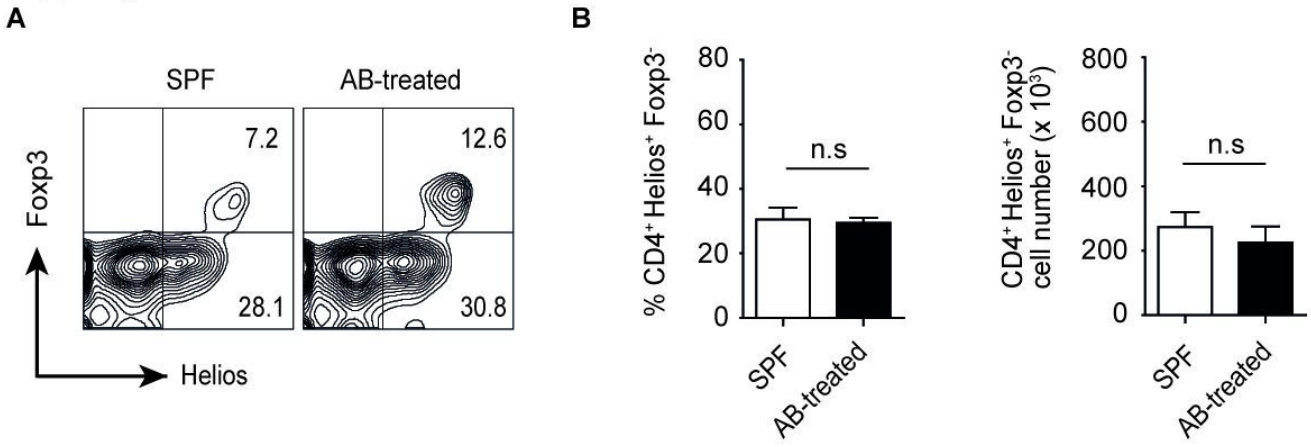
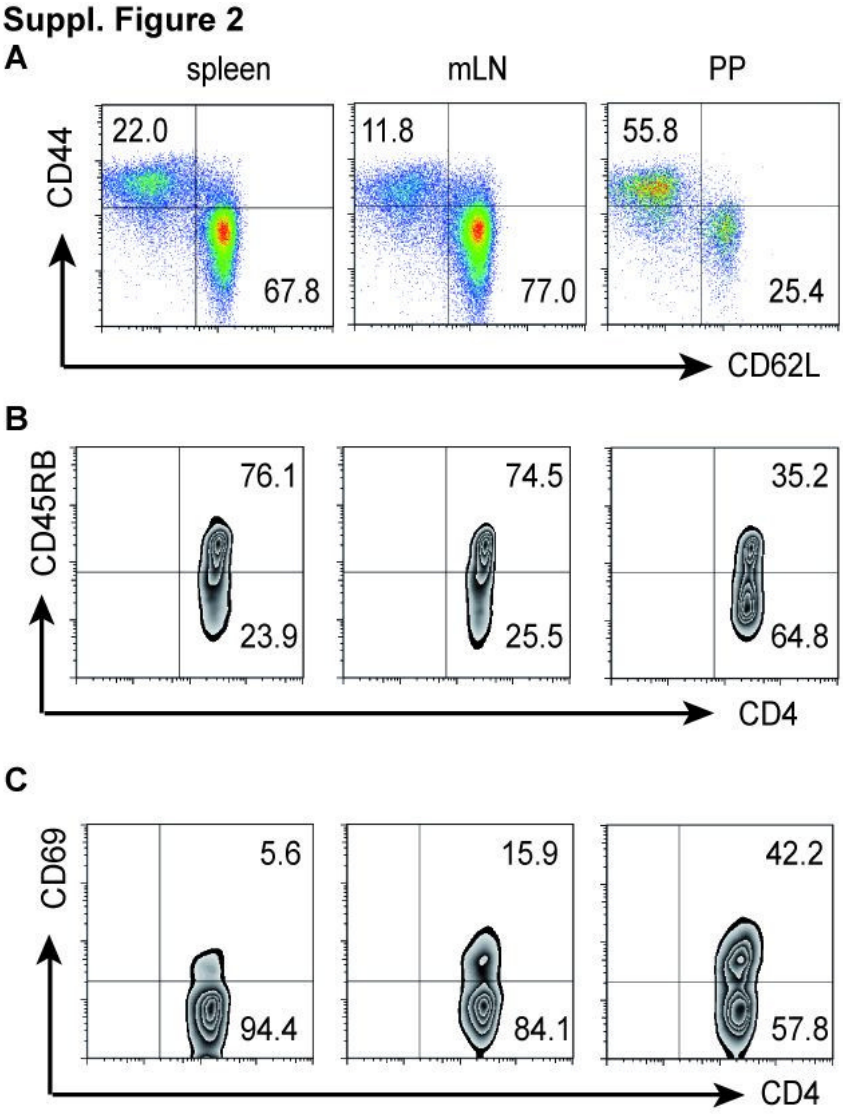
Suppl. Figure 1

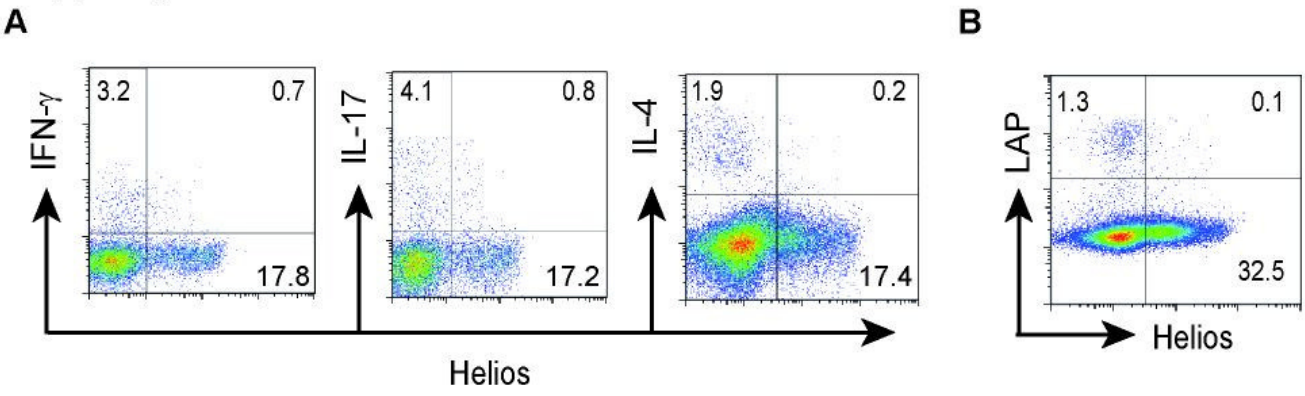


Supplementary Figure 1. Antibiotic treatment of adult mice has no impact on Helios$^+$Foxp3$^-$CD4$^+$ T cells in PP. (**A**) Comparison of percentages and (**B**) total cell numbers of PP Helios$^+$Foxp3$^-$CD4$^+$ T cells between SPF and ATB-treated mice fed ConvD. Effects of ATB treatment for 8 weeks on PP Helios$^+$Foxp3$^-$CD4$^+$ T cells were examined by FACS analysis. Two independent experiments were performed (n=6 mice/group). Data are mean ± SD. n.s. = not significant.

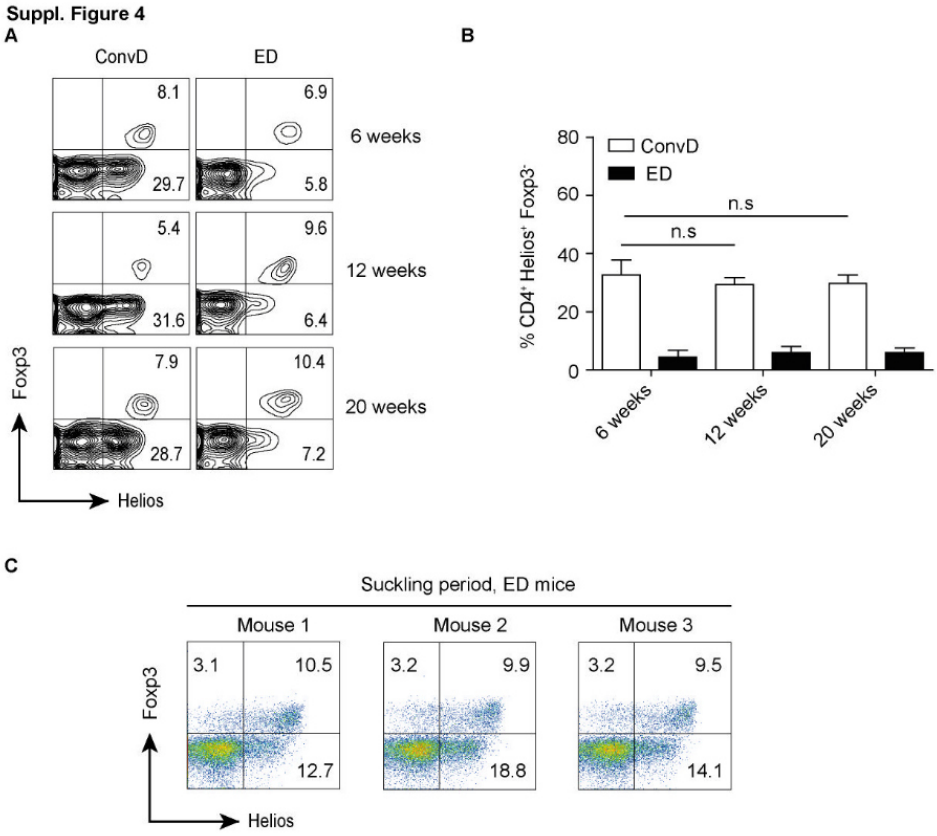


Supplementary Figure 2. Activation signature of CD4$^+$ T cells from spleen, mLN and PP of SPF mice. (**A, B** and **C**) Cell suspensions from spleen, mLN and PP of SPF mice were stained for CD4, CD44, CD62L, CD45RB and CD69, respectively. Cells were gated on CD4 and analyzed by FACS analysis. Representative staining from two independent experiments is shown.

Suppl. Figure 3

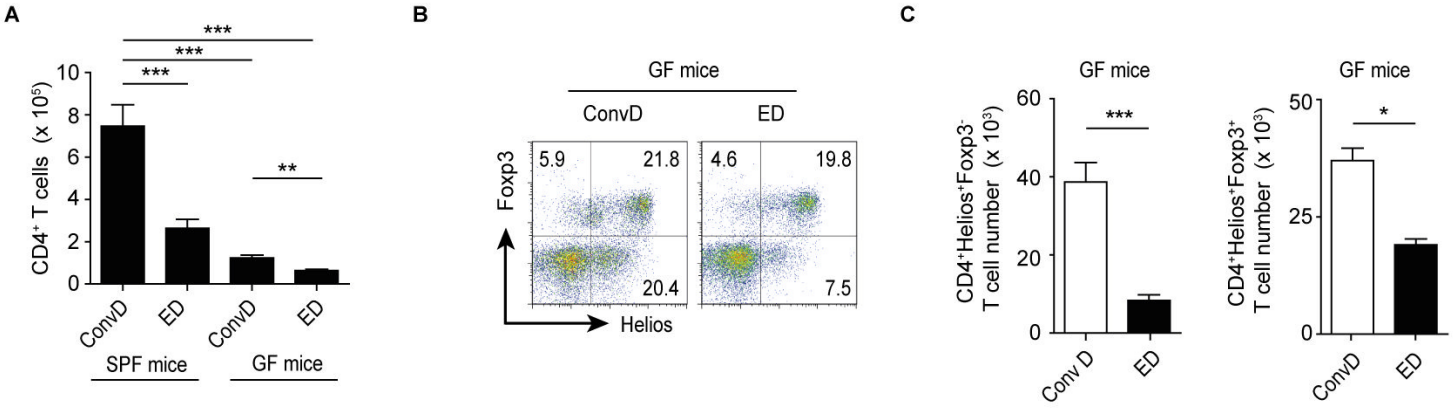


Supplementary Figure 3. Cytokine production by Helios^{+}CD4^{+} T cells in PP. (**A**) Percentages of IFN-γ-, IL-17- and IL-4-producing cells within Helios^{+} and Helios^{-}CD4^{+} T cells from PP after restimulation for 4hs with PMA/Ionomycin in the presence of Brefeldin A. Representative dot plots from three similar experiments are shown. (**B**) FACS analysis of expression of LAP and Helios in PP T cells (gated on CD4^{+}). Representative dot plot from two experiments is shown.

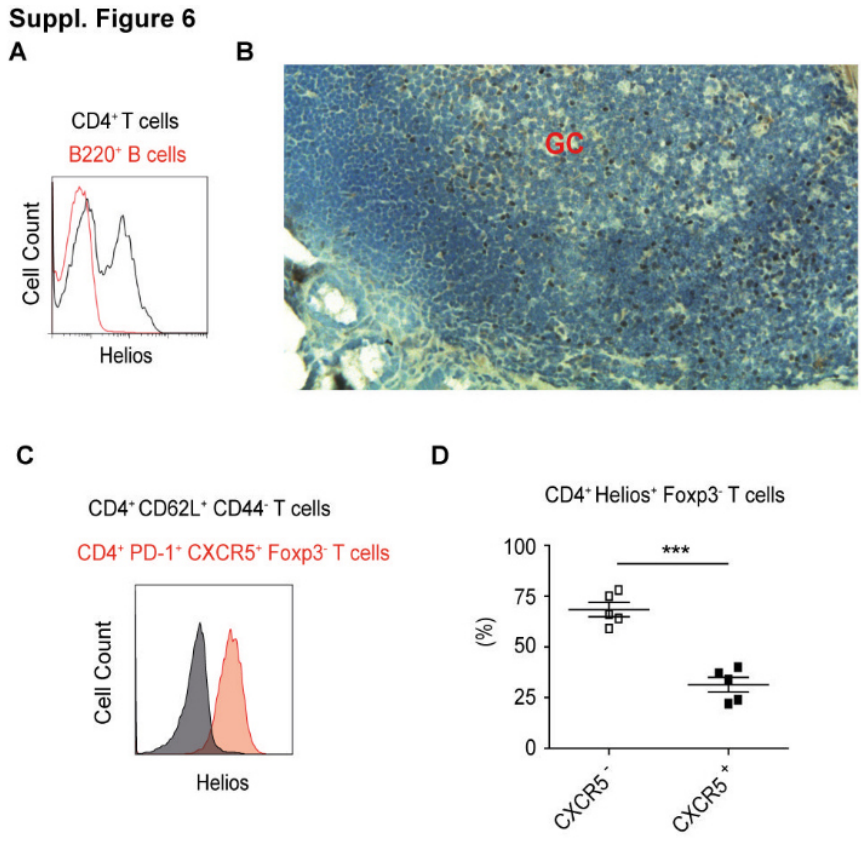


Supplementary Figure 4. Helios^{+}Foxp3^{-}CD4^{+} T cells in PP are aged independent. (**A** and **B**) Frequency of Helios^{+}Foxp3^{-}CD4^{+} T cells in WT mice fed ConvD or ED. 6-, 12-, and 20-week-old animals were analyzed by FACS (n=6 mice/group). Two independent experiments were performed. Error bars indicate mean ± SD. n.s. = not significant. (**C**) Frequency of Helios^{+}Foxp3^{-}CD4^{+} T cells of 25 day old pups prior to weaning from ED mothers.

Suppl. Figure 5

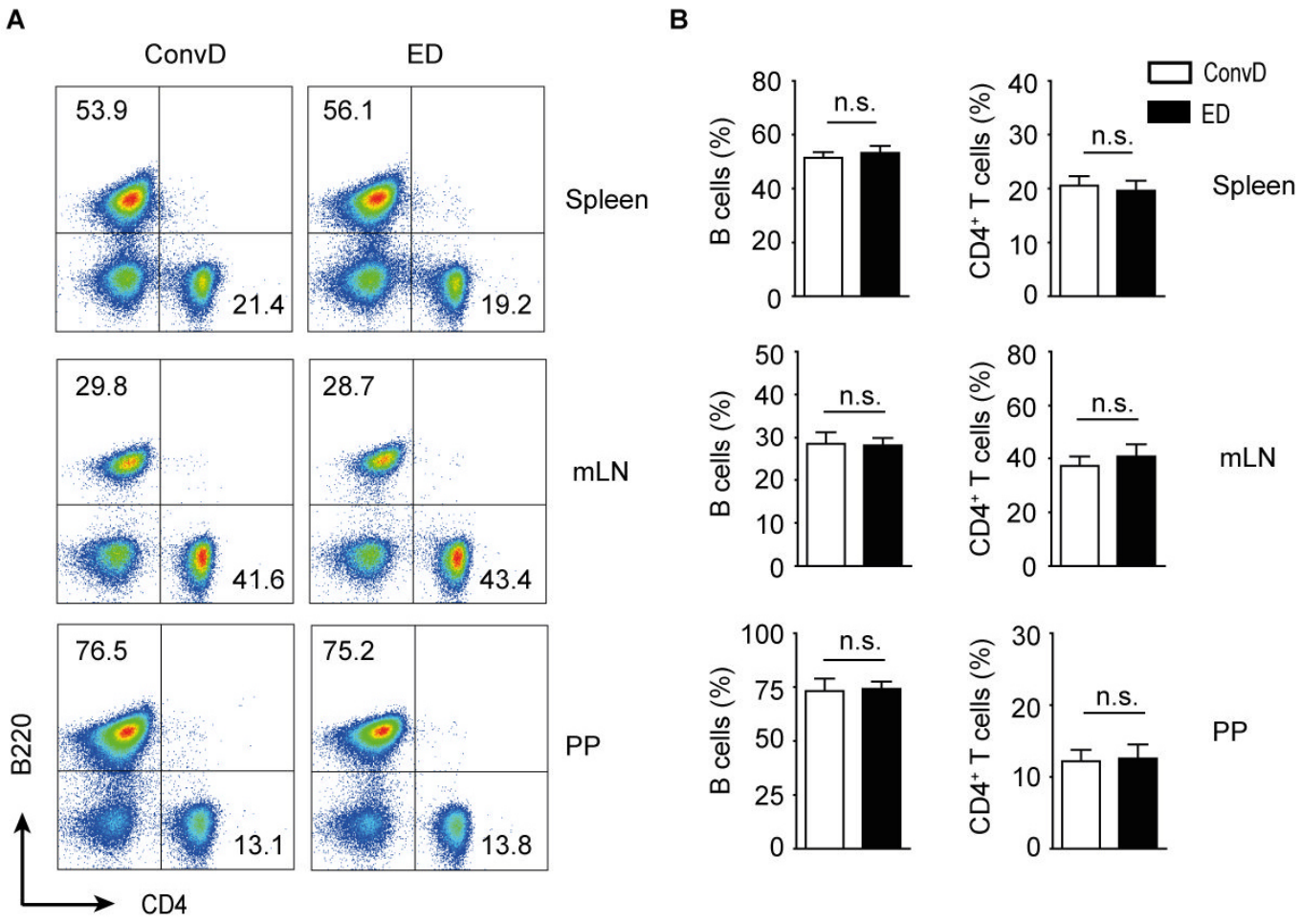


Supplementary Figure 5. Influence of microbiota and diet on CD4$^+$ T cells in PP. (**A**) Total numbers of CD4$^+$ T cells in PP of SPF or GF mice fed convD or ED. Results are expressed as mean ± SD of 5 mice per group. (**B**) Representative dot plots of Helios$^+$Foxp3$^-$ T cells in PP of GF mice with ConvD or ED. (**C**) Total cell numbers of Helios$^+$Foxp3$^-$CD4$^+$ T cells (left) and Tregs (right) in PP of ConvD or ED GF mice. Data are analyzed by student´s *t*-test, shown are means ± SD (n = 5 mice per group). *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.



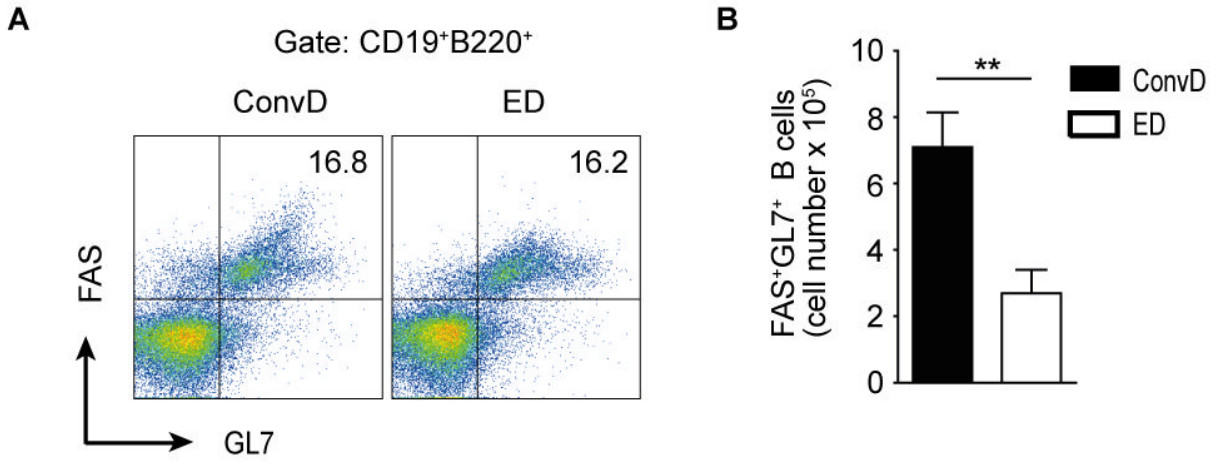
Supplementary Figure 6. Expression of Helios in PP cells of ConvD mice. (**A**) Helios expression in $B220^+$ B cells and $CD4^+$ T cells from the PP. (**B**) Immunohistochemical staining of Helios (black dots) in the PP. (**C**) Comparison of Helios expression in naïve $CD4^+$ (black) and Tfh cells (red) from PP. Data show representative staining from two independent experiments. (**D**) Percentage of CXCR5 positive and negative cells within the $CD4^+Helios^+Foxp3^-$ PP T lymphocytes (n=5), *** $P < 0.001$.

Suppl. Figure 7

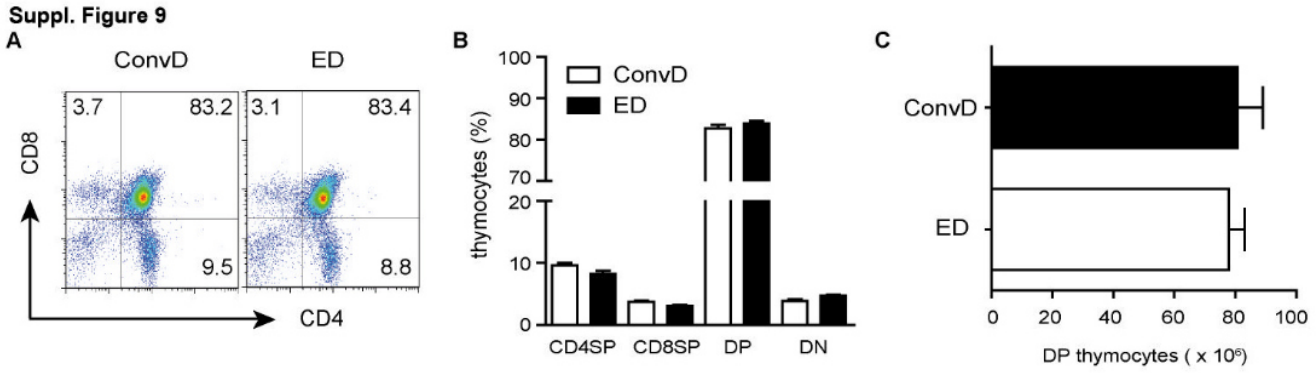


Supplementary Figure 7. Frequencies of T and B cells are not altered by dietary antigens. (**A**) Representative dot blot for $CD4^+$ and $B220^+$ staining (lymphocyte gate) in various organs of ConvD and ED mice. (**B**) Distribution of the frequencies of B and T cells from ConvD and ED mice, analyzed by student´s *t*-test (n=6 mice). Error bars indicate mean ± SD. n.s. = not significant.

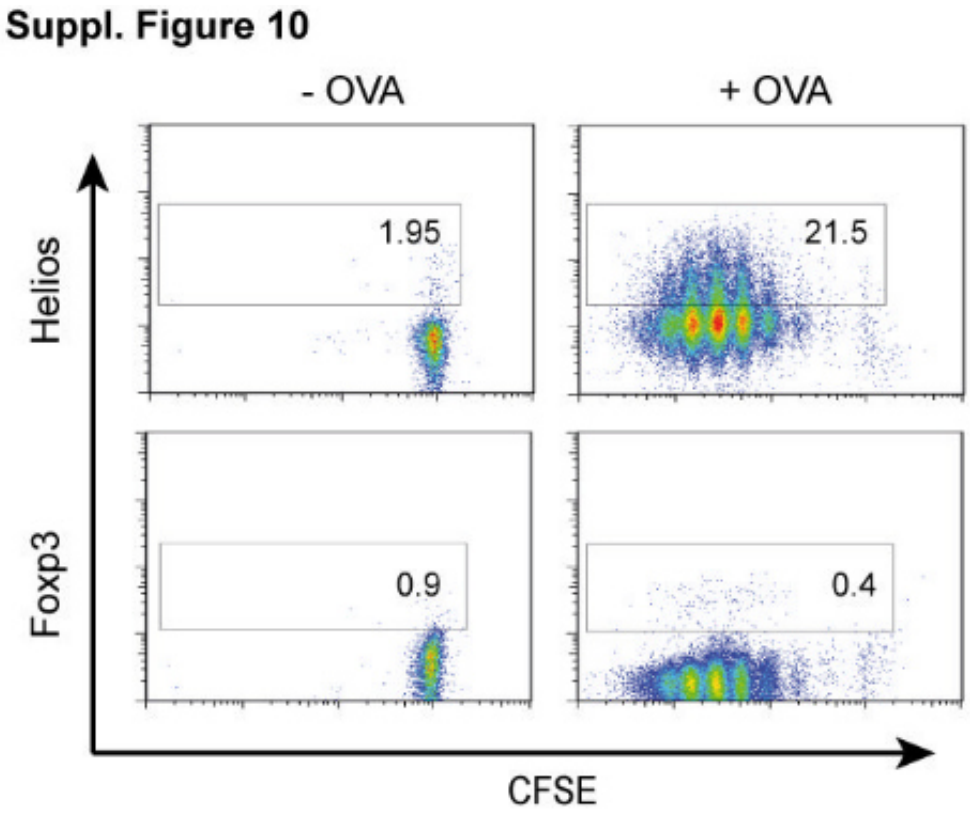
Suppl. Figure 8



Supplementary Figure 8. Influence of dietary antigens on germinal center B cells. (**A**) Representative dot blot (frequency) of GC B cells from PP of ConvD and ED mice ($CD19^+B220^+GL7^+Fas^+$). (**B**) Total numbers of GC B cells from PP of ConvD or ED animals. Data are means ± SD (n = 5 mice). **, $P < 0.01$.

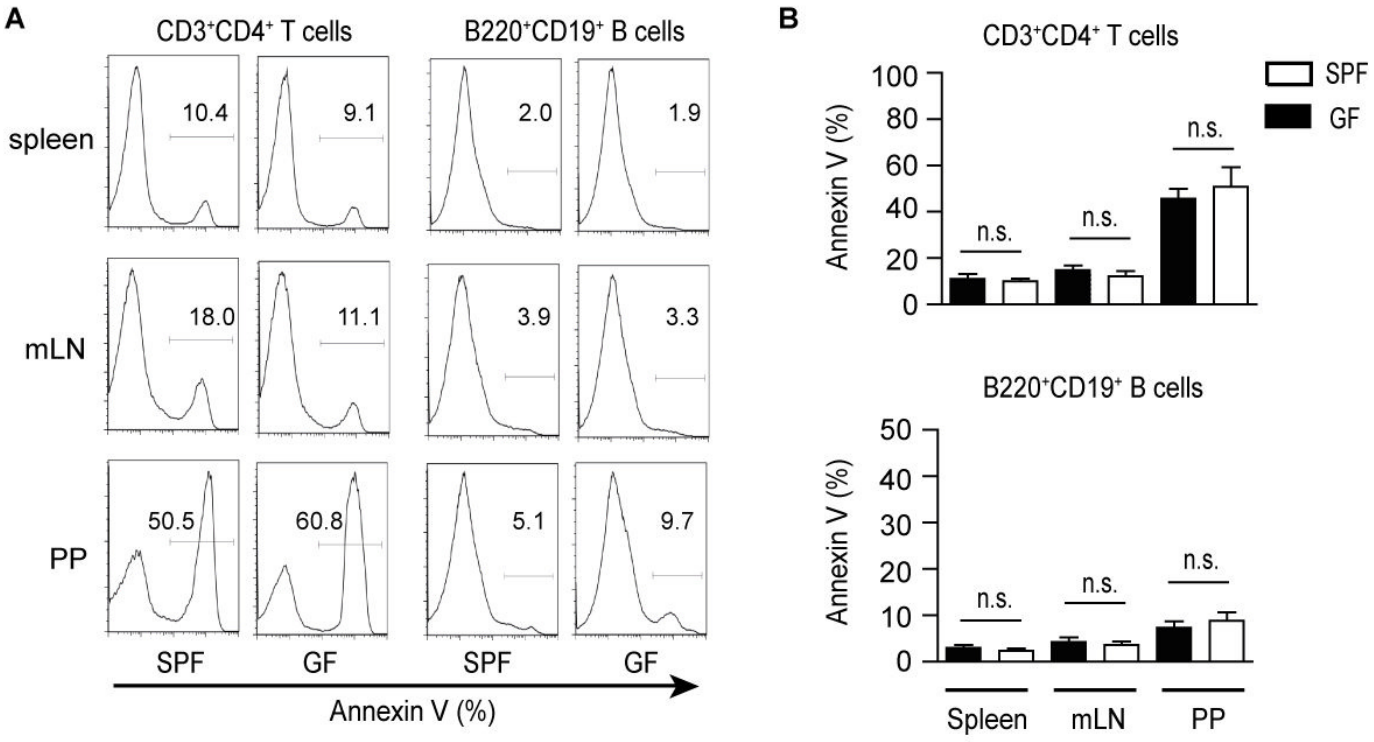


Supplementary Figure 9. Analysis of thymocytes in ConvD and ED mice. (**A**) Representative dot plots showing frequencies of $CD4^+$ and $CD8^+$ T cells in thymus of ConvD and ED mice. (**B**) Percentages of DP, DN and SP thymocytes in ConvD and ED mice. (**C**) Total cell numbers of $CD4^+CD8^+$ thymocytes in ConvD and ED mice. (**B**) and (**C**) Data are means ± SD (n = 5).

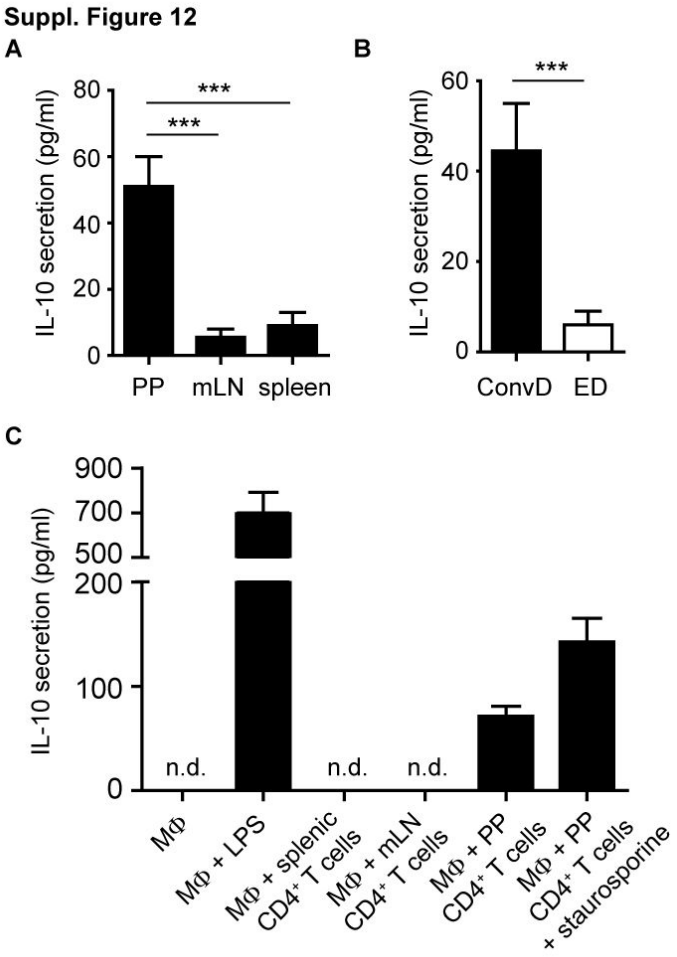


Supplementary Figure 10. Antigen-specific induction of Helios and Foxp3 in OT-II T cells. CFSE-labeled naïve $CD4^+$ T cells from OT-II mice were co-cultured with BMDC in the presence of 10^{-6} M OVA peptide (AA323-339). After five days, the proliferation of Helios and Foxp3 expressing $CD4^+$ T cells was measured.

Suppl. Figure 11

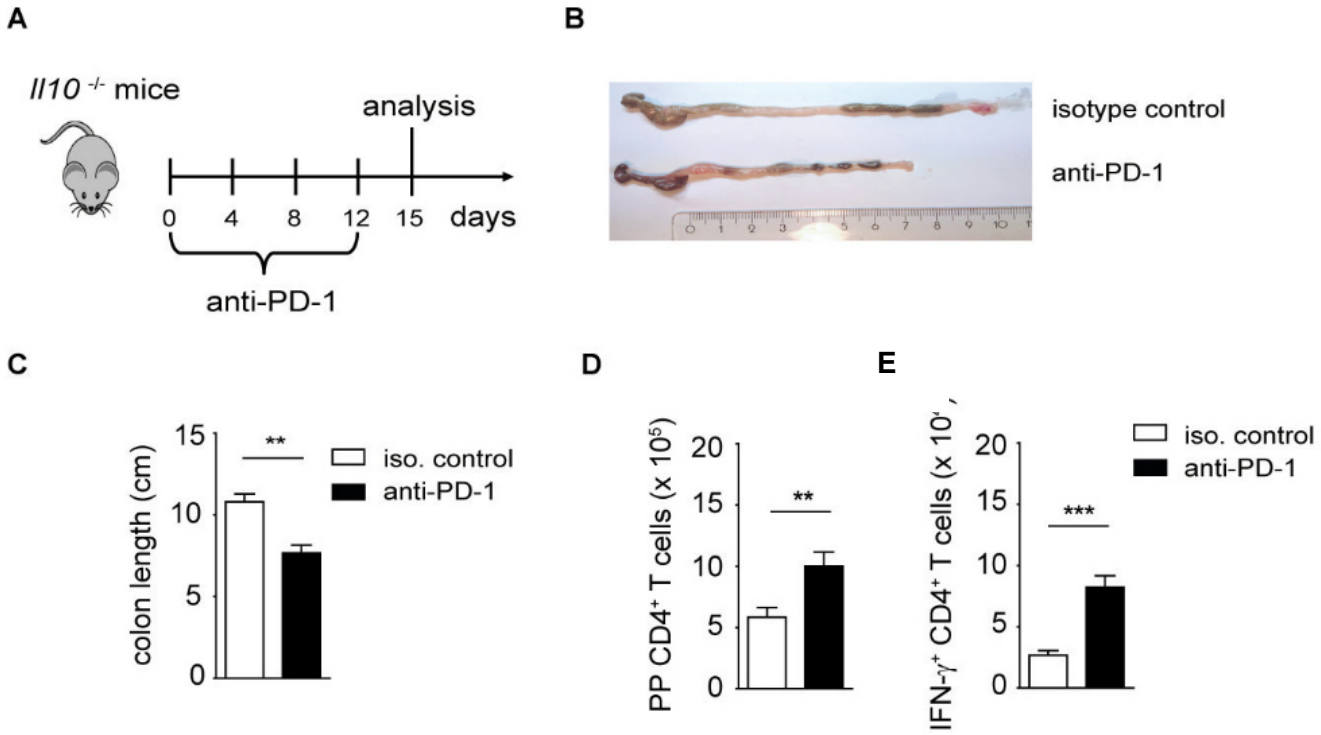


Supplementary Figure 11. Analysis of Annexin V$^+$ lymphocytes in SPF and GF mice. (**A**) Representative histogram of Annexin V staining for CD4$^+$ T and B lymphocytes from spleen, mLN and PP of SPF and GF mice kept on ConvD. (**B**) Frequencies of Annexin V$^+$ T and B cells in SPF and GF mice (n = 6) were analyzed by student´s *t*-test. Error bars indicate mean ± SD, n.s. = not significant.



Supplementary Figure 12. **Uptake of apoptotic cells induces IL-10**. (**A-C**) Single cell suspensions of indicated organs were cultured for 24 hours, and IL-10 in supernatants was measured by ELISA (n=5). (**A**) IL-10 secretion in spleen, mLN and PP of ConvD mice. (**B**) PP cells from ConvD and ED mice were cultured and analyzed for IL-10 secretion (n=5). (**C**) IL-10 from peritoneal macrophages cocultured with $CD4^+$ T cells from spleen, mLN and PP of ConvD mice. As positive control, macrophages were stimulated with LPS (0.5 µg/ml) or co-cultured with staurosporine-pretreated (0.5 µg/ml) PP $CD4^+$ T cells. Data were analyzed with student´s t-test, means ± SD are shown (n = 6), *** $P < 0.001$.

Suppl. Figure 13



Supplementary Figure 13. Anti-PD-1 treatment activates pro-inflammatory T cell responses in PP. (**A**) Scheme of anti-PD-1 (100µg, i.p.) treatment of IL-10 deficient mice. Controls were treated with isotype control Abs. (**B** and **C**) Effects of anti-PD-1 on colon length of IL-10 deficient mice. (**D**) Total number of PP $CD4^+$ T cells in mice treated with anti-PD-1 or isotype mAb. (**E**) Absolute numbers of IFN-γ^+CD4^+ T cells in PP of IL-10 deficient mice following treatment with anti-PD-1 or isotype controls. Results are representative of 2 independent experiments (n=6 mice). Error bars indicate mean ± SD. **P < 0.01; ***P < 0.001, student´s *t* test.

Supplemental Table 1

AZA, azathioprine; IFX, infliximab

Patients	**Healthy controls (n=9)**	**Crohn´s disease (n=9)**
Age	24-77 y, ø=50 y	18-64 y, ø=34 y
Sex [female/male]	5 / 4	6 / 3
Disease extension (Montreal classification)	no inflammation	7 x colon / ileum (L3) 2 x colon (L2)
Treatment	no treatment	1 x AZA 4 x IFX 2 x budesonide 1 x golimumab 1x newly diagnosed with no treatment