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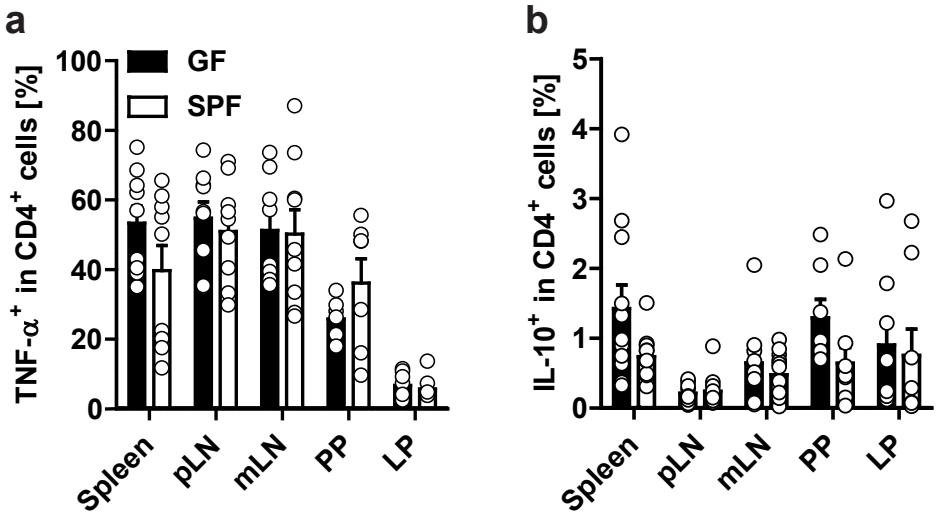
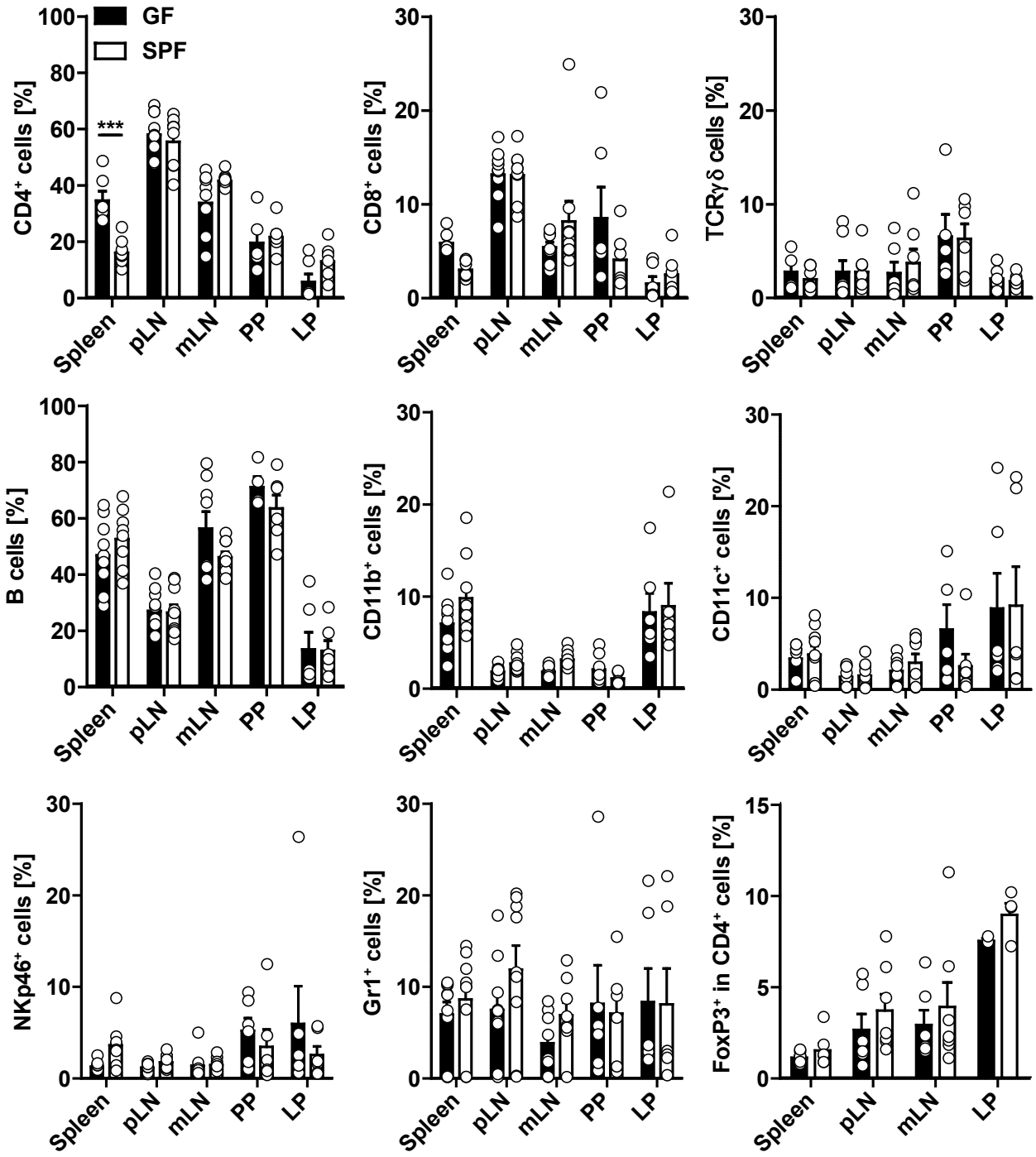
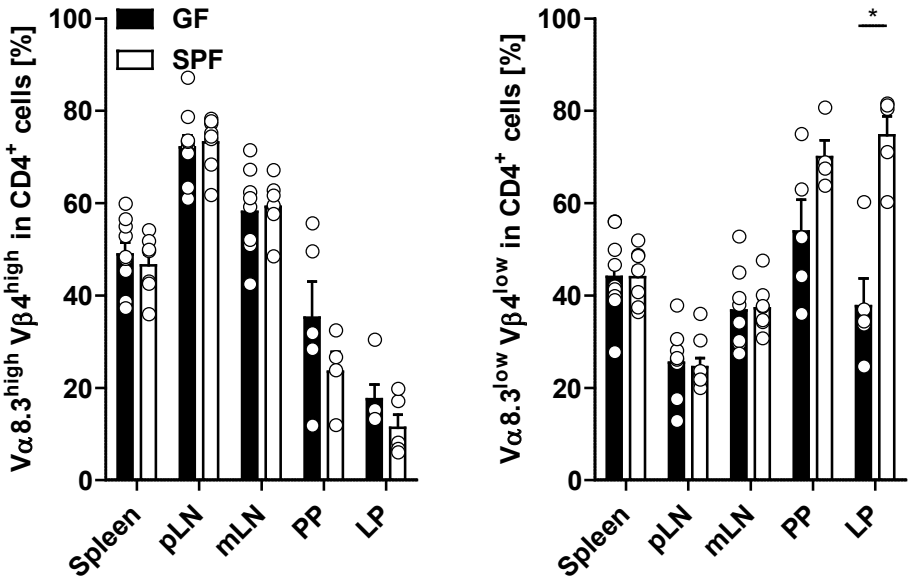


Supplementary Figure 1. Effect of microbiota on cytokine profiles of T cells in GALT. Frequencies of TNF-α (**a**) or IL-10 (**b**) producing T cells from spleen, pooled axillary and inguinal lymph nodes (pLN), mesenteric lymph nodes (mLN), Peyer's patches (PP) and small intestinal lamina propria (LP) of GF and SPF RR mice, measured by flow cytometry. Data represent the percentage of cytokine producing cells in the gated $CD4^+$ population. Each circle represents an individual mouse and bars depict mean ± s.e.m. *n* = 6-11 mice per group. Data were pooled from 4 independent experiments.

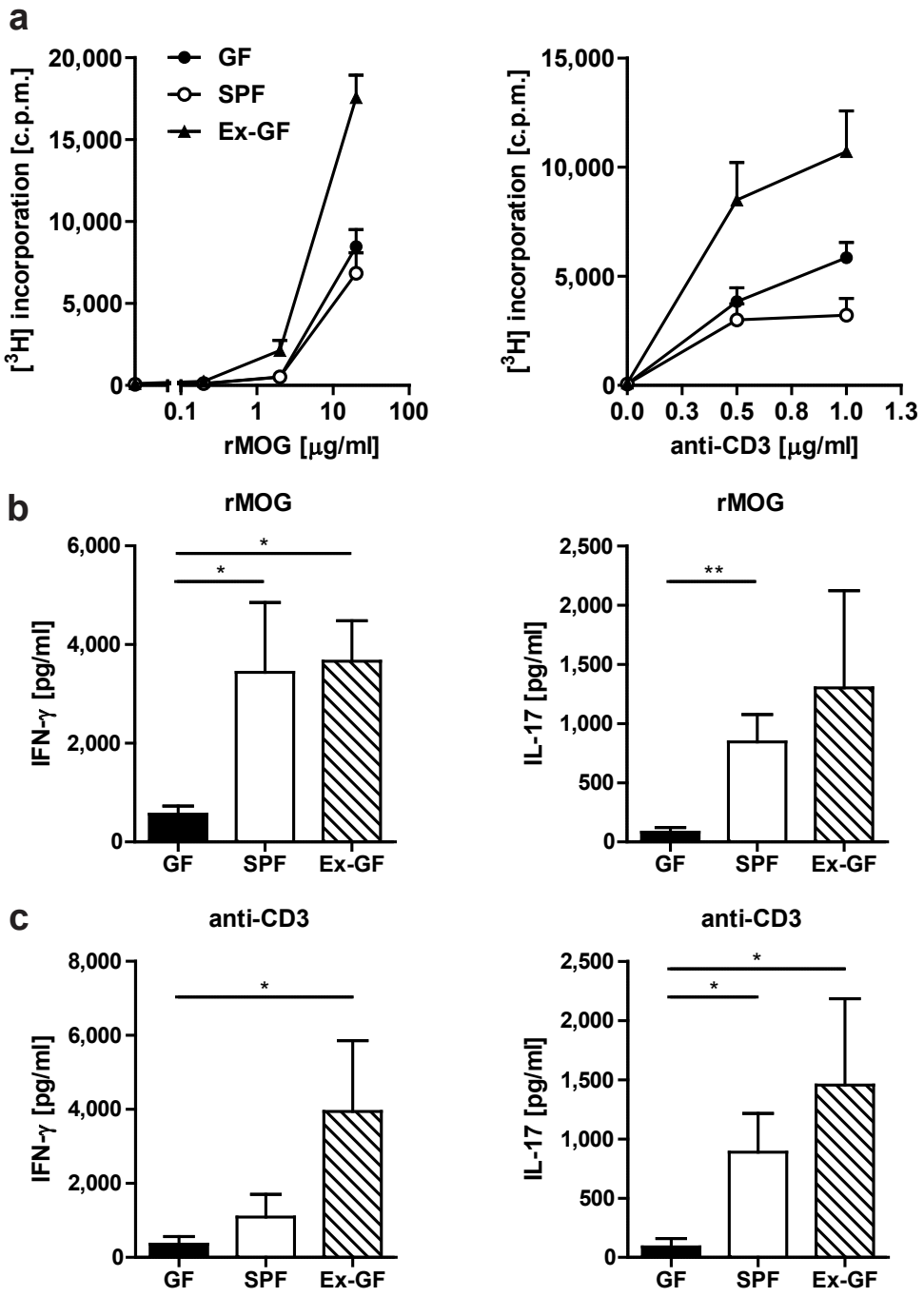


Supplementary Figure 2. Immune cell composition in lymphoid organs of GF and SPF RR mice.

Frequencies of $CD4^+$ T cells, $CD8^+$ T cells, $TCR\gamma\delta^+$ T cells, $B220^+$ B cells, $CD11b^+$ macrophages, $CD11c^+$ dendritic cells, $NKp46^+$ NK cells, $Gr1^+$ granulocytes and $Foxp3^+$ Tregs from spleen, pLN, mLN, PP and small intestinal LP of GF and SPF RR mice determined by flow cytometry. Data represent frequencies of positive cells in the live gate with the exception of $Foxp3^+$ cells where $CD4^+$ gate was used. Each circle represents an individual mouse and bars are shown as mean ± s.e.m. *n* = 3-9 mice per group. Data were pooled from 4 independent experiments.***P = 0.0007 (Mann-Whitney U test).

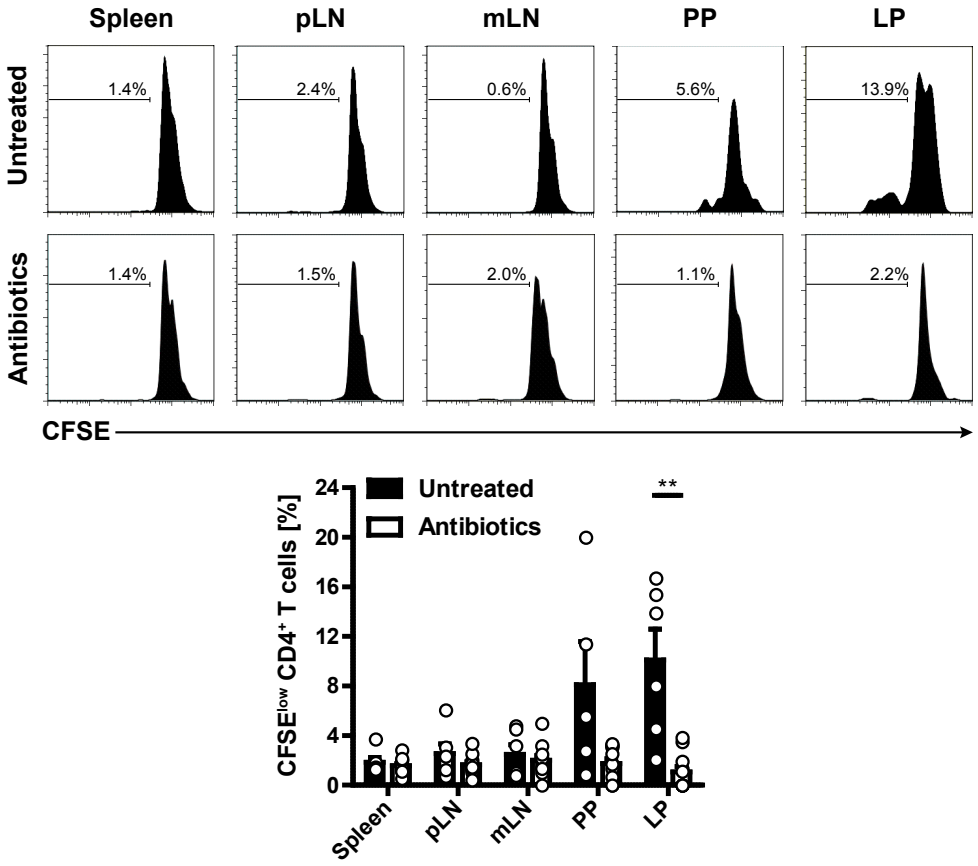


Supplementary Figure 3. TCR^{high} and TCR^{low} transgenic T cells in lymphoid tissues of GF and SPF RR mice. Frequencies of $V\alpha8.3/V\beta4^{high}$ (TCR^{high}) and $V\alpha8.3/V\beta4^{low}$ (TCR^{low}) T cells from spleen, pLN, mLN, PP and small intestinal LP of GF and SPF RR mice were determined by flow cytometry. Data represent frequencies of positive cells in the $CD4^+$ gate. Each circle represents an individual mouse and bars are shown as mean ± s.e.m. *n* = 5-7 mice per group. Data were pooled from 4 independent experiments. *P = 0.0160 (Mann-Whitney-U-test).

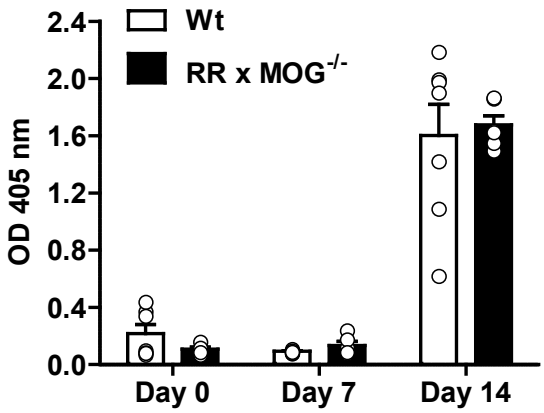


Supplementary Figure 4. Reduced cytokine production by T cells from GF RR mice.

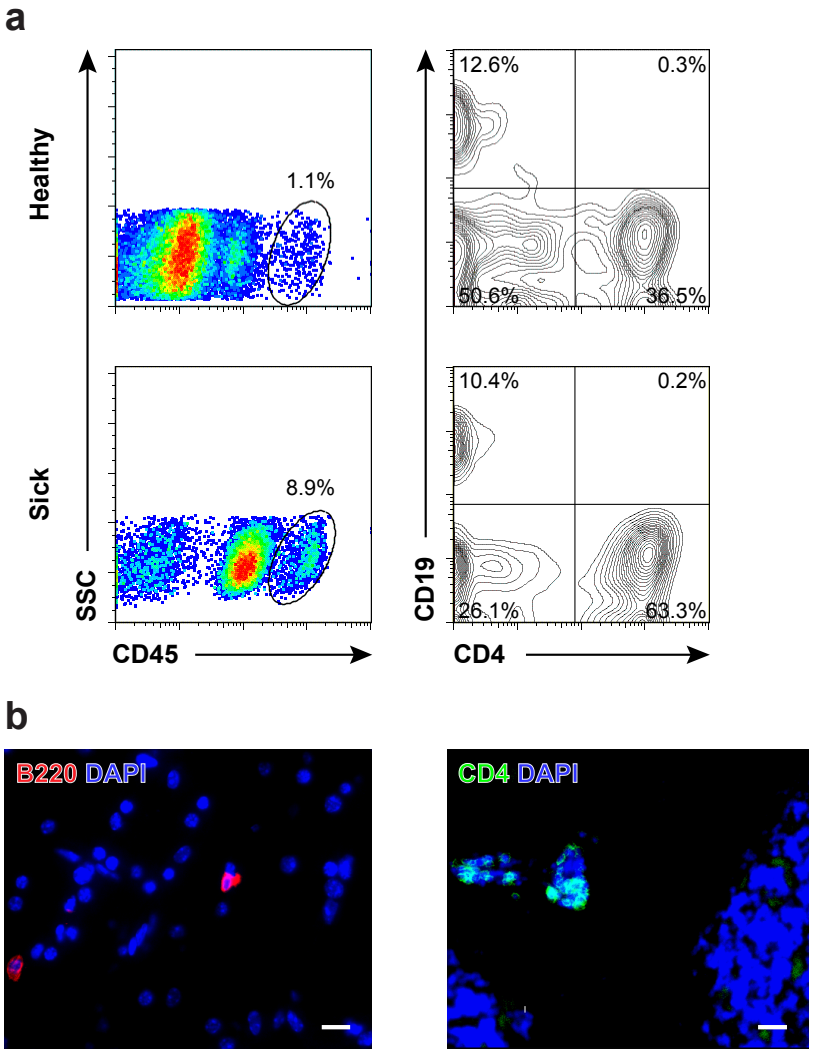
(**a**) Proliferation of splenic T cells from GF, SPF and Ex-GF RR mice cultured with increasing concentrations of rMOG or anti-CD3 antibody was measured by ^{3}[H]-thymidine incorporation. *n* = 3-6 per group. Data were pooled from 3 individual experiments. (**b - c**) Cytokine production of GF, SPF and Ex-GF RR mice splenocytes. Spleen cells stimulated for 48 h with either 20 µg/ml rMOG (**b**) or 1 µg/ml anti-CD3 antibody (**c**). IFN-γ and IL-17 levels in the supernatants were measured by ELISA. Bars depict mean ± s.e.m. *n* = 3-5 per group. **P* < 0.05; ***P* < 0.01 (unpaired t test).



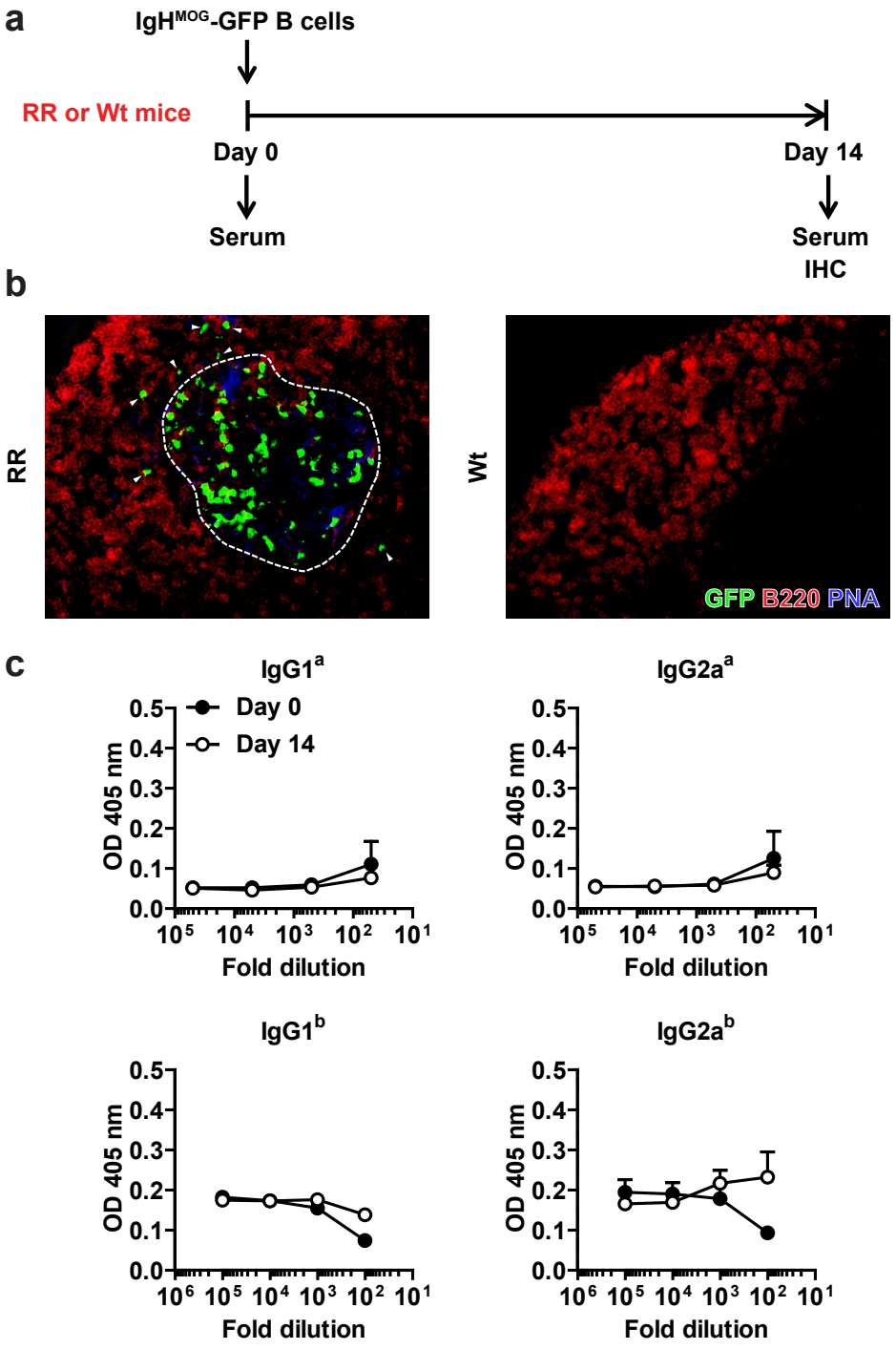
Supplementary Figure 5. Antibiotics treatment suppresses T cell activation induced by commensal flora. $CD4^+$ T cells from Wt mice were labeled with CFSE and transferred into untreated or antibiotics treated SPF Wt mice. Shown are the frequencies of $CFSE^{low}$ cells in the $CD4^+$ gate. Each circle represents an individual mouse and bars depict mean ± s.e.m. *n* = 6-12 per group. Data were pooled from 3 individual experiments. ***P* = 0.0087 (Mann-Whitney U test).



Supplementary Figure 6. Immunization of SPF RR x MOG$^{-/-}$ mice induces anti-MOG antibodies. Similar levels of anti-MOG antibodies in Wt (*n* = 7) and RR x MOG$^{-/-}$ (*n* = 6) mice immunized with rMOG in CFA. MOG specific IgG2a antibodies were measured by sandwich ELISA in sera collected on days 0, 7 and 14 after immunization. Values shown are the absorbances at 405 nm. Each circle represents an individual mouse and bars depict mean ± s.e.m. Data were pooled from 2 individual experiments.



Supplementary Figure 7. T and B cells infiltrate the CNS of RR mice before onset of clinical EAE. (**a**) CNS infiltrating cells from healthy (top) and sick (bottom) RR mice. Mice were perfused with PBS and CNS mononuclear infiltrates were isolated by percoll gradient. Distributions of cells stained with immune cell markers were determined by flow cytometry. Numbers in quadrants indicate percentage of stained cells. Data represent 3 animals analyzed in 2 independent experiments. (**b**) Immunofluorescent staining of brain sections from healthy RR mouse for infiltrating B ($B220^+$) and T ($CD4^+$) cells (CD4, green; B220, red; DAPI (cell nuclei), blue). Representative staining of >3 mice is shown. Scale bars, 25 µm.



Supplementary Figure 8. Homing behavior of MOG specific B cells. (**a**) Purified IgH^{MOG}-GFP B cells

were transferred into adult RR or Wt mice (day 0). Isotype switched serum anti-MOG antibody levels of the donor (a allotype) and recipients (b allotype) were measured before (day 0) and on day 14 after transfer. The distribution of transferred B cells (green) was documented 14 days after transfer via immunofluorescence in cLN sections. (**b**) MOG specific B cells home to the GC of brain draining cervical lymph nodes of RR but not Wt mice. B220 (red) and PNA (blue) label GC and naïve B cells, respectively. Arrow heads indicate transferred B cells. Dotted lines define boundaries of GCs. Representative immunofluorescence staining from two independent experiments consisting of 4-5 mice per group is shown. PNA (blue) and B220 (red) label GC and naïve B cells, respectively. Magnification: 20x. (**c**) Transgenic MOG specific B cells do not spontaneously isotype switch after transfer into Wt mice. Error bars represent s.e.m. *n* = 3-5 mice per group. Data were pooled from 2 independent experiments.

Supplementary Table 1: Activated MOG-specific T cells induce EAE in both GF and SPF mice.

TCR transgenic T cells were activated *in vitro* with rMOG for 2 days and transferred into GF and SPF mice and observed for clinical signs of EAE.

Experiment	Recipient	EAE affected/total	Day of onset (Mean ± SD)
Experiment-1	SPF	4/4	4 ± 0
	GF	4/4	6.25 ±0.5
Experiment-2	SPF	3/3	5 ± 0
	GF	4/4	5.75 ± 0.5