Cell Host & Microbe, Volume *14*

Supplemental Information

Intraluminal Containment of Commensal Outgrowth in the Gut during Infection-Induced Dysbiosis

Michael J. Molloy, John R. Grainger, Nicolas Bouladoux, Timothy W. Hand, Shruti Naik, Mariam Quinones, Amiran K. Dzutsev, Ji-Liang Gao, Giorgio Trinchieri, Philip M. Murphy, and Yasmine Belkaid

Supplemental Information Inventory

Supplemental Information includes six figures and one table:

- Figure S1, related to Figure 1.
- Figure S2, related to Figure 3.
- Figure S3, related to Figure 4.
- Figure S4, related to Figure 5.
- Figures S5, related to Figure 6.
- Table S1: sequences of the bacterial 16S rRNA gene primers used in this study.

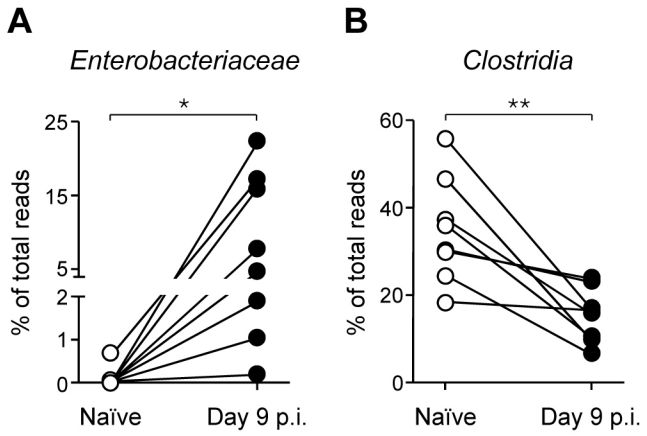


Figure S1 (related to Figure 1). Statistical analysis of the genomic alterations observed between naïve and *T. gondii*-infected mice. The 454 genomic analysis of fecal samples from naïve and acute *T. gondii* infected mice were found to have statistically significant increases in the proportion of *E. coli* (**A**) and reductions in Clostridia species (**B**). Each dot pair represents single mouse analyzed at the indicated time points (*$P<0.05$, **$P<0.01$). All data shown are representative of two independent experiments with similar results.

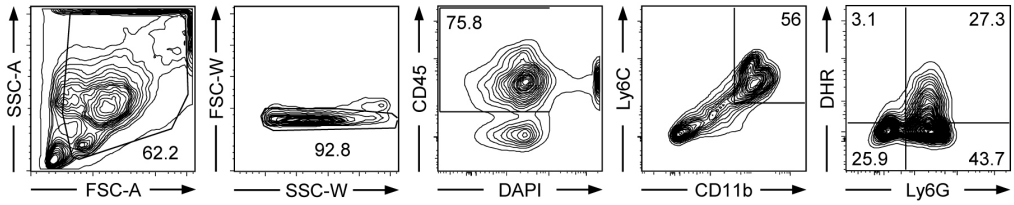


Figure S2 (related to Figure 3). Gating strategy used to analyze the leukocytes from the lumen of *T. gondii*-infected mice by flow cytometry.

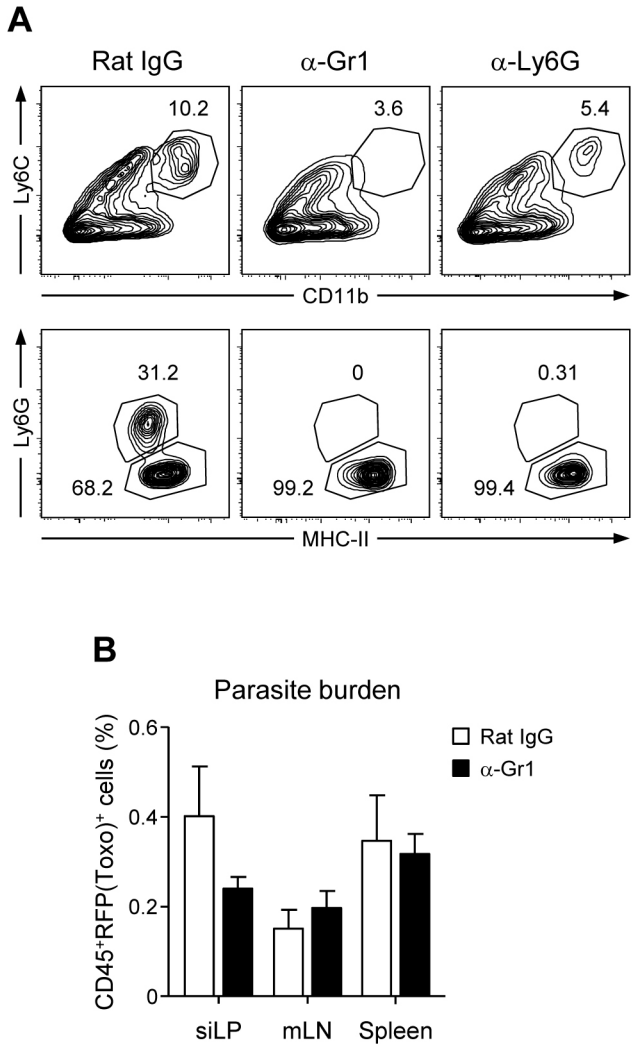


Figure S3 (related to Figure 4). Depletion of neutrophils and inflammatory monocytes by α-Gr1 and α-Ly6G and effects on parasite burden. (**A**) The percentage of inflammatory monocytes and neutrophils were determined in mice treated with either α-Gr1 or α-Ly6G. (**B**) The parasite burden of rat IgG and α-Gr1 treated *T. gondii*-infected mice was determined at day 9 p.i. by analyzing the percentage of infected $CD45^+$ cells. All data shown are representative of two independent experiments with similar results. Each bar represents the mean ± SEM of three to four mice analyzed. All data shown are representative of two independent experiments with similar results.

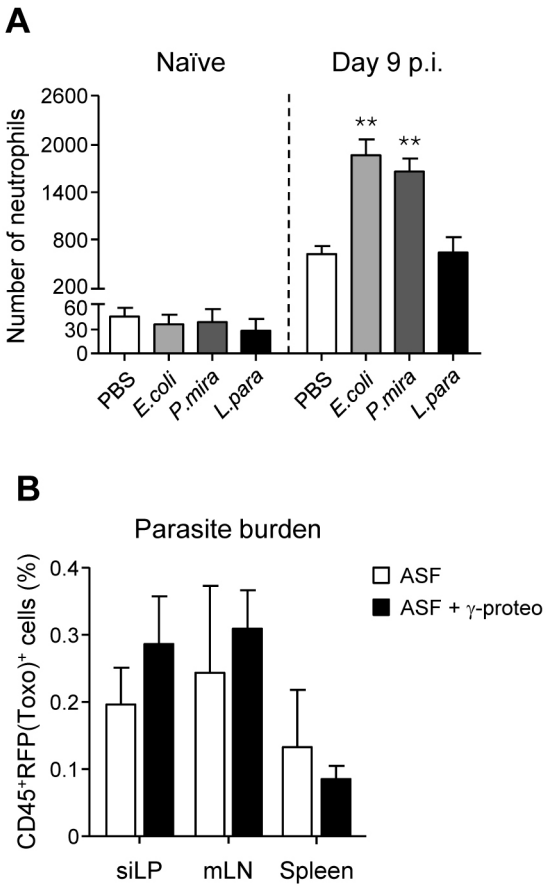
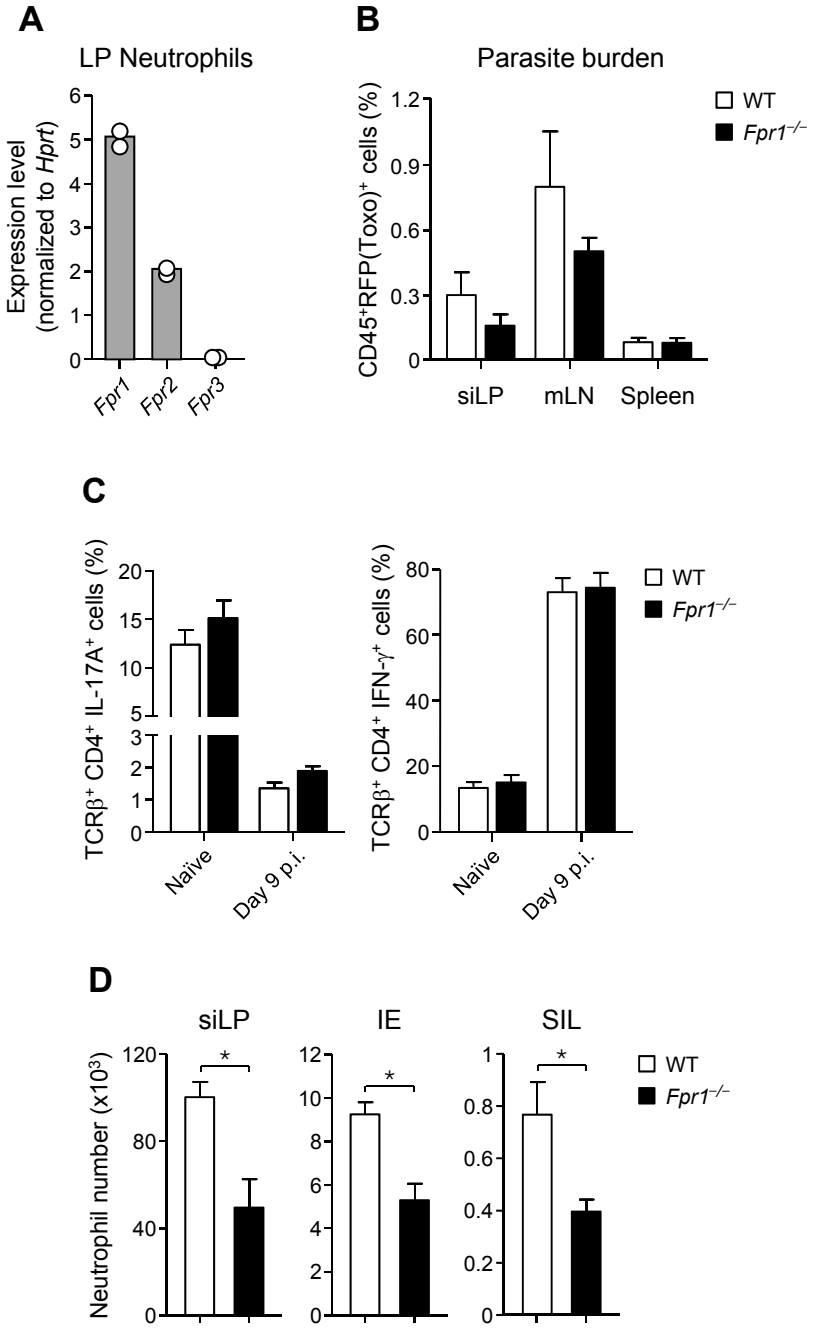


Figure S4 (related to Figure 5). Gavage of γ-proteobacteria results in increased luminal recruitment of neutrophils into *T. gondii* infected mice but not in naïve mice. (A**)** Mice were infected with 15 *T. gondii* cysts and gavaged at day 6 p.i. with either *E. coli*, *P. mirabilis* or *L. paracasei*. Bar graphs show the number (mean ± SEM) of luminal neutrophils isolated at day 9 p.i. (***P<0.01*). (**B**) The parasite burden of mice colonized with ASF or ASF + γ-proteobacteria was determined at day 9 p.i. by analyzing the percentage of infected CD45+ cells. Each bar represents the mean ± SEM of three to four mice analyzed. Data shown are representative of a single experiment.



Molloy et *al.* Figure S5

Figure S5 (related to Figure 6). Analysis of WT and *Fpr1*$^{-/-}$ mice for alterations in immune function at steady state and during *T. gondii* infection. (A) Small intestine *lamina propria* neutrophils express high levels of *Fpr1*. Neutrophils were FACS purified from the small intestine *lamina propria* of mice on day 8 after oral infection with 15 *T. gondii* cysts. Cells were resuspended in TRIzol and mRNA isolated. *Fpr1*, *Fpr2* and *Fpr3* expression levels were then analyzed by RT-PCR. Circles represent the relative expression for each sample after normalization to the housekeeping gene *Hprt* and bars the mean relative expression. (**B**) Parasite burden from WT and *Fpr1*$^{-/-}$ mice infected with *T. gondii* for 9 days. (**C**) Naïve and *T. gondii*-infected WT and *Fpr1*$^{-/-}$ mice were analyzed for differences in TCRβ^{+}CD4^{+} producing IFN-γ and IL-17A. (**D**) Total neutrophils in the siLP, IE and lumen (SIL) of WT and *Fpr1*$^{-/-}$ mice on day 11 p.i. Each bar represents the mean $\pm$ SEM of three to four mice analyzed (*$P<0.05$). All data shown are representative of two independent experiments with similar results.

Table S1: Bacterial 16S rRNA gene primers used in this study.

16S rRNA gene	Forward Primer	Reverse Primer
Eubacteria (Universal)	ACTCCTACGGGAGGCAGCAGT	ATTACCGCGGCTGCTGGC
Enterobacteriacae	GTGCCAGCMGCCGCGGTAA	GCCTCAAGGGCACAACCTCCAAG
Escherichia coli	CATGCCGCGTGTATGAAGAA	CGGGTAACGTCAATGAGCAAA
Bacteroides	GGTTCTGAGAGGAGGTCCC	GCTGCCTCCCGTAGGAGT
Eubacterium rectale/Clostridium coccoides group (EREC)	ACTCCTACGGGAGGCAGC	GCTTCTTAGTCAGGTACCGTCAT
Segmented Filamentous Bacteria (SFB)	GACGCTGAGGCATGAGAGCAT	GACGGCACGGATTGTTATTCA
Lactobacillus/Lactococcus group	AGCAGTAGGGAATCTTCCA	CACCGCTACACATGGAC