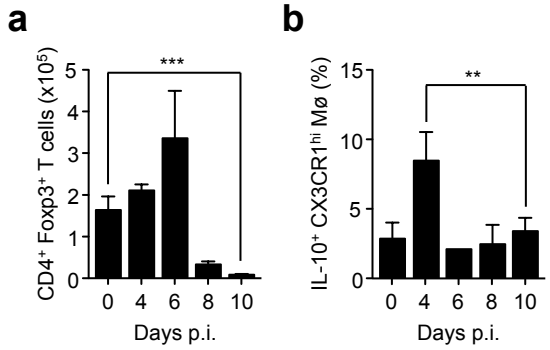
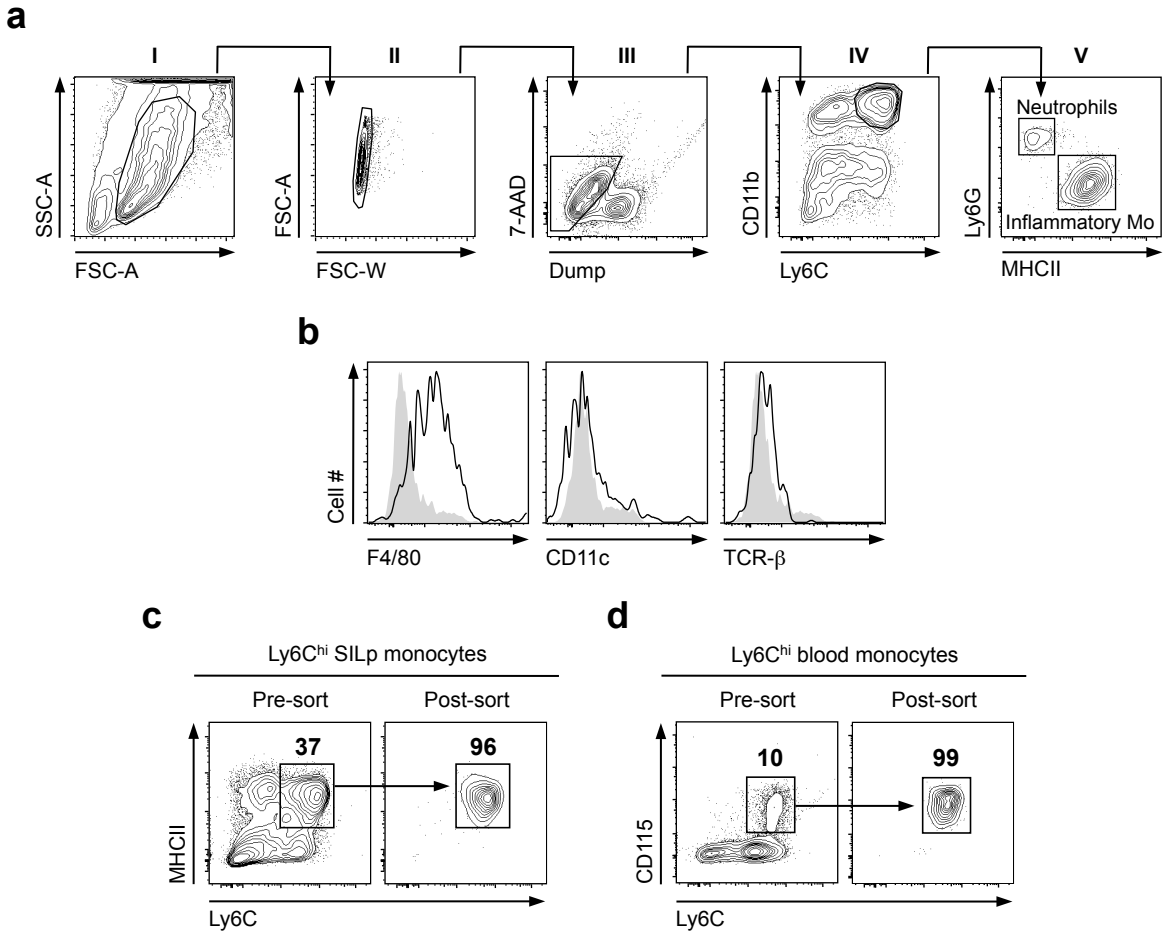
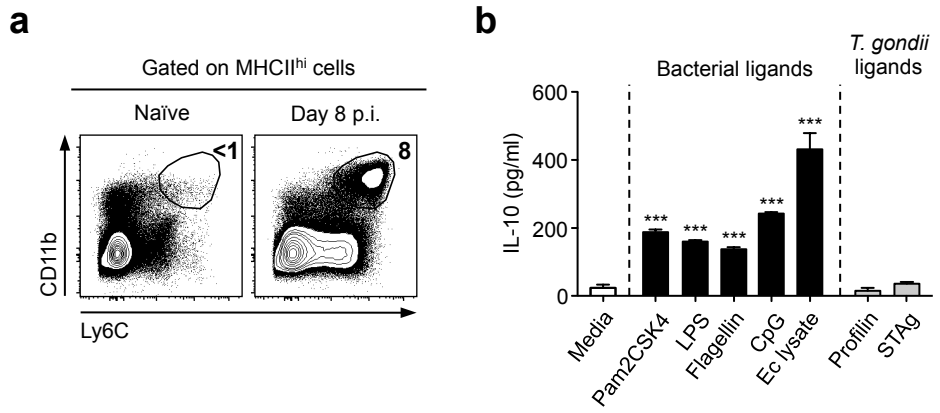


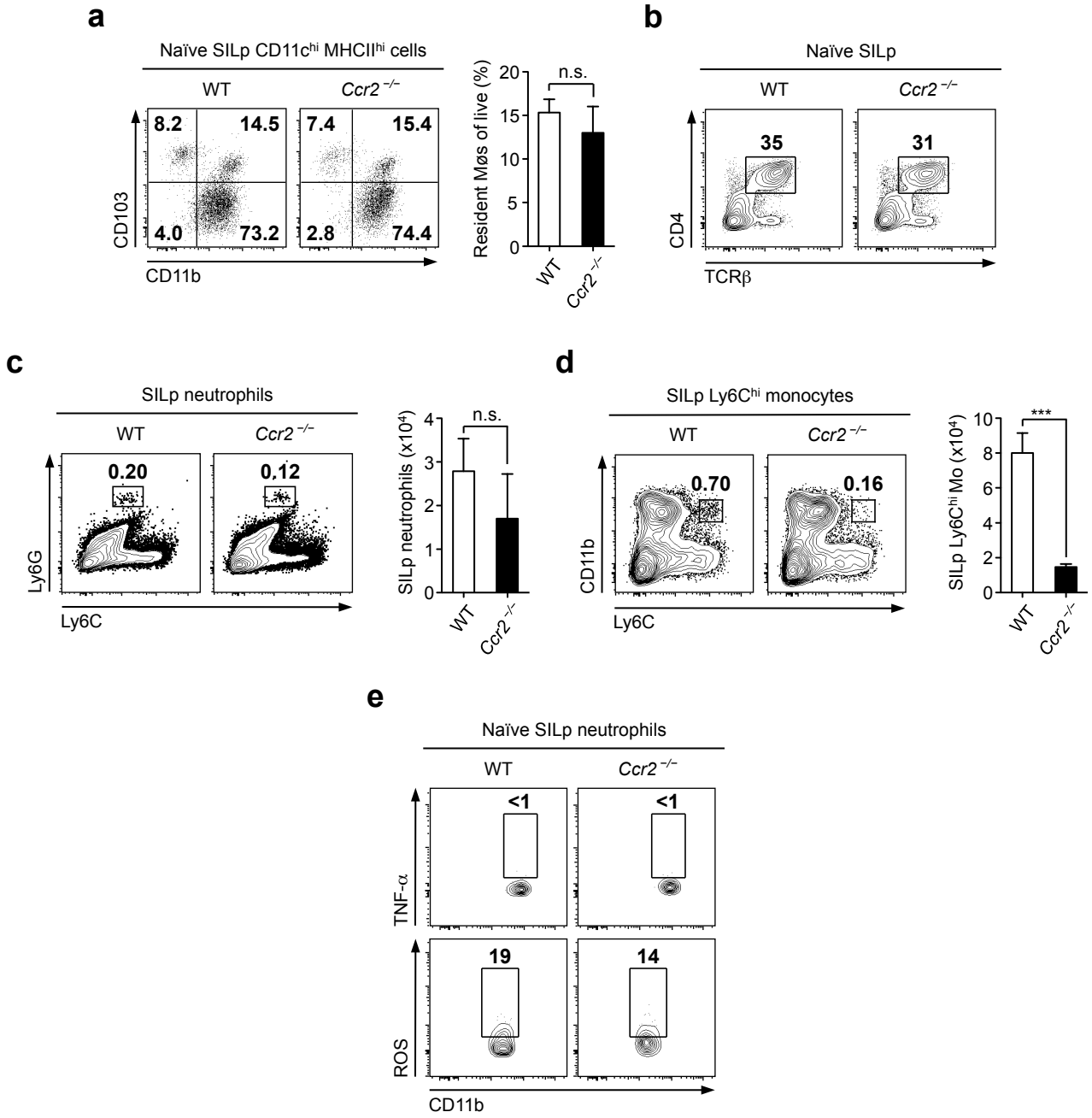
Supplementary Figure 1: Collapse of Foxp3$^+$ Tregs, and reduced IL-10 production by CX3CR1^{hi} resident macrophages during low-dose *T. gondii* infection. C57BL/6 mice were orally infected with *T. gondii*. At various time points after infection the phenotype and function of specific immune cell subsets was analysed by flow-cytometry. (**a**) Absolute number of TCR-β^+CD4$^+$Foxp3$^+$ cells present in the small intestine Lp over the course of infection. (**b**) Assessment of the frequency of IL-10-producing CX3CR1^{hi}CD11c^{hi}MHCII^{hi} resident macrophages by intracellular cytokine staining following 3 hr culture of single cell Lp suspensions in brefeldin A. Histograms represent the mean of individual animals ± SEM (n=3-4). Data are representative of two similar experiments. Statistical comparisons were performed using the Student's t test (*p<0.05, **p<0.01, ***p<0.001).



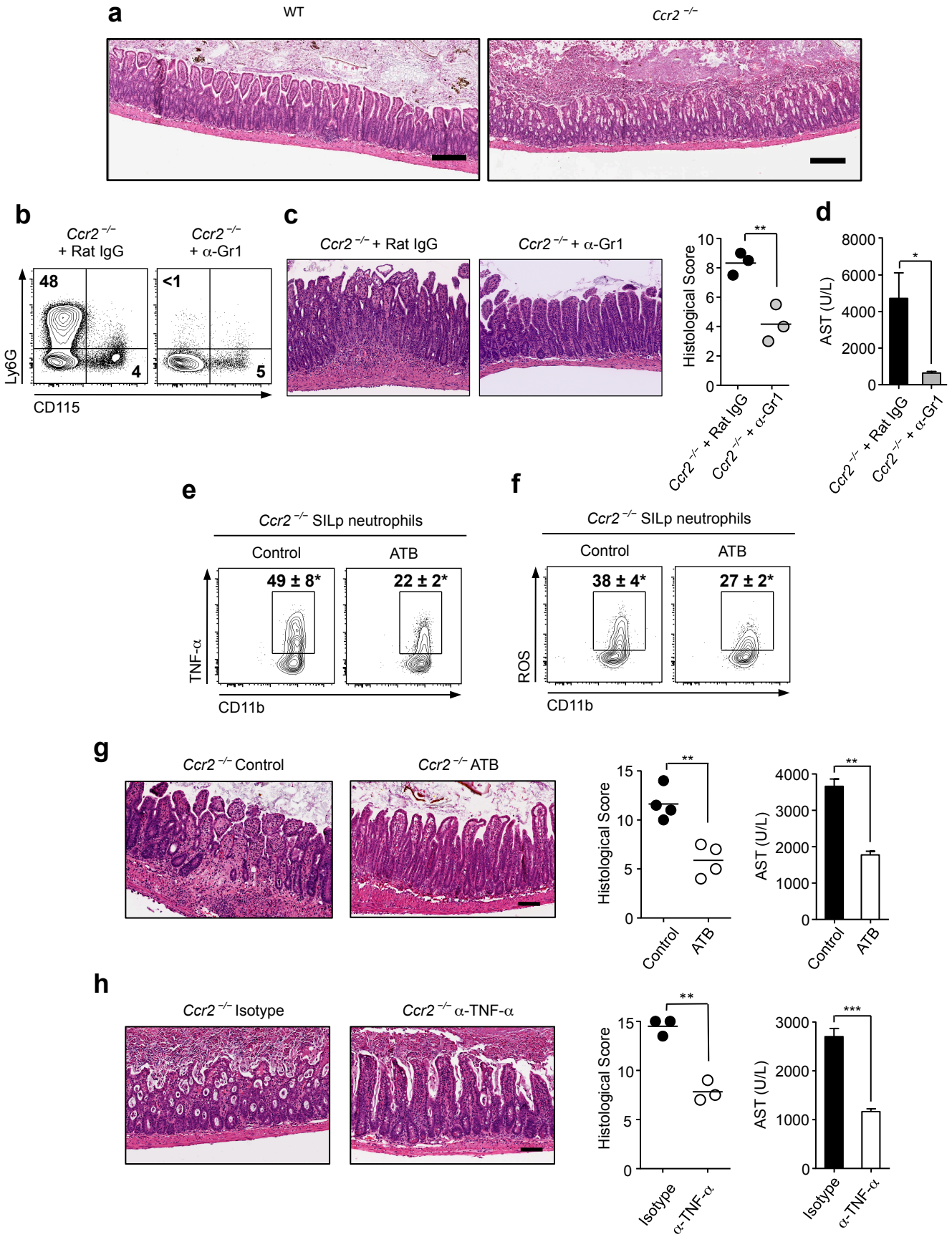
Supplementary Figure 2: Gating, phenotyping, and sorting, of blood and small intestine Ly6C^{hi} monocytes. (**a**) Flow-cytometry gating strategy for Ly6C^{hi} inflammatory monocytes (Mo) in single cell suspensions isolated from day 8 *T. gondii* infected small intestine *lamina propria* (SILp). Cells were identified as follows: (**I**) SSC-A versus FSC-A to remove cellular debris. (**II**) FSC-A versus FSC-W to exclude doublets. (**III**) 7-AAD versus Dump (CD3/B220) to identify live cells not expressing lymphocyte markers. (**IV**) CD11b versus Ly6C to gate recruited inflammatory cells. (**V**) Ly6G versus MHCII to distinguish neutrophils from Ly6C^{hi} Mo. (**b**) Surface phenotyping of Ly6C^{hi} Mo for F4/80, CD11c, and TCR-β. Gray shading, isotype control antibody. (**c,d**) Analysis of the purity of Ly6C^{hi}(MHCII^{hi}) small intestine Lp Mo, and Ly6C^{hi} (CD115^{+}) blood monocytes before, and after FACS isolation. Gated on live cells. Plots are of representative of animals from more than six experiments.



Supplementary Figure 3: Ly6C^{hi}MHCII^{hi} cells are present in the spleen during *T. gondii* infection. (**a**) Flow-cytometric staining of Ly6C^{hi}MHCII^{hi} monocytes in the spleen of C57BL/6 mice infected orally with *T. gondii* at day 8 after infection. Numbers refer to percentage of cells within gate. Plots are representative of animals from at least three separate experiments. (**b**) On day 8 p.i. Ly6C^{hi} Mo were sorted by FACS and cultured for 18 hrs, alone, or in the presence of *T. gondii* ligands (left histogram) or bacterial ligands (right histogram). TNF-α was measured in triplicate supernatants by ELISA. Data are presented as mean ± SEM. Statistical comparisons were performed using the Student's t test (*p<0.05, **p<0.01, ***p<0.001) compared to media alone.

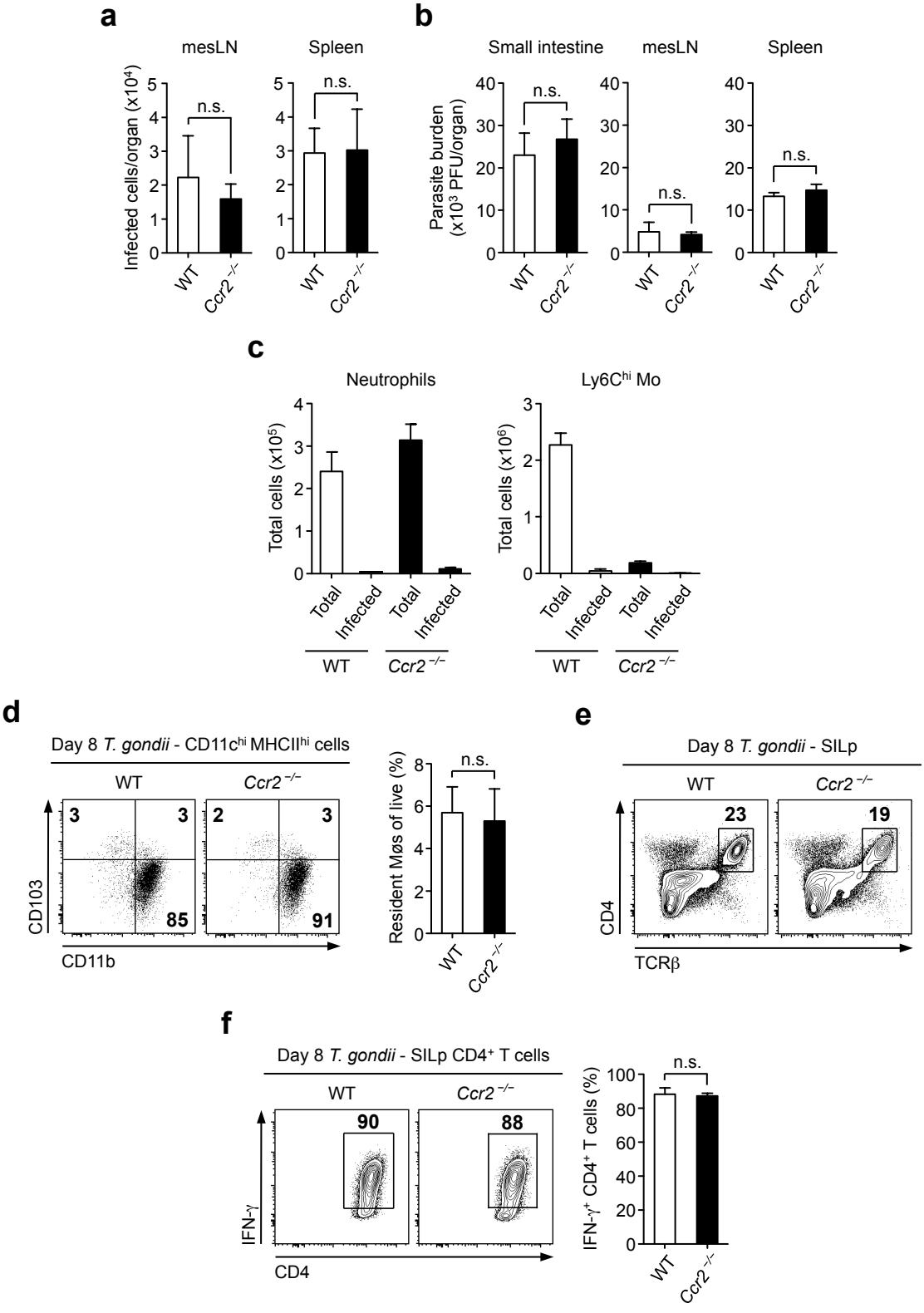


Supplementary Figure 4: Steady-state assessment of SILp immune cell composition in *Ccr2*$^{-/-}$ mice. (a) Frequencies of CD11b^{+}CD103^{+} DCs, CD11b^{-}CD103^{+} DCs and CD11b^{+}CD103^{-} resident Møs, within the total CD11c^{hi}MHCII^{hi} gate were assessed by flow-cytometry in naïve wild-type (WT) and *Ccr2*$^{-/-}$ animals. Graphical representation of frequency of resident Møs within the total live cell gate of FACS plots. **(b)** Flow-cytometric assessment of frequencies of CD4^{+}TCRβ^{+} T cells within the live cell gate of naïve WT and *Ccr2*$^{-/-}$ animals. **(c)** Frequency of neutrophils (Ly6G^{+}Ly6C^{int}) within the total live cell gate in naïve WT and *Ccr2*$^{-/-}$ animals. Graphical representation of absolute number of neutrophils in SILp of WT or *Ccr2*$^{-/-}$ animals. **(d)** Frequency of Ly6C^{hi} monocytes (Ly6C^{hi}CD11b^{hi}) in naïve WT and *Ccr2*$^{-/-}$ animals. Graphical representation of absolute number of Ly6C^{hi} monocytes in SILp of WT or *Ccr2*$^{-/-}$ animals of FACS plots. **(e)** Representative plots of TNF-α production and ROS production by neutrophils was assessed by flow-cytometry in WT and *Ccr2*$^{-/-}$ animals (n=3). Data are presented as mean ± SEM. Statistical comparisons were performed using the Student's t test (*p<0.05, **p<0.01, ***p<0.001). Data are representative of two independent experiments.



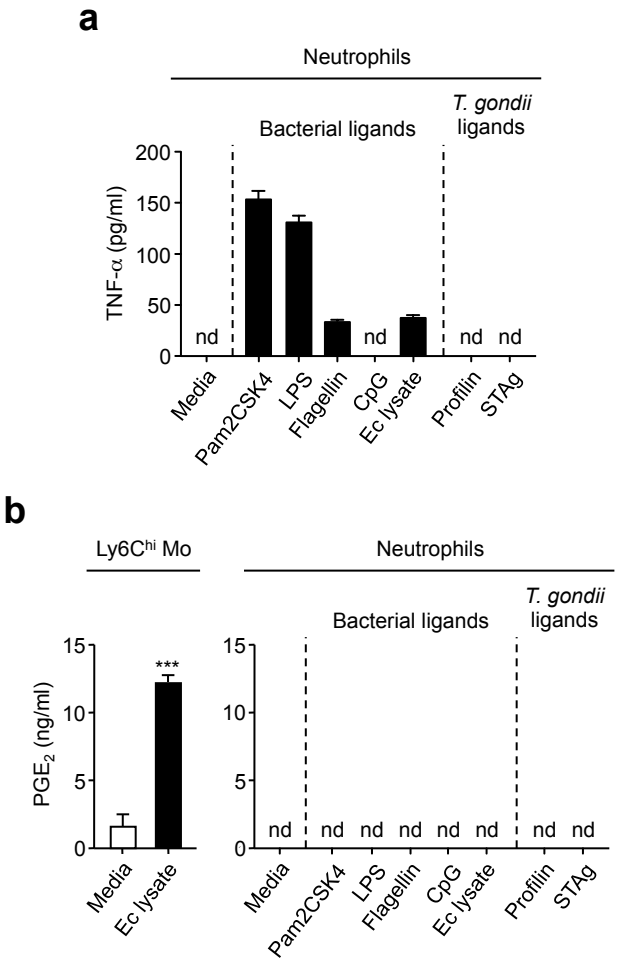
Grainger et *al.* Supplementary Figure 5

Supplementary Figure 5: Commensal driven pathology in *Ccr2*$^{-/-}$ mice is neutrophils mediated. (a) Representative H&E stained section of small intestine from day 8 infected WT and *Ccr2*$^{-/-}$ animals (scale bar 200uM) **(b-d)** Wild-type (WT) or *Ccr2*$^{-/-}$ mice were orally infected with *T. gondii*. On day 5 post infection (p.i.) *Ccr2*$^{-/-}$ animals were treated with anti-Gr1 antibody or isotype control. WT mice were treated with isotype only. **(b)** Representative FACS plots showing frequencies of neutrophils (Ly6G^{+}CD11b^{+}) and monocytes (CD115^{+}) of total live cells in antibody-treated animals day 9 p.i. **(c)** Representative H&E stained section of small intestine in control and anti-Gr1 treated animals with graph depicting histological scoring. **(d)** AST sera levels were measured day 9 p.i. in control and anti-Gr1 treated animals. **(e-g)** *Ccr2*$^{-/-}$ mice were pre-treated with antibiotics (ATB) or not (control) and orally infected with *T. gondii*. Neutrophil activation and pathology were examined day 9 p.i. **(e)** SILp were treated with BFA for 3 hrs, and neutrophils were assessed for TNF-α production by intracellular cytokine staining. Numbers represent the percentage of cells in each quadrant. Bar graph summarizes the average mean fluorescence intensity (MFI) of TNF-α in neutrophils (n=3). **(f)** SILp neutrophils were assessed for the production of reactive oxygen species (ROS) by flow-cytometry. Bar graphs summarize the mean percentage of ROS^{+} cells, and MFI of ROS, ± SEM. (n=3). **(g)** Representative H&E stained section of small intestine from day 9 infected control and antibiotic (ATB) treated *Ccr2*$^{-/-}$ animals. AST sera levels were measured at day 9 p.i. from control or ATB treated *Ccr2*$^{-/-}$ animals with graph depicting histological scoring. **(h)** *T. gondii* orally infected *Ccr2*$^{-/-}$ mice were treated with anti-TNF-α antibody or isotype control on day 5 and 8 p.i. Representative H&E stained section of small intestine from day 9 infected control or anti-TNF-α treated animals with graph depicting histological scoring. AST sera levels were measured at day 9 p.i. of control or anti-TNF-α treated animals (n=3). Data are presented as mean ± SEM. Data are representative of two independent experiments. Statistical comparisons were performed using the Student's t test (*p<0.05, **p<0.01, ***p<0.001).

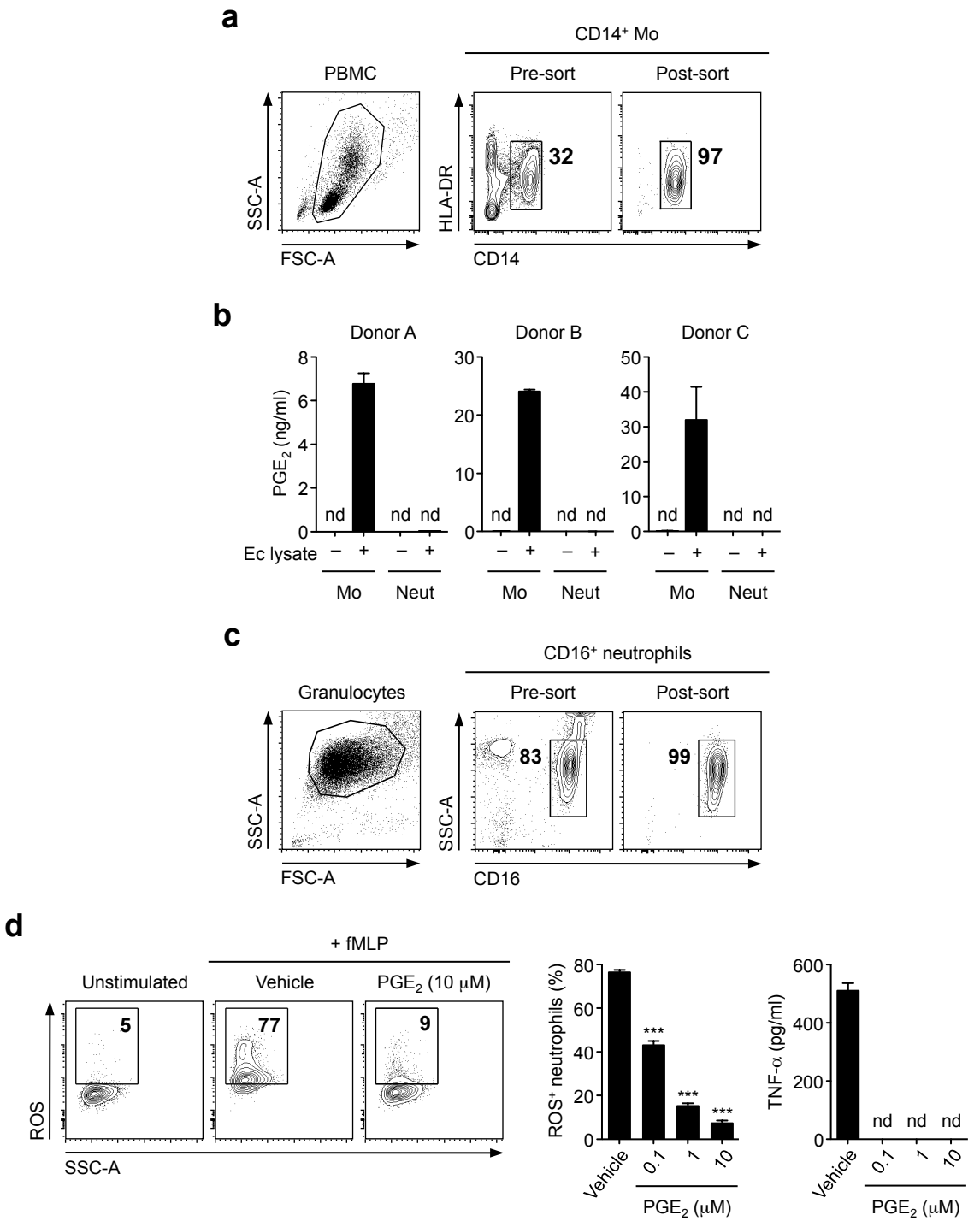


Grainger et *al.* Supplementary Figure 6

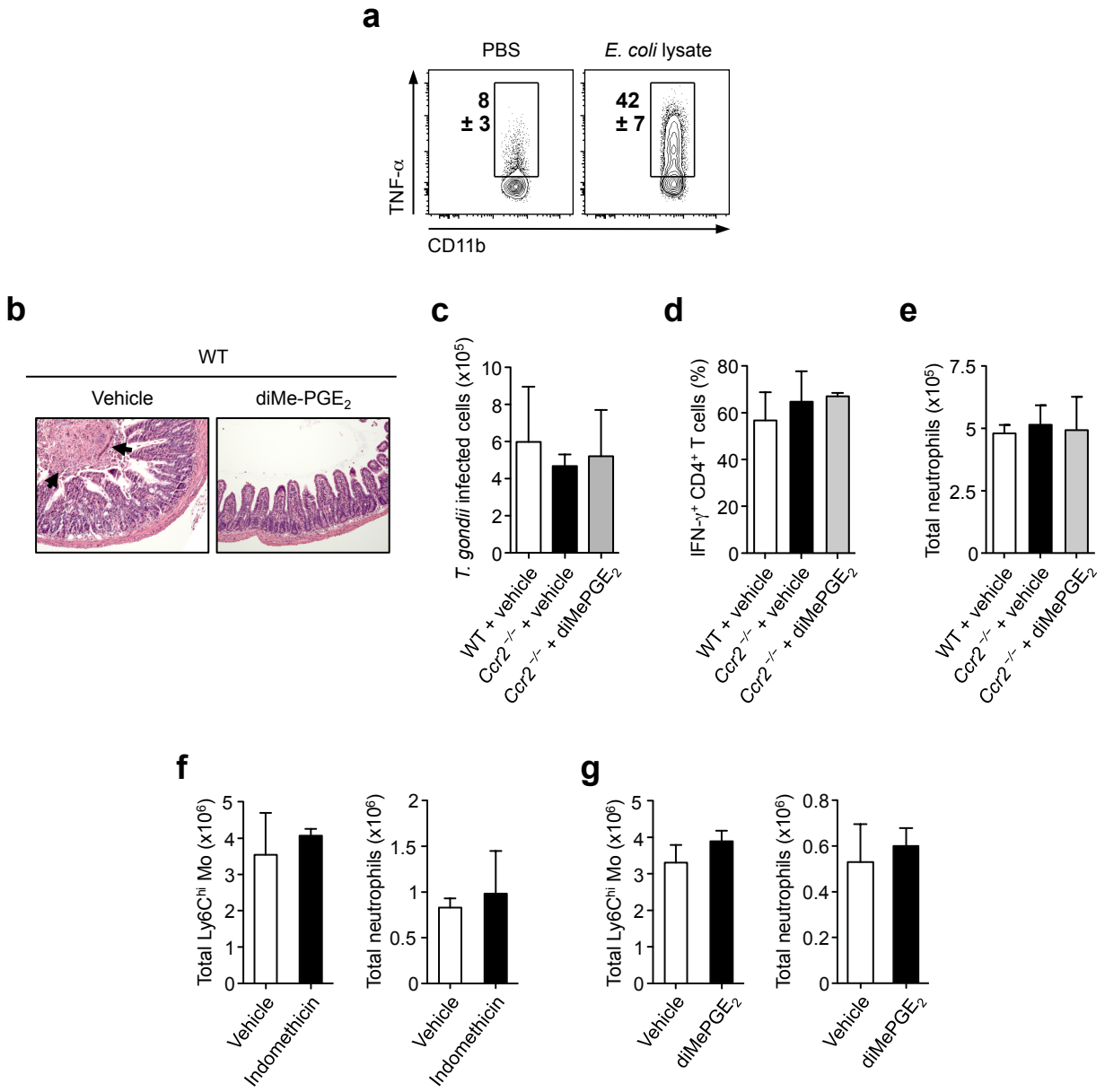
Supplementary Figure 6: Total numbers of neutrophils, parasite load and T_H1 responses are unaltered in *Ccr2*$^{-/-}$ mice. **(a)** Absolute number of *T. gondii* infected cells in mesLN and spleen of wild-type (WT) and *Ccr2*$^{-/-}$ animals, assessed by flow-cytometry day 8 p.i. **(b)** Parasite burden assessed by plaque assay in the SILp, mesLN, and spleen day 8 p.i. **(c)** Absolute numbers, and total numbers of infected cells, of neutrophils and Ly6C^{hi} Mo in SILp of WT and *Ccr2*$^{-/-}$ day 8 p.i. Parasite burden was assessed by flow-cytometry. **(d)** Representative FACS plots showing frequencies of CD103^{+} DCs, and resident Møs within the CD11c^{hi}MHCII^{hi} gate of infected mice. Bar graphs summarize the mean percentage of resident Møs as a proportion of live cells. **(e)** Representative FACS plots showing proportion of CD4^{+} T cells of total live cells at day 8 p.i. **(f)** SILp was restimulated for 3 hrs with PMA and ionomycin in the presence of BFA and CD4^{+} production of IFN-γ was assessed by intracellular cytokine staining. Representative FACS plots are shown (n=3). Bar graph summarizes the mean percentage of IFN-γ^{+} cells, ± SEM (n=3). Data are presented as mean ± SEM. Statistical comparisons were performed using the Student's t test (*p<0.05, **p<0.01, ***p<0.001). Data are representative of two independent experiments.



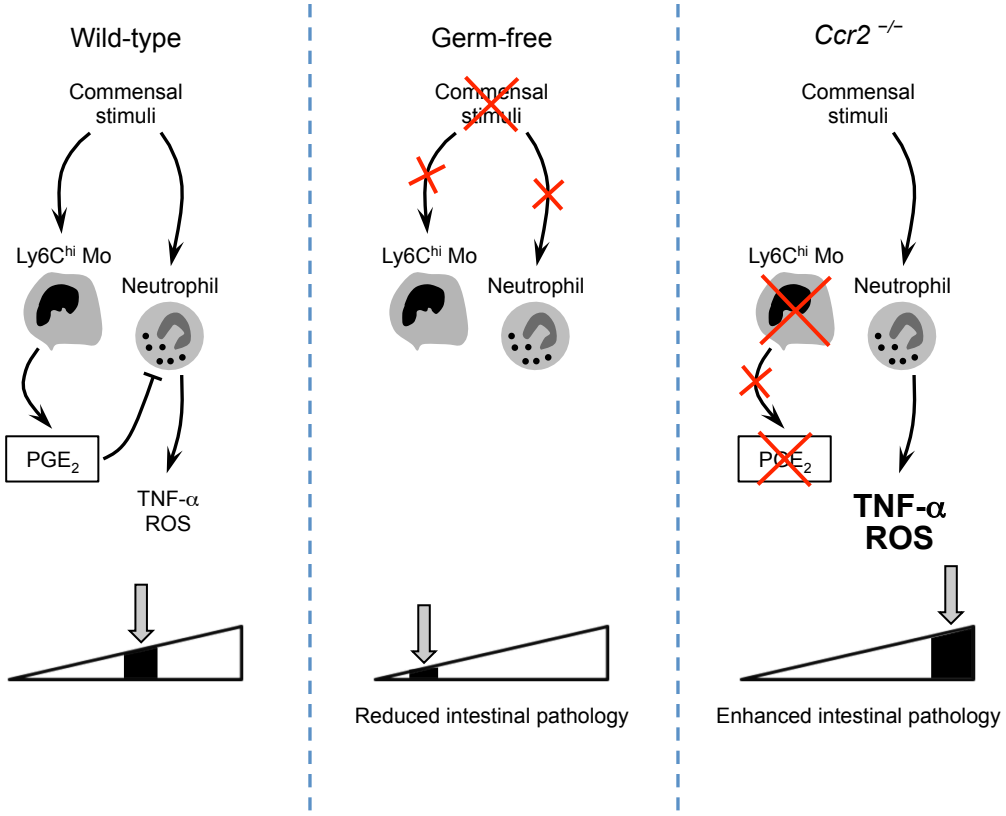
Supplementary Figure 7: Neutrophils produce TNF-α but not PGE_2 in response to bacterial products. (**a**) On day 8 p.i. $Ly6G^+$ neutrophils were sorted by FACS from the SILp and cultured for 18 hrs, alone, or in the presence of *T. gondii* ligands or bacterial ligands. TNF-α was measured in triplicate supernatants by ELISA. Data are presented as mean ± SEM. (**b**) Levels of PGE_2 as measured by EIA in supernatant from overnight culture of neutrophils or $Ly6C^{hi}$ Mo. Data are representative of three independent experiments, results shown as mean ± SEM. Statistical comparisons were performed using the Student's t test (*p<0.05, **p<0.01, ***p<0.001).



Supplementary Figure 8: Human CD14⁺ monocytes, but not neutrophils, produce PGE_2 in response to commensal stimuli. (a) Highly pure human CD14⁺ monocytes (Mo) were FACS sorted from peripheral blood mononuclear cells (PBMC) of healthy donors. **(b)** Purified populations from individual donors were cultured alone or in the presence of *E. coli* lysate for 18 hrs and PGE_2 measured in triplicate supernatants by EIA. **(c)** Neutrophils (CD16⁺) were FACS sorted from the granulocyte fraction of the same donors. **(d)** Purified neutrophils were treated with fMLP, for 30 minutes, and ROS-production was assessed by flow-cytometry. Alternatively, purified neutrophils were treated with *E. coli* lysate without or in the presence of PGE_2 for 18 hrs. TNF-α was measured in triplicate supernatants by ELISA. (n.d., not detected.) Results are shown as ± SEM (n=3). Statistical comparisons were performed using the Student's t test (*p<0.05, **p<0.01, ***p<0.001). Data are representative of three individual donors.



Supplementary Figure 9: Commensal stimuli drive neutrophil activation in intraperitoneal *T. gondii* infection and manipulation of PGE_2 does not impact T cell activation or cell recruitment during infection. (a) C57BL/6 mice were intraperitoneally (i.p) infected with 20 cysts *T. gondii*. To activate neutrophils mice were i.p. injected on day 4 p.i. with *E. coli* lystate or PBS. Peritoneal lavage cells were harvested and cultured in the presence of BFA for 3 hrs and neutrophil production of TNF-α was assessed by intracellular cytokine staining. Representative FACS plots of TNF-α production by PEC neutrophils ($Ly6G^+CD11b^{hi}$). **(b)** *T. gondii* infected WT mice were treated with 16,16-dimethyl (diMe) PGE_2 or vehicle control from days 6-8 p.i. Histological analysis of ileums from infected WT mice treated with $diMePGE_2$ or vehicle control. **(c-e)** WT and *Ccr2*$^{-/-}$ mice were orally infected with *T. gondii* and treated with $diMePGE_2$ or vehicle control and SILp was assessed for **(c)** parasite burden, **(d)** frequency of IFNγ producing $CD4^+$ T cells and **(e)** absolute number of $Ly6G^+$ neutrophils were assessed by flow cytometry. Histograms represent the mean of individual animals ± SEM (n=3-4). **(f-g)** WT mice were orally infected with *T. gondii* and treated with either **(f)** indomethicin or **(g)** $diMePGE_2$ or appropriate vehicle controls from days 6-8 p.i. Absolute numbers of $Ly6C^{hi}$ monocytes and $Ly6G^+$ neutrophils from the SILp. Histograms represent the mean of individual animals ± SEM (n=3-4). Statistical comparisons were performed using the Student's t test (*p<0.05, **p<0.01, ***p<0.001). Data are representative of two independent experiments.



Supplementary Figure 10: Commensal-driven PGE_2 production by Ly6C^{hi} Mo regulates neutrophil activation in acute gastrointestinal infection. During acute gastrointestinal infection, as a result of mucosal damage and enhanced exposure to commensals Ly6C^{hi} Mo and neutrophils are exposed to commensal products. In response to commensal ligands, neutrophils release pro-inflammatory factors including TNF-a and reactive oxygen species (ROS). Ly6C^{hi} Mo produce regulatory factors such as the lipid mediator PGE_2 in response to commensals. PGE_2 potently suppresses neutrophil activation and as a result of this regulatory loop neutrophil responses are controlled, limiting immunopathology. Infection of germ-free animals results in limited neutrophil responses due to the absence of commensal signals, and intestinal pathology is reduced as a result. In the absence of Ly6C^{hi} Mo as occurs in *Ccr2*$^{-/-}$ animals PGE_2 levels are reduced, leading to dysregulated neutrophil responses towards the commensal microbiota and increased immunopathology.