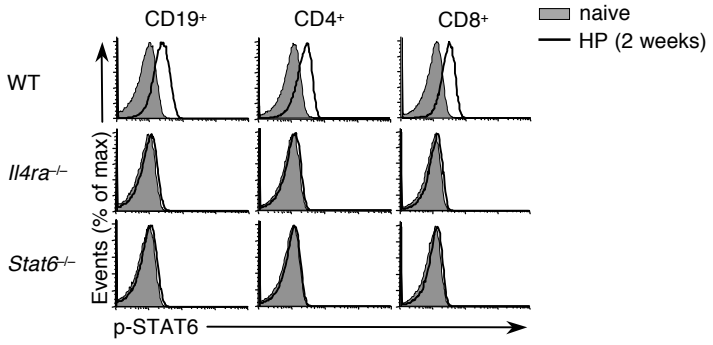
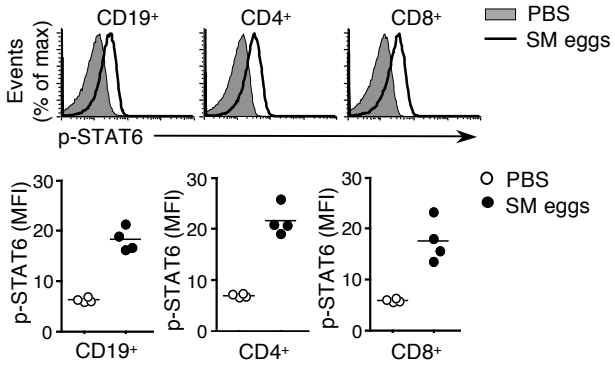
Supplementary figures S1 - S9

Figure S1



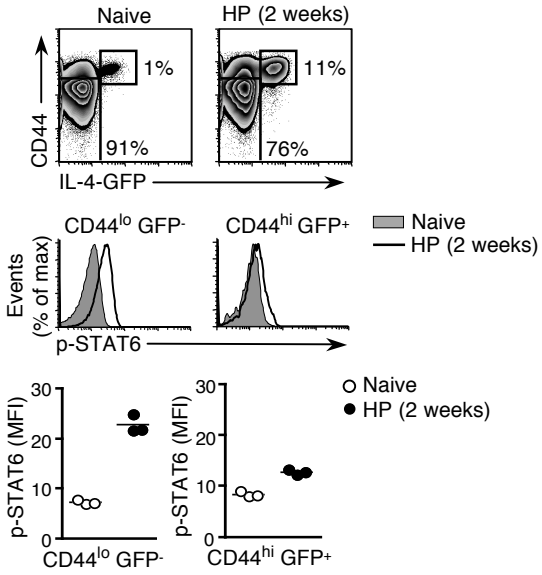
Supplementary Figure 1. Absence of STAT6 phosphorylation in naïve mice. Wild-type, *Il4ra*$^{-/-}$ and *Stat6*$^{-/-}$ mice were infected or not with *H. polygyrus* (HP) and, 2 weeks later, STAT6 phosphorylation in lymphocyte subsets of the mesLN was assessed by flow cytometry. Data are representative of four separate experiments using 3-5 mice per group.

Figure S2



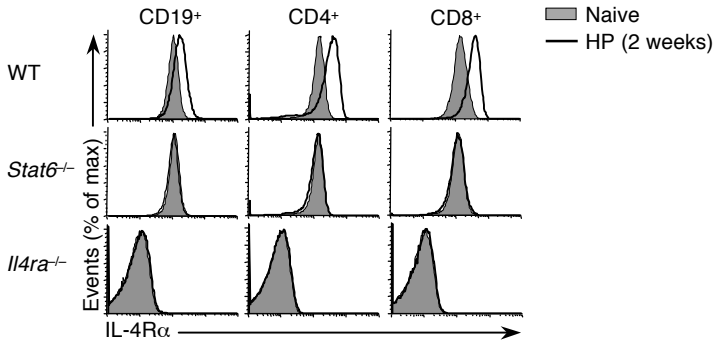
Supplementary Figure 2. Ubiquitous STAT6 phosphorylation in the reactive lymph node is elicited in response to *Schistosoma mansoni*. 4get mice were injected subcutaneously into the footpad with *S. mansoni* eggs (SM eggs) or PBS. 7 days later, draining popliteal lymph nodes were harvested and STAT6 phosphorylation was assessed by flow cytometry. Data shown are gated on GFP$^-$ cells and each data point represents an individual mouse. Data are representative of 4 mice per group in two independent experiments.

Figure S3



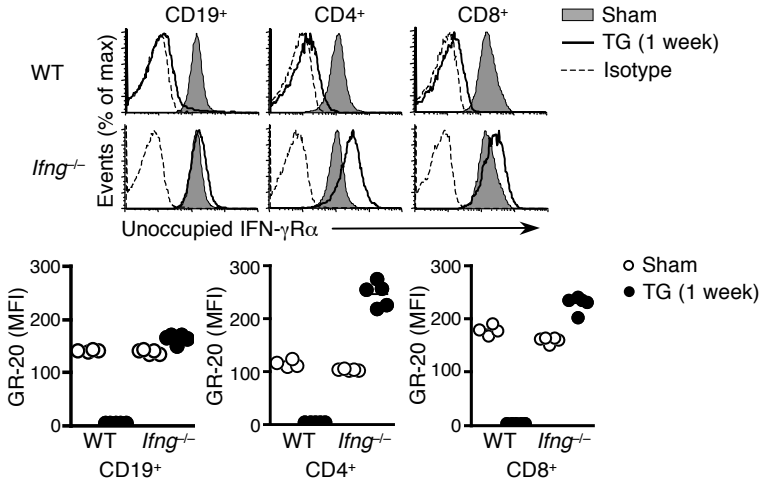
Supplementary Figure 3. STAT6 phosphorylation in bystander CD4$^+$ cells in the Th2 reactive lymph node. 4get mice were infected with *H. polygyrus* and mesLN cells harvested 2 weeks later. STAT6 phosphorylation was assessed by flow cytometry. Data shown are gated on CD4$^+$ cells and each data point represents an individual mouse. Data are representative of five independent experiments using 3-4 mice per group.

Figure S4



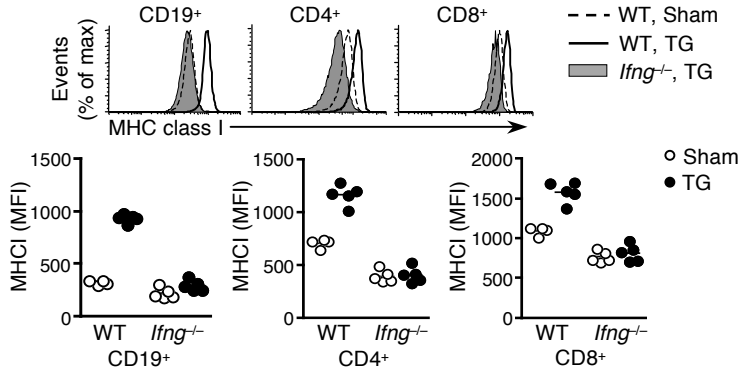
Supplementary Figure 4. IL-4Rα upregulation is STAT6 dependent. WT, *Stat6*$^{-/-}$ and *Il4ra*$^{-/-}$ mice were infected or not with *H. polygyrus* and, 2 weeks later, IL-4Rα expression on lymphocyte subsets of the mesLN was measured by flow cytometry. Data are representative of four separate experiments with 3-5 mice per group.

Figure S5



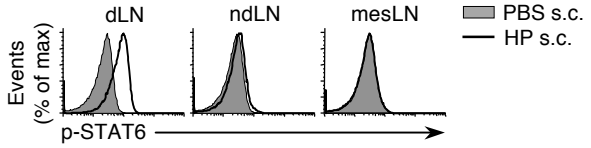
Supplementary Figure 5. In the absence of IFN-γ, GR-20 staining is not reduced by *T. gondii* infection. Lymphocytes from mesLN of *T. gondii*- or sham-infected wild-type and *Ifng*$^{-/-}$ mice were stained with the GR-20 mAb, whose binding to the IFN-γ receptor α chain is blocked by receptor ligation of IFN-γ. Data shown is gated on the indicated lymphocyte subsets. An isotype control antibody was used as a negative staining control. Each data point represents an individual mouse. Data are representative of two independent experiments with 4-5 mice per group.

Figure S6



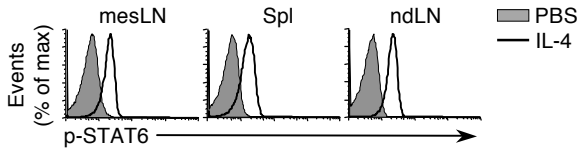
Supplementary Figure 6. MHC class I upregulation in a Th1 reactive lymph node is IFN-γ dependent. MHC class I (H-2K^b) expression on lymphocyte subsets of WT and *Ifng*$^{-/-}$ mesLN, 1 week after *T. gondii*- or sham- infection. Graphed data depict the geometric mean fluorescence intensity (MFI) and each data point represents an individual mouse. Data are representative of four separate experiments using 4-5 mice per group.

Figure S7



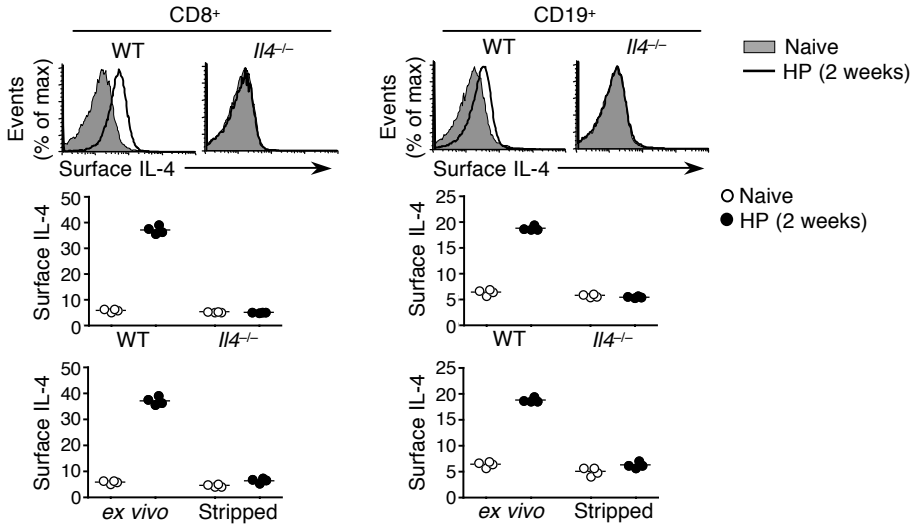
Supplementary Figure 7. STAT6 can be phosphorylated in a Th2 reactive lymph node distinct from the mesLN. 4get mice were immunized subcutaneously into the footpad with *H. polygyrus* larvae or PBS. 7 days later, the draining popliteal lymph nodes (dLN), the non-draining contralateral popliteal lymph nodes (ndLN) and mesenteric lymph nodes (mesLN) were harvested and STAT6 phosphorylation was assessed by flow cytometry. Data shown are gated on $CD4^+$ GFP^- cells and are representative of 3 mice per group in each of three independent experiments.

Figure S8



Supplementary Figure 8. Exogenous IL-4 triggers systemic STAT6 phosphorylation in naïve mice. Naïve 4get mice were injected intravenously with either recombinant murine IL-4 or a PBS carrier control and, 1 hour later, the indicated tissues harvested and assessed for STAT6 phosphorylation by flow cytometry. Data shown are gated on $CD4^+$ GFP^- cells and are representative of two independent experiments.

Figure S9



Supplementary Figure 9. IL-4 binds to all CD8$^+$ and CD19$^+$ lymphocytes in the Th2 reactive lymph node. mesLN cells were harvested from naïve or *H. polygyrus* -infected wild-type 4get or *Il4*$^{-/-}$ mice. IL-4 bound to the cell surface was detected using an anti-IL-4 antibody (clone BVD6) and assessed by flow cytometry either immediately or following incubation with an IL-4 neutralizing antibody (clone 11B11) for 60min (Stripped). Data shown are gated on CD8$^+$ or CD19$^+$ cells, as indicated. Each data point represents an individual mouse and all data are representative of three separate experiments with 4 mice per group.