

Composition of the gut microbiota modulates the severity of malaria

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Plasmodium infections result in clinical presentations that range from asymptomatic to severe malaria, resulting in ~1 million deaths annually. Despite this toll on humanity, the factors that determine disease severity remain poorly understood. Here, we show that the gut microbiota of mice influences the pathogenesis of malaria. Genetically similar mice from different commercial vendors, which exhibited differences in their gut bacterial community, had significant differences in parasite burden and mortality after infection with multiple *Plasmodium* species. Germfree mice that received cecal content transplants from “resistant” or “susceptible” mice had low and high parasite burdens, respectively, demonstrating the gut microbiota shaped the severity of malaria. Among differences in the gut flora were increased abundances of *Lactobacillus* and *Bifidobacterium* in resistant mice. Susceptible mice treated with antibiotics followed by yogurt made from these bacterial genera displayed a decreased parasite burden. Consistent with differences in parasite burden, resistant mice exhibited an elevated humoral immune response compared with susceptible mice. Collectively, these results identify the composition of the gut microbiota as a previously unidentified risk factor for severe malaria and modulation of the gut microbiota (e.g., probiotics) as a potential treatment to decrease parasite burden.

Plasmodium | gut microbiome | severe malaria

Infection by *Plasmodium* species remain a global health burden causing over 200 million cases of malaria and around 1 million deaths annually, with the vast majority of fatalities being children under the age of 5 y living in sub-Saharan Africa (1). Many *Plasmodium* infections are either asymptomatic or cause only mild malaria. However, some infections progress to severe malaria that most often manifests as impaired consciousness (cerebral malaria), respiratory distress, and severe anemia (2). The best correlate of disease severity following *Plasmodium falciparum* infection in humans is parasite density (3, 4).

The gut microbiota has an impact on multiple facets of host physiology (5), including shaping susceptibility to numerous diseases (6–14). The effects of the gut microbiota on the host are strongly influenced by the collective composition of the bacterial populations (15), and commensal flora are known to affect local pathogen burdens and host immunity (16–18). In addition to influencing local gut immunity, the gut microbiome affects host immunity to extra-gastrointestinal tract viral infections (19).

Recent studies also support that the gut microbiome modulates *Plasmodium* infections in humans. Anti- α -gal Abs, induced by the gut pathobiont *Escherichia coli* O86:B7, cross-react with sporozoites from human and rodent *Plasmodium* species that impair transmission of the parasite between the vector and vertebrate host; however, this cross-reactive immunity did not affect blood stage parasite burden (20). Additionally, the stool bacteria composition of Malian children correlated prospectively with risk of *P. falciparum* infection, but not progression to febrile malaria (21). Importantly, it remains unclear whether the gut microbiome also contributes to the development of severe malaria. Using the murine model of malaria,

these data demonstrate that the gut microbiome affects blood stage parasite burden and the subsequent severity of malaria.

Results

Mice from Different Vendors Exhibit Differential Susceptibility to Malaria. Genetically similar inbred strains of mice (C57BL/6) maintained by different vendors [Jackson Laboratory (Jax) and Taconic (Tac)] have differences in their gut bacterial communities (22, 23). To determine whether these differences had any effect on *Plasmodium* infections, C57BL/6 mice from Jax, Tac, National Cancer Institute/Charles River (NCI), and Harlan (Har) were infected with *Plasmodium yoelii*. Following infection, profound differences in parasitemia (the fraction of RBCs infected with *P. yoelii*) were observed between the four groups of mice (Fig. 1 A and B). Whereas resistant mice (Jax and Tac) exhibited a maximum of ~10% parasitemia, they had no signs of morbidity (weight loss) or mortality, which was in contrast to the substantial weight loss and mortality observed in susceptible mice (NCI and Har), where parasitemia was >60% (Fig. 1 C and D). Moreover, NCI and Har mice exhibited more profound and longer lasting anemia

Significance

Plasmodium infections cause >200 million cases of malaria and ~1 million deaths annually. Although these infections result in disease states that range from asymptomatic to life-threatening, factors that contribute to disease severity remain poorly defined. This report demonstrates that the assemblage of microbes in the gut can modulate the severity of malaria. Mice from different vendors with differences in their gut microbiome showed significant differences in pathology after infection with *Plasmodium*. Among the bacterial populations that were different between “resistant” and “susceptible” mice were *Lactobacillus* and *Bifidobacterium*, and treatment of mice with *Lactobacillus* and *Bifidobacterium* resulted in decreased *Plasmodium* burden. These results identify both a previously unidentified risk factor for severe malaria and a potential new avenue of treatment.

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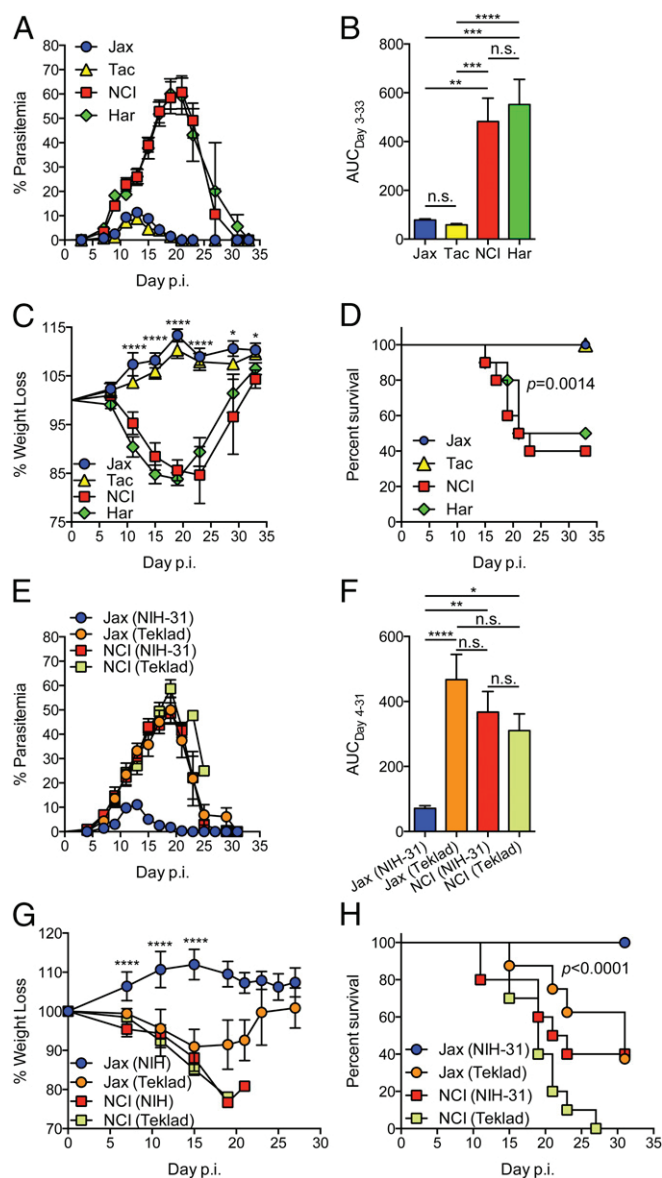


Fig. 1. *Plasmodium* parasite burden, morbidity, and mortality vary by mouse vendor and diet. C57BL/6 mice were infected with *P. yoelii* parasitized RBCs. (A) Fraction of RBCs infected with *P. yoelii* (percentage of parasitemia). (B) AUC analysis. Data were analyzed by one-way ANOVA and Tukey's multiple comparison posttest. (C) Percentage of weight loss following infection. Data were analyzed by one-way ANOVA. (D) Survival of mice following infection. Survival curves were analyzed by log-rank (Mantel-Cox) test. (E–H) Mice were fed either NIH-31 or Teklad 22/5 diet before and after *P. yoelii* infection. (E) Percentage of parasitemia following *P. yoelii* infection. (F) AUC analysis. Data were analyzed by one-way ANOVA and Tukey's multiple comparison posttest. (G) Percentage of weight loss following infection. Data were analyzed by one-way ANOVA. (H) Survival of mice following infection. Survival curves were analyzed by log-rank (Mantel-Cox) test. Data (mean $\pm$ SE) in A–F and H are cumulative results ($n = 8$ –10 mice per group) from two experiments. Data (mean $\pm$ SD) in G are from four to five mice per group from one experiment. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. n.s., not significant; p.i., postinfection.

(loss of RBCs per milliliter) compared with Jax and Tac mice (SI Appendix, Fig. 1A). Additionally, when the total number of RBCs per milliliter was used to derive total pathogen burden, similarities were noted between the parasite burden as detected by parasitemia or parasitized RBCs (pRBCs) per milliliter of blood (SI Appendix, Fig. 1B–E). Of note, mice infected with different doses of *P. yoelii* pRBCs showed similar parasitemia kinetics between the

different doses and susceptibility to infection (SI Appendix, Fig. 2), suggesting mice from different vendors are differentially susceptible to progression to severe malaria but not to blood stage infection.

To determine the broader applicability of these data, another mouse strain and two *Plasmodium* species were tested. BALB/c mice from Jax, Tac, Charles River (CR), and Har were infected with *P. yoelii*. Mice were purchased from CR in lieu of NCI. Of note, C57BL/6 mice purchased from CR exhibit similar parasitemia and morbidity as NCI mice following infection with *P. yoelii* (SI Appendix, Fig. 3). Consistent with *P. yoelii* infections in C57BL/6 mice (SI Appendix, Fig. 3), BALB/c mice from Jax and Tac exhibit reduced *P. yoelii* parasitemia compared with mice from CR and Har (SI Appendix, Fig. 4A and B). Furthermore, C57BL/6 mice from Jax and Tac exhibited reduced parasitemia compared with mice from CR and Har following *Plasmodium chabaudi* infection (SI Appendix, Fig. 4C and D). Finally, we assessed the development of experimental cerebral malaria in C57BL/6 mice infected with *Plasmodium berghei* ANKA. Jax and Tac mice trended toward reduced parasitemia compared with NCI and Har mice at early time points; moreover, there was a significant ($P = 0.04$) difference in survival between these groups of mice (SI Appendix, Fig. 4E and F). In sum, these data indicate the severity of malaria was dependent on the source of mice.

Diet is a strong modulator of organismal health as well as the gut microbiome and its function (24). To determine whether the diet could shape the severity of malaria, Jax and NCI mice were fed one of two commercially available rodent diets, either NIH-31 (used in Fig. 1A–D) or Teklad 22/5. Parasitemia in NCI mice was unaffected; however, Jax mice had high levels of parasitemia when fed Teklad 22/5 (Fig. 1E and F). Consistent with the parasitemia data, Jax mice fed Teklad 22/5 also exhibited substantial weight loss and elevated mortality compared with Jax mice fed NIH-31 (Fig. 1G and H). Because these diets had no effect on parasite burden in NCI mice, the changes in parasitemia in Jax mice were unlikely due to a direct effect of these diets on the parasite burden. Moreover, high parasite burdens in NCI mice fed NIH-31 suggest this diet supported the proliferative expansion of *P. yoelii*. When Jax and NCI mice were placed on the reciprocal vendor-specific diet and then infected with *P. yoelii*, we noted a modest increase in parasite burden in Jax mice fed the NCI in-house diet but no effect of the Jax in-house diet on NCI mice (SI Appendix, Fig. 5). Collectively, these datasets led to the hypothesis that the gut microbiota influenced *Plasmodium* infections.

Gut Bacterial Community Structure and Function Are Different in Resistant and Susceptible Mice. To test directly for differences in the gut microbiome, sections of the gastrointestinal tract from resistant (Jax and Tac) and susceptible (NCI and Har) mice were collected and the bacterial communities were characterized using 16S rRNA gene analysis (SI Appendix, Table 1). There was a high degree of similarity between the microbial community assemblages found within the cecum and colon of mice from the same vendor (SI Appendix, Fig. 6A), whereas there were clear differences between the microbial communities of these regions compared with the distal half of the small intestine in mice from the same vendor. Moreover, significant differences between mice from all vendors were apparent in the nonmetric multidimensional scaling analysis of population structure within the cecum, with the susceptible NCI and Har libraries showing a comparative overlap with each other yet distinct differences compared with the resistant Jax and Tac communities (SI Appendix, Fig. 6B). Analysis of the cecal bacterial communities at the family level revealed substantial differences, with Clostridiaceae, Erysipelotrichaceae, Lactobacillaceae, and Peptostreptococcaceae (members of the Firmicutes phylum) being proportionally more abundant in resistant (Jax and Tac) mice, whereas Bacteroidaceae and Prevotellaceae (members of Bacteroidetes phylum) and Sutterellaceae (member of Proteobacteria phylum) were proportionally more abundant in susceptible (NCI and Har) mice (Fig. 2A and B). Finally, dietary changes are capable of inducing significant changes in the gut microbiome (25) that reach steady state within 3–4 d in mice (26). Consistent with these reports, we observed defined changes in the gut bacterial

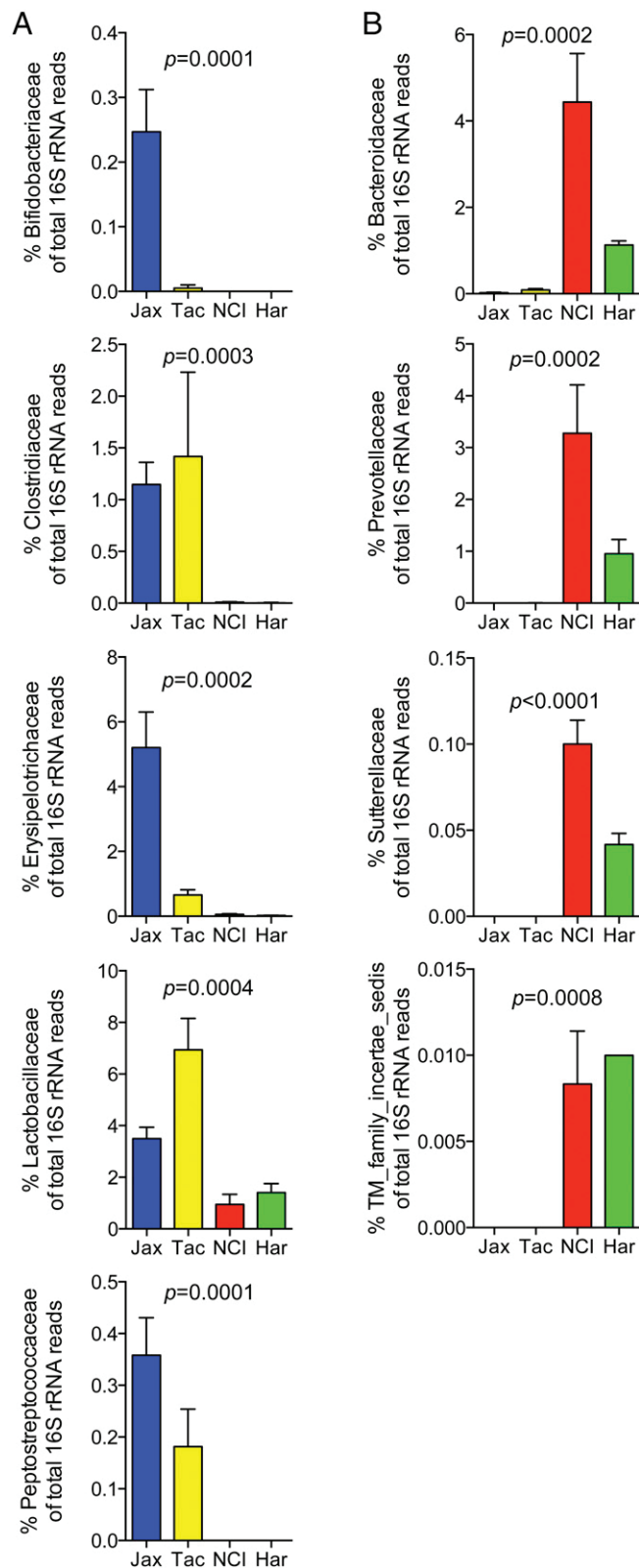


Fig. 2. Susceptibility to malaria correlates with differences in cecal bacteria populations. (A) Bacterial families that were identified as being significantly enriched in Jax or Tac mice. (B) Bacterial families identified as being significantly enriched in NCI or Har mice. Data (mean $\pm$ SE) in A and B are from six mice per group and extracted from analysis in *SI Appendix*, Fig. 6C. Data were analyzed by the Kruskal–Wallis test.

communities in Jax mice fed Teklad 22/5 or NIH-31 (*SI Appendix*, Figs. 7 and 8). In Jax mice fed the Teklad diet, there was a noted decrease in Peptostreptococcaceae below the levels observed in either Jax or Tac mice, resulting in an increased similarity to the susceptible NCI and Har mice (*SI Appendix*, Fig. 7C). These changes coincide with a shift in the severity of malaria between these two groups of mice (Fig. 1 E–H).

Consistent with changes in the gut bacterial community, analysis of metabolites in the small intestine, cecum, and plasma of Jax and NCI mice revealed differential expression between each tissue (*SI Appendix*, Fig. 9A). An *F* test of partial least squares discriminant analysis (27) used to probe variation between metabolite profiles in Jax and NCI mice on a per tissue basis confirmed that the means of the variate-1 (component 1), which differentiated Jax from NCI mice in all tissues, were significantly different ($P \leq 0.0003$, $P \leq 0.0001$, $P \leq 0.0001$) for the small intestine, cecum, and plasma, respectively, (*SI Appendix*, Fig. 9 B–D and Table 2). Several metabolites exhibited large (≥ 1.5 fold) and statistically significant ($P \leq 0.1$) differences between Jax and NCI mice, with the top 25% of metabolites associated with distinct metabolic pathways (*SI Appendix*, Fig. 9 E and F and Tables 3–5). Therefore, differences in the gut bacterial populations and metabolites support the hypothesis that the severity of malaria was modulated by differences in gut bacterial communities.

Differences in the Gut Microbiome Shape Susceptibility to Malaria.

To test this hypothesis directly, genetically identical germfree (GF) C57BL/6 mice received cecal content transplants from either Jax or NCI mice. Of note, GF C57BL/6J mice exhibited no difference in parasitemia compared with conventional C57BL/6J mice following infection with *P. yoelii nigeriensis* (28). Sequence analyses demonstrated the bacterial communities in colonized, GF mice reflected the bacterial communities of the donor communities and were different from the communities in GF mice exposed to only environmental microbes (Fig. 3A). Furthermore, there was only a slight decrease in community diversity between the respective donor and colonized GF mice (*SI Appendix*, Fig. 10). Following *P. yoelii* infection, GF mice that received either Jax or NCI cecal transplants had parasite burdens similar to control Jax and NCI mice (Fig. 3 B and C). Both NCI control mice and GF mice that received NCI cecal transplants also had decreased survival compared with the Jax control mice and GF mice that received Jax cecal transplants (Fig. 3D). Collectively, these data provided a direct demonstration that the severity of malaria was modulated by the gut microbiota.

Decreased Parasite Burden in Mice Treated with *Lactobacillus* and *Bifidobacterium*.

To identify individual microbial phylotypes that may shape the severity of malaria, a deeper analysis was performed on the bacterial communities in the cecum. When pooled by resistance (Jax/Tac) or susceptibility (NCI/Har) to *P. yoelii*, several phylotypes (referred to here as operational taxonomic units) emerged from a linear discriminant analysis effect size (LEfSe)-driven analysis (29) as biomarkers of the resistant or susceptible phenotype. Among those differences, *Lactobacillus* and *Bifidobacterium* were overly abundant in the resistant mice compared with susceptible mice (*SI Appendix*, Fig. 11), with differences in *Lactobacillus* being the greatest driver of the differential community structure between resistant and susceptible mice (*SI Appendix*, Fig. 11B).

To evaluate the linkage between *Lactobacillus* and *Bifidobacterium* toward resistance to severe malaria, Jax and NCI mice were treated with laboratory-cultured yogurt supplemented with probiotics that contained *Lactobacillus* and *Bifidobacterium* species before and following infection with *P. yoelii*. DNA sequencing of *Lactobacillus* isolated from fecal pellets from Jax and NCI mice or laboratory-cultured yogurt demonstrated phylogenetic congruence (*SI Appendix*, Fig. 12). Consumption of *Lactobacillus* and *Bifidobacterium* can modulate the gut microbial community structure (30) or function (31). Following infection with *P. yoelii*, both Jax and NCI mice treated with yogurt had a modest, but significant (Jax: $P < 0.0001$, NCI: $P = 0.0418$), decrease in parasite burden compared with control untreated mice (*SI Appendix*, Fig. 13). Jax and NCI mice treated with

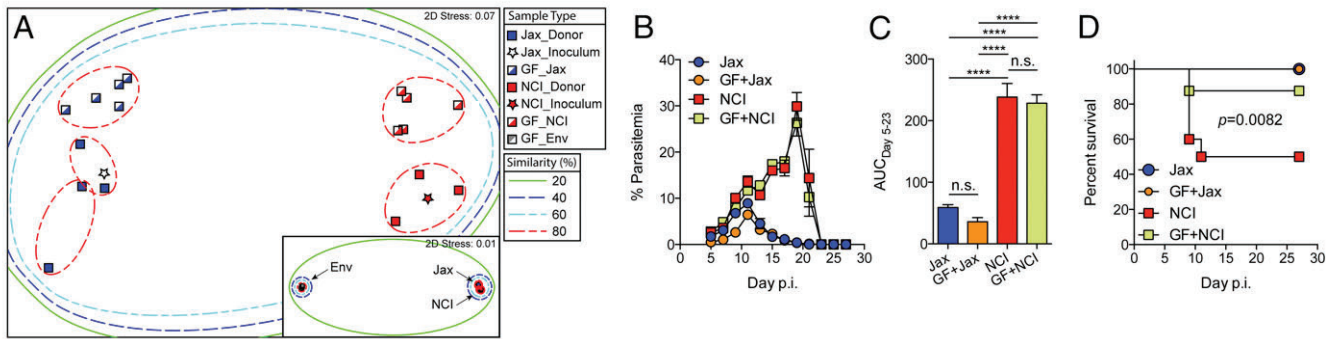


Fig. 3. Gut microbiome shapes susceptibility to severe malaria. GF mice were colonized with cecal contents from Jax or NCI mice. (A) Bacterial population analysis was performed using nonmetric multidimensional scaling, as described in *SI Appendix, Fig. 6*. (B–D) Colonized GF mice and control Jax and NCI mice were infected with *P. yoelii*. (B) Percentage of parasitemia following *P. yoelii* infection. (C) AUC analysis. Data (mean $\pm$ SE) in B and C from four to five mice per group are representative of two experiments. Data were analyzed by one-way ANOVA and Tukey's multiple comparison posttest. (D) Survival of mice following infection. Data are cumulative results ($n = 8$ –10 mice per group) from two experiments. Survival curves were analyzed by log-rank (Mantel–Cox) test. **** $P < 0.0001$. Env, environment.

milk used to make the yogurt showed a similar parasite burden as control Jax and NCI mice [mean area under the parasitemia curve (AUC) on days 5–34 (AUC_{Day 5–34}): Jax control ($n = 4$): 107.2 ± 11.39 (SD) vs. Jax milk ($n = 4$): 83.55 ± 24.83 (SD), $P = 0.13$; NCI control ($n = 4$): 447.1 ± 85.65 (SD) vs. NCI milk ($n = 3$): 384.8 ± 73.08 (SD), $P = 0.36$]. However, when mice were treated with antibiotics before yogurt treatment, we observed a profound decrease (14-fold) in parasite burden in the susceptible NCI mice (Fig. 4A and B), and no weight loss in those mice was noted compared with the other NCI groups (Fig. 4C). These data support the ability of *Lactobacillus* and *Bifidobacterium* to contribute toward the modulation of *Plasmodium* parasite burden, yet other constituents of the gut microbiota may also contribute to regulating the severity of malaria.

Severity of Malaria Correlates with the Magnitude of the Host Immune Response. The gut microbiota can shape host immunity to systemic viral infections (19), and T follicular helper (Tfh)-produced IL-21 is required for germinal center (GC) B-cell help and clearance of murine *Plasmodium* infections (32). Consistent with these observations, resistant Jax mice exhibited elevated *P. yoelii*-specific CD4⁺ T-cell [CD49d^{hi}CD11a^{hi} (33)], Tfh cell, and GC B-cell responses compared with susceptible NCI mice (Fig. 5A–C and *SI Appendix, Fig. 14*). Jax and NCI mice had similar titers of IgM specific for the 19-kDa fragment of merozoite surface protein 1 (MSP1₁₉) from *P. yoelii* (Fig. 5D), suggesting similar activation of B cells in both groups. In contrast, Jax mice exhibited accelerated Ab class switching from MSP1₁₉-specific IgM to IgG isotypes, four- to 10-fold higher titers at day 14 postinfection, compared with NCI mice (Fig. 5D). Thus, one mechanism by which

the gut microbiome shapes the severity of malaria following *P. yoelii* infection may be through modulation of the host immune response.

Discussion

This study demonstrates that the murine gut microbiome influences the parasite burden of *Plasmodium* rodent species and modulates the severity of malaria in mice. Importantly, parasite burden is currently the best-known correlate of disease severity following *P. falciparum* infection in humans (3, 4). An association between the gut microbial community and *Plasmodium* parasites has been previously recognized in the mosquito vector (34–38). Interestingly, the unique assemblage of skin bacteria on human skin has also been shown to have an impact on the attractiveness of *Anopheles* mosquitoes to particular individuals (39, 40).

Two recent publications further support that the gut microbiota affects mammalian stages of the *Plasmodium* life cycle. The first study demonstrated that specific gut bacteria could have an impact on the transmission of *P. berghei* sporozoites from mosquitoes to mice (20). The authors showed that the gut pathobiont *E. coli* O86:B7 induced the production of anti- α -gal Abs. When *Plasmodium*-infected mosquitoes injected sporozoites into the dermal tissue during a blood meal, the anti- α -gal Abs bound to the *Plasmodium* sporozoites, which prevented their migration to the liver (20). These results also extended to humans, where the presence of anti- α -gal IgM Abs correlated with protection against *P. falciparum* infection. The effect of *E. coli* O86:B7 on *Plasmodium* infection was limited to transmission of sporozoites because there was no effect of the anti- α -gal Abs on the symptomatic blood stage of the infection. Consistent with these findings, a second report

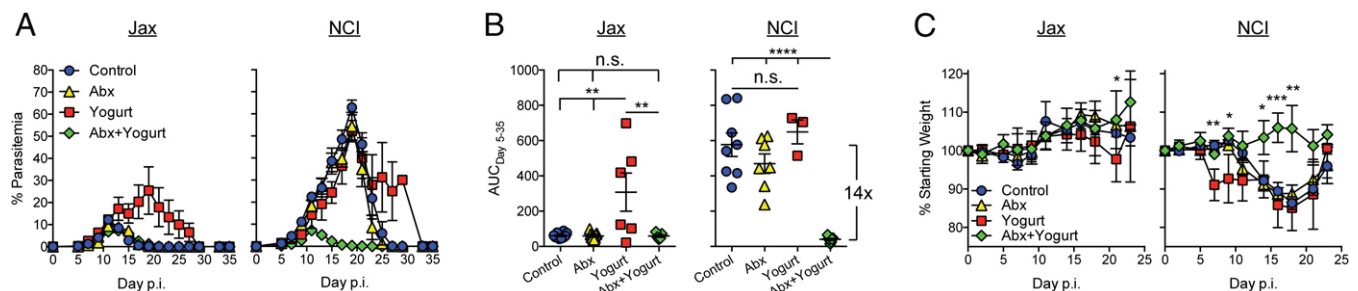


Fig. 4. Susceptible mice treated with yogurt have decreased parasitemia and morbidity. Jax and NCI mice were left untreated (control), treated with antibiotics for 3 wk and then left untreated for an additional 3 wk (Abx), left untreated for 3 wk followed by treatment with yogurt five times per week for 3 wk (Yogurt), or treated with antibiotics for 3 wk followed by treatment with yogurt five times per week for 3 wk (Abx+Yogurt). Mice were then infected with *P. yoelii*. Yogurt-treated mice continued to receive yogurt five times per week following infection. (A) Percentage of parasitemia following *P. yoelii* infection. (B) AUC analysis. Data were analyzed by one-way ANOVA and Tukey's multiple comparison posttest. (C) Percentage of weight loss following infection. Data were analyzed by one-way ANOVA. Data (mean $\pm$ SE) in A–C are cumulative results ($n = 3$ –10 mice per group) from two experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

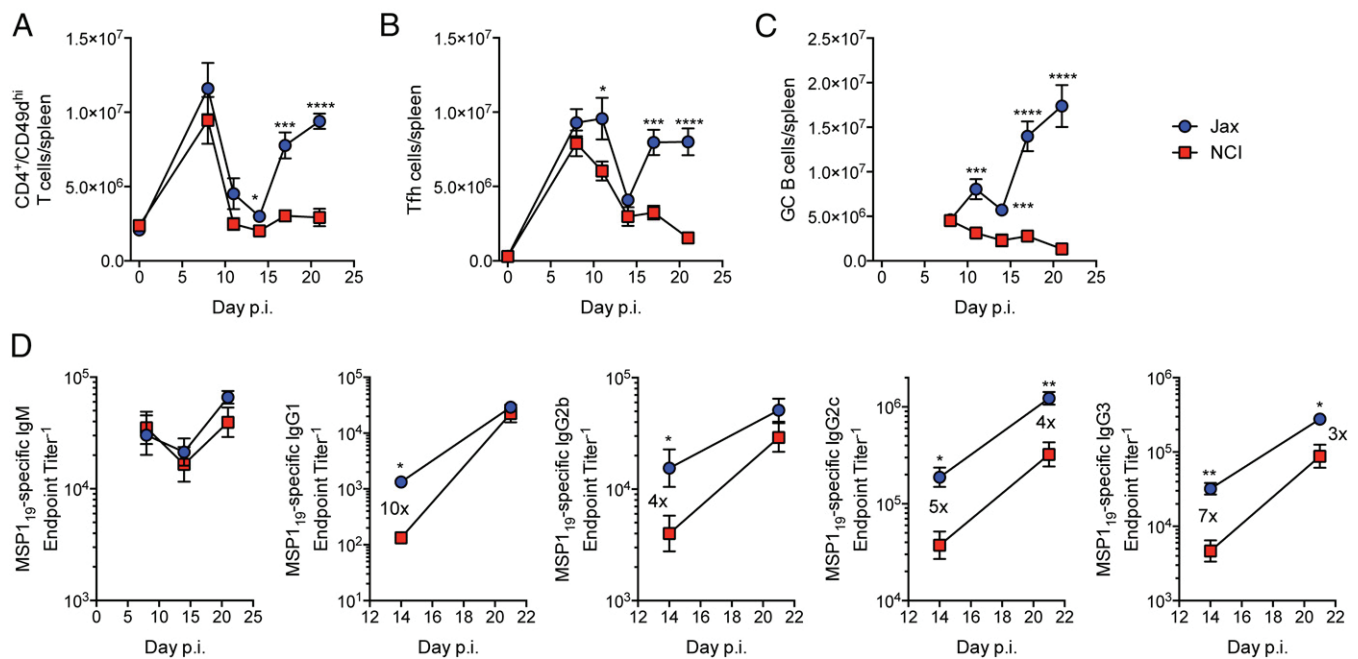


Fig. 5. Resistant Jax mice have an elevated cellular and humoral immune response to *Plasmodium*. Jax and NCI mice were infected with *P. yoelii*. Total number of CD4⁺CD11a^{hi}CD49d^{hi} cells (A), Tfh cells (B), and germinal center (GC) B cells (C) per spleen on the indicated day. Data (mean ± SE) are cumulative results ($n = 5-10$ mice per data point) from three experiments. (D) Serum MSP1₁₉-specific Ab end-point titers. Data (mean ± SE) are cumulative results ($n = 3-7$ mice per data point) from two experiments. Numbers in the panels represent the fold difference between the means of the Jax and NCI mice. Data were analyzed by an unpaired two-tailed *t* test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

demonstrated that the unique composition of stool bacteria in Malian children correlated with prospective risk of *P. falciparum* infection, although not progression to febrile malaria (21). Although the mechanism responsible for this observation is unknown, the similarities between these two studies (i.e., susceptibility to infection but not severity of blood stage infection) suggest the prospective risk of *P. falciparum* infection differentiated by stool bacteria composition may be attributed to differences in anti- α -gal IgM Abs. In contrast to these two publications, we show that the gut microbiota modulates the severity of *P. yoelii* blood stage infections in mice, implying a different mechanism. Moreover, our findings show that the influence of the gut microbiome on *Plasmodium* infections is broad and not limited to the transmission of the parasite. Taken together, our observations and the findings of Yilmaz et al. (20) result in the intriguing speculation that the human intestinal microbiota might have an impact on different stages of the *Plasmodium* life cycle in humans. Clearly, this area is ripe for future research.

One potential mechanism by which the gut microbiota regulates the severity of malaria is a direct effect on the parasite itself, where gut microbiota-derived products either promote or inhibit its growth. Although this possibility has not been formally excluded, we observe similar parasitemia expansion kinetics, when plotted on a log scale, between days 5 and 11 postinfection in both resistant and susceptible mice. This observation suggests that the gut microbiota does not have a direct effect on the parasite. Consequently, it is more likely that the gut microbiota has an impact on the severity of malaria by modulating the host immune response to *Plasmodium*. Consistent with this possibility, resistant Jax mice exhibited an elevated anti-*Plasmodium* immune response compared with susceptible NCI mice. Although these data correlate with the parasite burden in these mice, further experiments will be necessary to demonstrate whether the differential immune response is responsible for the difference in severity, and if so, how the gut microbiota modulates the host immune response to this extragastrintestinal infection. It has been previously shown that the gut microbiome provides signals to monocytes/macrophages that primed those cells to respond to and help control systemic lymphocytic choriomeningitis virus infections

(19). Whether the gut microbiome modulates host immunity to *Plasmodium* through similar or different effects on the host immune system remains to be determined.

As mentioned above, diet has a major role in shaping the composition and activity of the gut microbiota (25, 41, 42). Consequently, manipulating the structure and function of these complex communities through the diet provides an opportunity to manipulate the host immune system (41). In our study, we identified that *Lactobacillus* and *Bifidobacterium* species in cecal content could have a protective role by modulating the parasite burden and attenuating the severity of the disease. It is also possible that these bacterial genera correlate with decreased parasitemia through niche competition that decreases the abundance of bacterial genera that cause elevated parasitemia. Because antibiotic treatment followed by yogurt treatment triggered a 14-fold reduction in parasite burden in susceptible mice, the results suggest that through optimization (e.g., identifying and treating with the most effective “protective” bacterial species or eliminating bacteria that contribute to high parasitemia), modulating the gut microbiome has the potential to be a novel prophylaxis to prevent severe malaria. Consistent with this possibility, prior work has shown that children in a rural African village in Burkina Faso have an enrichment of the Bacteroidetes phylum and a depletion of the Firmicutes phylum, which contains *Lactobacillus*, compared with European children (43). This bacterial assemblage resembles the community structure in susceptible mice that have increased Bacteroidetes and reduced Firmicutes compared with resistant mice (SI Appendix, Fig. 11). Therefore, the commonality between the bacterial community structure in African children and *Plasmodium*-susceptible mice suggests the possibility that probiotic modulation of the gut microbiota in mice to control severe malaria may work in humans.

This report demonstrates that the severity of malaria in mice is profoundly affected by the composition of the gut microbiota. The data lead to the hypothesis that differences in the gut microbiota may explain why some humans infected with *Plasmodium* progress to severe disease and others do not. The results also support the possibility that manipulating the gut microbiota has the potential to control the severity of malaria in humans. Whereas modulating the

gut microbiota may not prevent *Plasmodium* infections, altering the gut microbiome has the potential to ameliorate severe disease and save thousands of lives annually.

Materials and Methods

Mice and Infections. Conventionally housed mice were purchased from Jax, NCI, CR, Har, and Tac. GF mice were purchased from the National Gnotobiotic Rodent Resource Center at the University of North Carolina at Chapel Hill. The University of Tennessee and University of Louisville Institutional Animal Care and Use Committees reviewed and approved animal experiments. Mice were fed NIH-31 Modified Open Formula Mouse/Rat Irradiated Diet (Harlan 7913), unless otherwise noted, in which case mice were fed Teklad 22/5 Rodent Diet (Harlan 8640), Jax in-house diet (5K67; Cincinnati Lab & Pet Supply, Inc.), or NCI in-house diet (5L79 Cincinnati Lab & Pet Supply, Inc.). GF mice received diluted cecum material administered by oral gavage. After transplants, mice were housed using conventional conditions. Mice were infected with *P. yoelii* 17XNL, *P. chabaudi* AS, and *P. berghei* ANKA. Blood samples were taken from the tail at regular intervals from 3 to 35 d postinfection. Parasitemia, percentage of RBCs infected with *Plasmodium*, was assessed by evaluation of thin blood smears or flow cytometry. Yogurt was made using a starter culture (Yogurt Starter Culture no. 2; Custom Probiotics) enriched with a probiotic powder supplement containing numerous *Lactobacillus* and *Bifidobacterium* species (11 Strain Probiotic Powder; Custom Probiotics). Mice were treated with an oral antibiotic mixture consisting of ampicillin, vancomycin, metronidazole, neomycin sulfate, and gentamycin sulfate. Cellular immune response was measured by flow cytometry, and MSP1₉-specific Abs were measured by ELISA.

Gut Microbiota Analysis. The distal half of the small intestine, cecum, and colon was excised from mice and flash-frozen in liquid nitrogen. DNA was extracted from samples using the MoBio PowerSoil DNA Isolation Kit. Bacterial 16S rRNA

genes were amplified using bacteria-specific PCR primers targeting the V4 region. DNA sequencing was completed using the MiSeq (Illumina) platform at Hudson Alpha Institute for Biotechnology, Huntsville, AL. Sequences have been deposited in the NCBI Sequence Read Archive under Bioproject PRJNA289122. The Mothur software package was used to process sequences, to cluster sequences for phylogenetic classification, and to sort sequences into groups based on digestive tract regions. The PRIMER-E software package was used to interrogate the relationships between phylotypes across samples and to derive correlations between phylotype presence/abundance and other parameters. Detection of “biomarker” sequences was performed using the software package LEfSe (huttenhower.sph.harvard.edu/galaxy/).

Statistical Analysis. Descriptive and comparative statistical analyses of data, except the gut microbiota and metabolomics data, were done using GraphPad Software (Prism, version 6). The AUC was estimated for each group following the trapezoidal rule with the following equation:

$$AUC_{t_1-t_{last}} = 0.5 \sum (Y_i + Y_{i+1}) * (t_{i+1} - t_i),$$

where “t” is sampling time and “Y” is the observed outcome (e.g., percentage of parasitemia).

Additional details are available in *SI Appendix, Materials and Methods*.

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1 **Supplementary Information:**

2

3 **Materials and Methods**

4 **Animals and housing.** Conventionally housed mice: Female C57BL/6J and BALB/cJ
5 mice (6-8 weeks old) were purchased from The Jackson Laboratory (Bar Harbor, Maine);
6 C57BL/6N and BALB/cN mice (6-8 weeks old) were purchased from the National
7 Cancer Institute (Frederick, MD), Charles River Laboratories (Wilmington, MD), Harlan
8 Laboratories (Indianapolis, IN) and Taconic (Hudson, NY). Mice were acclimatized for
9 a minimum of 7 days prior to starting experiments.

10 Germ-free mice: Female C57BL/6J mice (8-10 weeks old) were purchased from The
11 National Gnotobiotic Rodent Resource Center at the University of North Carolina-Chapel
12 Hill. Upon arrival at the University of Tennessee the mice were kept in the transporting
13 box until fecal transplant was done, then mice were housed in conventional (specific
14 pathogen free) conditions. Animal experiments were carried out at The University of
15 Tennessee adhering to the local and national regulations of laboratory animal welfare.
16 Procedures involving the care or use of mice were reviewed and approved by The
17 University of Tennessee Institutional Animal Care and Use Committee.

18

19 **Diets.** Unless noted otherwise mice were fed NIH-31 Modified Open Formula
20 Mouse/Rat Irradiated Diet (Harlan 7913) (Harlan, Indianapolis, IN). In some
21 experiments mice were fed Teklad 22/5 Rodent Diet (Harlan 8640; Harlan, Indianapolis,
22 IN), Jackson Laboratory in-house diet (5K67; Cincinnati Lab & Pet Supply, Inc.;
23 Cincinnati, OH) or NCI/CR in-house diet (5L79 Cincinnati Lab & Pet Supply, Inc.;

Cincinnati, OH). Mice were placed on the respective diets upon arrival at the facility and maintained on the different diets for at least one week prior to *P. yoelii* infection or removal of intestinal tissues for bacterial population analysis.

Plasmodium infection. Mice were infected with *P. yoelii* 17XNL, *P. chabaudi* AS, or *P. berghei* ANKA. For *P. yoelii* or *P. berghei* ANKA infections mice received 1×10^5 parasitized red blood cells (intravenously) prepared from frozen/thawed stabilites. For *P. chabaudi* infections, experimental mice were infected intraperitoneally with freshly prepared 1×10^5 parasitized red blood cells after one *in vivo* passage in C57BL/6 mice.

Evaluation of parasitemia. Blood samples were taken from the tail at regular intervals from 3 to 35 days post-infection. Parasitemia was assessed by evaluation of thin blood smears or flow cytometry. Thin blood smears—at least 5 high-power (1000x) fields were assessed for each sample. Total red blood cells and parasitized red blood cells were counted in each field. Flow cytometry—about 5-10 μ l of blood was added to PBS and then fixed with 0.00625%% glutaraldehyde. Cells were stained with CD45.2-APC (clone 104; Biolegend; San Diego, CA), Ter119-APC/Cy7 (clone TER-119; Biolegend; San Diego, CA), dihydroethidium (Sigma Aldrich; St. Louis, MO), and Hoechst 33342 (Sigma Aldrich; St. Louis, MO). Red blood cells (CD45.2⁻Ter119⁺) were gated on and *Plasmodium* infected cells were subsequently identified as dihydroethidium⁺Hoechst 33342⁺. Parasitemia represents the percentage of red blood cells infected with *P. yoelii*, *P. chabaudi* or *P. berghei*.

Enumeration of red blood cells and parasitized red blood cells. Blood samples were collected and used to quantify red blood cells per mL using a hemocytometer, and percent parasitemia. Red blood cells per mL and percent parasitemia were used to calculate the number of parasitized red blood cells per mL of blood.

Cecum transplant. Cecal content was squeezed from donor mice (n=3-5 mice per group) into a sterile petri dish. Immediately after harvesting the cecum material, it was diluted with sterile saline (2 mL) and mixed gently for ~30 seconds. Each germ-free mouse received the diluted cecum material (200 μ L/mouse) administered by oral gavage. For each mouse, a new sterile oral gavage needle was used. After the cecal transplant, mice were housed using conventional conditions. Mice were infected with *P. yoelii* 3 weeks after the cecal transplant.

Gut microbiota analysis.

Tissue collection. The distal half of the small intestine, cecum, and colon were excised from mice and flash frozen in liquid nitrogen. Samples were then stored at -80°C.

DNA isolation. DNA was extracted from samples using the MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA) according to manufacturer's protocols. No effort was made to separate mouse tissue from the bacterial component so as not to select against bacteria that were in close association with mouse tissue.

Ribosomal 16S sequencing. For amplification of bacterial 16S rRNA genes, bacteria specific PCR primers targeting the V4 region (bases 515-806) were used.

Amplification, barcoding and sequencing were completed by the Genome Sequencing Center at the Hudson-Alpha Institute for Biotechnology (Huntsville, AL) using the MiSeq platform with 150 bp paired-end reads.

Community analysis. We used the Mothur software package (version 1.33.1) (42) to process sequences of sufficient length and quality similarly to the Schloss MiSeq SOP (http://www.mothur.org/wiki/MiSeq_SOP). Mothur was also used to cluster sequences into phylotypes and for phylogenetic classification and to sort our sequences into groups based on the region of the digestive tract from which the DNA was extracted.

The Primer-E software package (Version 6; (43)) was used to interrogate the relationships between phylotypes across samples and to derive correlations between phylotype presence/abundance and other parameters. The “.shared” file (a matrix file containing phylotype abundances for each sample), created by Mothur, was imported directly into the Primer-E software package. All phylotype abundances were standardized to the total number of sequences per barcoded library (proportional abundances). Standardized abundances were square-root transformed to partially deemphasize more highly abundant phylotypes. A Bray-Curtis similarity matrix was constructed and used to perform non-metric multidimensional scaling analysis (NMDS) for visualization of community structure relationships between the different samples. Detection of “biomarker” sequences was performed using the software package LEfSe (<http://huttenhower.sph.harvard.edu/galaxy/>) (26).

Metabolomics analysis.

91 Collection of small intestine and cecum content samples. Immediately after euthanasia,
92 the abdominal cavity was exposed and the small intestine and the cecum were dissected
93 from the other intestinal sections. The small intestine and cecum contents were collected
94 into petri dishes by squeezing the tissues from the proximal to distal ends of the organs.
95 In addition, extraction solvent (1mL) (40:40:20 HPLC grade methanol, acetonitrile, water
96 with 0.1% formic acid) was used to flush the small intestine and the cecum using a needle
97 and syringe. The intestine contents were then transferred into separate cryotubes and
98 flash frozen in liquid nitrogen. The samples were kept on ice for approximately one hour
99 until extraction.

100 Collection of plasma samples. Blood was collected from the peri-orbital sinus using
101 heparinized capillary tubes under general anesthesia with isoflurane. Immediately after
102 collection, blood samples were transferred to centrifuge tubes and centrifuged for 10
103 minutes at 2,000 x g at room temperature. Following centrifugation, plasma samples
104 were transferred to cryotubes and flash frozen in liquid nitrogen. The samples were kept
105 on ice for approximately one hour until extraction.

106 Sample extraction and analysis. Extraction of samples was allowed to proceed at -78°C
107 for 20 minutes. Samples were then centrifuged for 5 minutes (16.1 rcf) at 4°C. The
108 supernatant was transferred to new vials and the sample pellet was resuspended in 200
109 µL of chilled (4°C) extraction solvent. The extraction was allowed to proceed for 15 min
110 at -20°C at which time the samples were centrifuged for 5 min (16.1 rcf) at 4°C. The
111 supernatant was transferred to vials and another 50 µL of extraction solvent was added to
112 the sample pellet where the extraction was repeated once more. Vials containing all of
113 the collected supernatant were dried under a stream of N₂ until the extraction solvent had

evaporated. Solid residue was resuspended in 300 μ L of sterile water and transferred to autosampler vials. Samples were immediately placed in autosampler trays for mass spectrometric analysis.

Ultra Performance Liquid Chromatography—High Resolution Mass Spectrometric (UPLC-MS) Analysis. Samples were placed in autosampler trays and kept at 4°C. A 10 μ L aliquot of each was injected through a Synergi 2.5 micron Hydro-RP 100, 100 x 2.00 mm LC column (Phenomenex) kept at 25°C. The eluent was introduced into the MS via an electrospray ionization source conjoined to a Thermo Scientific Exactive Plus Orbitrap MS through a 0.1 mm internal diameter fused silica capillary tube, and the MS was run in fullscan mode with negative mode ionization using a method adapted from Rabinowitz (44). Briefly, the samples were run with a spray voltage was 3 kV. The nitrogen sheath gas was set to a flow rate of 10 psi with a capillary temperature of 320°C. AGC target was set to 3e6. The samples were analyzed with a resolution of 140,000 and a scan window of 85 to 800 m/z for from 0 to 9 minutes and 110 to 1000 m/z from 9 to 25 minutes. Solvent A consisted of 97:3 water:methanol, 10 mM tributylamine, and 15 mM acetic acid. Solvent B was methanol. The gradient from 0 to 5 minutes was 0% B, from 5 to 13 minutes was 20% B, from 13 to 15.5 minutes was 55% B, from 15.5 to 19 minutes was 95% B, and from 19 to 25 minutes was 0% B with a flow rate of 200 μ L/min.

Metabolite feature extraction and data processing. The RAW files generated by the instrumental data collection software Xcalibur (Thermo Scientific) were converted to the mzML format (45) using the ProteoWizard package (46). The MAVEN software package (Princeton University) was used to automatically align the total ion

chromatograms using the retention times of annotated metabolites and other dominant features from each sample (47, 48). Metabolites were manually identified and integrated using known masses (± 5 ppm mass tolerance) and retention times ($\Delta \leq 1.5$ min). Metabolite ion counts were normalized via mass for cecum and small intestine samples, and fold changes were calculated between samples. The resulting data were clustered using Cluster 3.0 (49) and heatmaps were then generated from clustered data using Java Treeview (50) software. PCA analyses were performed and figures were generated using the statistical package R along with the ggplot2 (51) and ggbiplot (52) packages. PLS-DA plots were also generated via R along with the mixOmics (53) package using metabolite areas as the predictors and mouse type as the discrete outcomes with a tolerance of 1×10^{-6} and a max iteration of 500.

Yogurt treatment. Antibiotic treated mice were treated orally with an antibiotic cocktail consisting of ampicillin (0.5 mg/ml) (Sigma Aldrich; St. Louis, MO), vancomycin (0.25 mg/ml) (Amnesco; Solon, OH), metronidazole (0.5 mg/ml) (Spectrum Chemical Mfg. Comp.; Gardena, CA), neomycin sulfate (0.5 mg/ml) (EMD Millipore; Billerica, MA), and gentamycin sulfate (0.5 mg/ml) (Corning; Manassas, VA). Mice were treated *ad libitum* for 3 weeks. Water bottles containing the antibiotic cocktail were changed weekly. After 3 weeks antibiotic treatment ceased and half of the antibiotic treated mice were then treated with yogurt for 3 weeks prior to the *P. yoelii* infection. Yogurt was made using a starter culture containing *Lactobacillus bulgaricus*, *L. acidophilus*, *L. lactis*, *Bifidobacterium lactis*, and *Streptococcus thermophilus* (Yogurt Starter Culture #2, Custom Probiotics, Glendale, CA). In addition, yogurt was enriched with a probiotic

powder supplement containing *L. acidophilus*, *L. rhamnosus*, *L. salivarius*, *L. plantarum*,
L. casei, *L. lactis*, *B. breve*, *B. infantis*, *B. longum*, *B. bifidum*, and *B. lactis* (11 Strain
Probiotic Powder, Custom Probiotics, Glendale, CA). Fresh yogurt was made for each
treatment by adding the starter culture (1.2 grams) and the probiotic supplement (1.2
grams) to 15 mL of 2% reduced fat milk (Mayfield, Athens, TN) or organic 2% reduced
fat milk–antibiotic pretreatment experiment (Organic Valley; La Farge, WI). The yogurt
was then incubated at 37°C for 6-8 hours before administration. Mice were treated with
yogurt as described in figure legends with 0.2 ml administered by oral gavage.

Isolation and sequencing of *Lactobacillus*. Fecal pellets from Jackson and NCI mice
were processed separately. Pellets were homogenized in buffered saline solution and
plated onto lactobacillus selective MRS agar. Plates were subsequently incubated at
37°C in anaerobic culture jars. Single colonies were re-streaked three times to purify the
strains. DNA was extracted from bacterial colonies, lab-cultured yogurt, and probiotic
powder using the MoBio Powersoil DNA extraction kit (MoBio, Carlsbad, CA)
according to the manufacturer’s protocols. An approximately 1500 bp segment of the
16S rRNA gene was amplified from isolate DNA, via PCR, using the 9F-1522R primer
set. PCR products were purified using the Qiaquick PCR purification kit (Qiagen,
Valencia, CA) and sequenced at the University of Tennessee Molecular Biology
Resource Facility using Sanger sequencing.

Cellular immune response. Spleens were disrupted to generate single-cell suspensions
in Hyclone RPMI 1640 media (Thermo Fisher Scientific Inc., Waltham, MA)

supplemented with 10% fetal bovine serum (FBS) (Atlanta Biologicals, Inc., Lawrenceville, GA). RPMI 1640 was also supplemented with 1.19 mg/ml HEPES (Thermo Fisher Scientific Inc., Waltham, MA), 0.2 mg/ml L-glutamine (Research Products International Corp., Mt. Prospect, IL), (0.05 units/ml & 0.05 mg/ml) penicillin/streptomycin (Invitrogen, Grand Island, NY), 0.05 mg/ml gentamicin sulfate (Invitrogen, Grand Island, NY), and 0.05 μ M 2-Mercaptoethanol (Thermo Fisher Scientific Inc., Waltham, MA). Single cell suspensions were treated with ammonium chloride potassium to lyse red blood cells. Spleens were harvested as indicated at the number of days post infection.

Cells were stained with Fc block (anti-CD16/32; clone 2.4G2) and the following fluorescence-conjugated antibodies (CD45.2; clone 104, CD4; clone RM4-5, CD8; clone 53-6.7, CD3; clone 17A2, CD19; clone 6D5, Ter119; clone Ter-119, CD11a; clone M17/4, CD49d; clone R1-2, PD-1; clone 29F.1A12, CD95; clone Jo2, GL7; clone GL7, b10-12, CXCR5; clone 2G8, CD44; clone IM7) purchased from Biolegend (San Diego, CA) and BD Biosciences (San Diego, CA). For CXCR5 staining cells were stained with biotinylated-CXCR5 for 30 minutes at room temperature prior to staining with fluorescence-conjugated streptavidin. Antibodies were resuspended in FACS buffer (1x PBS, 1% FBS, 0.02% sodium azide) and cells were stained for 30 minutes at 4°C. Following staining cells were fixed and permeabilized with Fixation Buffer (Biolegend, San Diego, California). Cells were acquired through an LSR II (BD Biosciences). Data were analyzed by FlowJo software (Tree Star, Ashland, OR).

Detection of *P. yoelii* MSP1₁₉-specific antibodies. Serum was collected on the indicated days post *P. yoelii* infection and stored at -20°C. MaxiSorp Immuno plates (Thermo Scientific) were coated with 1 µg/ml recombinant MSP1₁₉ (The following reagent was obtained through the MR4 as part of the BEI Resources Repository, NIAID, NIH: *Plasmodium yoelii* yP.y.MSP1-19(XL)/VQ1, MRA-48, deposited by DC Kaslow.) Dilutions of serum were added to wells. Total MSP1₁₉-specific IgM, IgG1, IgG2b, IgG2c, and IgG3 antibodies were detected with horseradish peroxidase–conjugated goat anti–mouse IgM, IgG1, IgG2b, IgG2c, and IgG3, respectively, (Jackson ImmunoResearch) and 3,3',5,5'-tetramethylbenzidine substrate (Arcos Organics). Reactions were stopped by addition of 2M H₂SO₄. Results are presented as average endpoint titers with absorbance readings below 0.1 (absorbance at 450 nm).

Statistical analysis. Descriptive and comparative statistical analyses of data, except the gut microbiota and metabolomics data, were done using GraphPad Software version 6 (La Jolla, CA, USA). The area under the parasitemia curve (AUC) was estimated for each group following the trapezoidal rule with the following equation (54, 55).

$$AUC_{t1-t_{last}} = 0.5 \sum (Y_i + Y_{i+1}) * (t_{i+1} - t_i)$$

Where “t” was sampling time and “y” the observed outcome (e.g., % parasitemia).

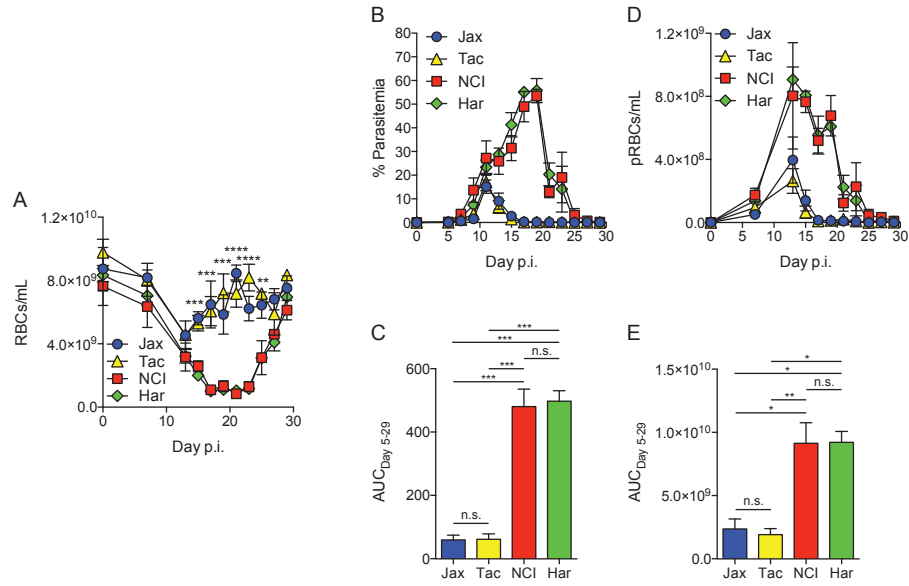


Fig. S1. Differential anemia and circulating parasitized red blood cells between mice from different vendors. C57BL/6 mice were infected with 1×10^5 *P. yoelii* parasitized red blood cells. (A) Number of red blood cells (RBCs) per mL of blood. (B) Fraction of red blood cells infected with *P. yoelii* (% parasitemia). (C) Percent parasitemia AUC analysis. (D) Number of parasitized red blood cells (pRBCs) per mL of blood. (E) pRBCs/mL of blood AUC analysis. (A-E) Data (mean $\pm$ S.E.) are from 3-4 mice/group. (A,C, and E) Data were analyzed by one-way ANOVA and Tukey's multiple comparison post-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, n.s. = not significant.

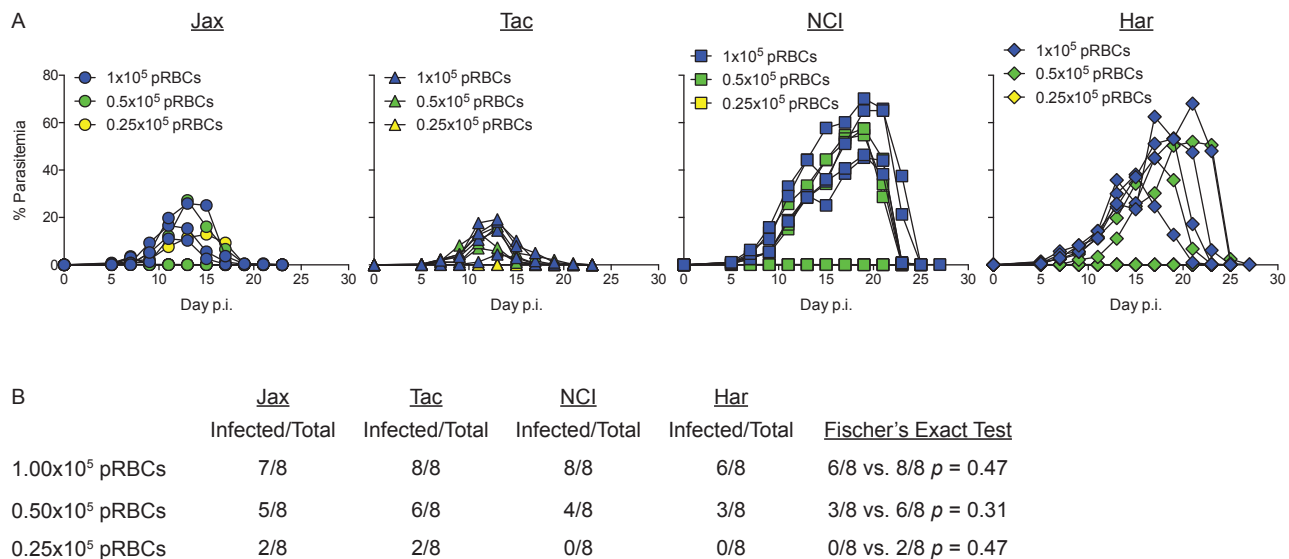


Fig. S2. Similar susceptibility to parasitized red blood cell infection between C57BL/6 mice from different vendors. C57BL/6 mice from the indicated vendors were infected with the indicated number of *P. yoelii* parasitized red blood cells (pRBCs). (A) Fraction of RBCs infected with *P. yoelii* (% parasitemia). Each line represents parasitemia kinetics from an individual mouse (n=4 mice/dose/vendor). Data are representative of two experiments. (B) Number of mice from each vendor infected with the indicated dose of pRBCs and the total number of mice injected. Data are cumulative results from two experiments. Susceptibility to infection at the different doses between vendors was analyzed by two-tailed Fisher's Exact Test.

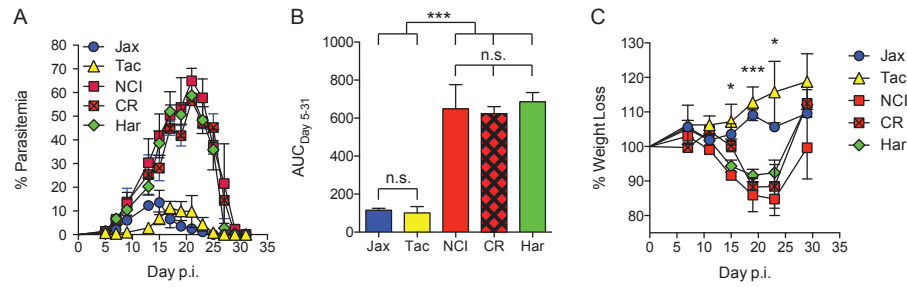


Fig. S3. C57BL/6 mice from Charles River are susceptible to high *P. yoelii* parasite burden. C57BL/6 mice were infected with 1×10^5 *P. yoelii* parasitized red blood cells. (A) Fraction of red blood cells infected with *P. yoelii* (% parasitemia). (B) Area under the parasitemia curve (AUC) analysis. Data were analyzed by one-way ANOVA and Tukey's multiple comparison test. (C) Percent weight loss following infection. Data were analyzed by one-way ANOVA. (A-C) Data (mean $\pm$ S.E.) are from 3-5 mice/group and representative of two experiments. * $p < 0.05$, *** $p < 0.001$, n.s. = not significant.

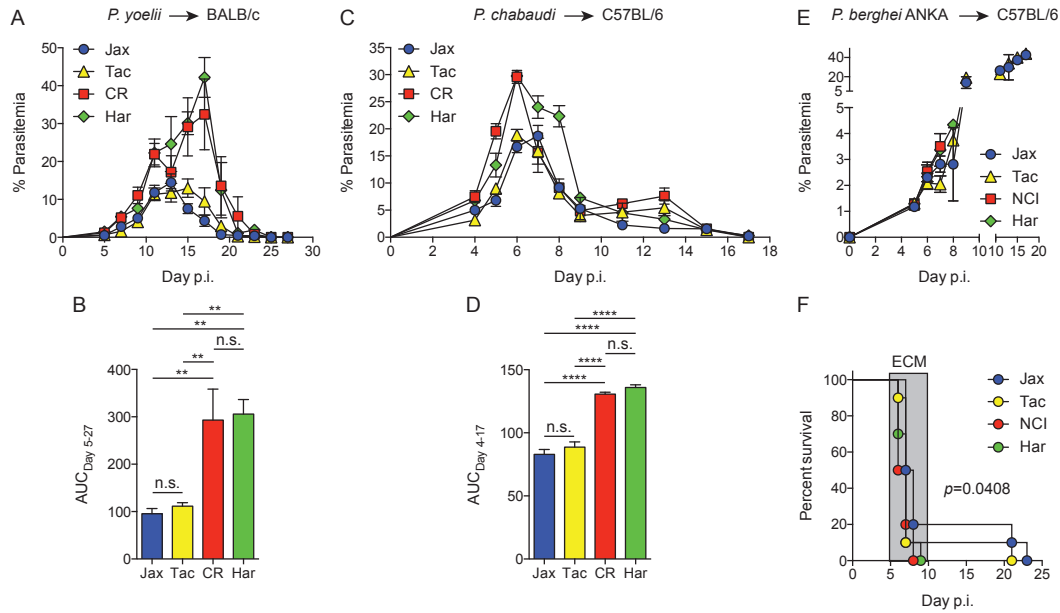


Fig. S4. Modulation of malaria pathogenesis by the gut microbiota is generalizable to another mouse strain and *Plasmodium* species. (A-B) BALB/c mice were infected with 1×10^5 *P. yoelii* parasitized red blood cells. (A) Fraction of red blood cells infected with *P. yoelii* (% parasitemia). (B) Area under the parasitemia curve (AUC) analysis. Data were analyzed by one-way ANOVA and Tukey's multiple comparison test. (A-B) Data (mean $\pm$ S.E.) are cumulative results (n=8 mice/group) from two experiments. (C-D) C57BL/6 mice were infected with 1×10^5 *P. chabaudi* parasitized red blood cells. (C) Fraction of red blood cells infected with *P. chabaudi* (% parasitemia). (D) Area under the parasitemia curve (AUC) analysis. Data were analyzed by one-way ANOVA and Tukey's multiple comparison test. (C-D) Data (mean $\pm$ S.E.) are from 3-4 mice/group and representative of two experiments. (E-F) C57BL/6 mice were infected with 1×10^5 *P. berghei* ANKA parasitized red blood cells. (E) Fraction of red blood cells infected with *P. berghei* (% parasitemia) from surviving mice. Data (mean $\pm$ S.E.) are cumulative results (n=10 mice/group) from two experiments. (F) Survival of mice following infection (n=10 mice/group). Survival curves were analyzed by Log-rank (Mantel-Cox) test. ** $p < 0.01$, **** $p < 0.0001$, n.s. = not significant.

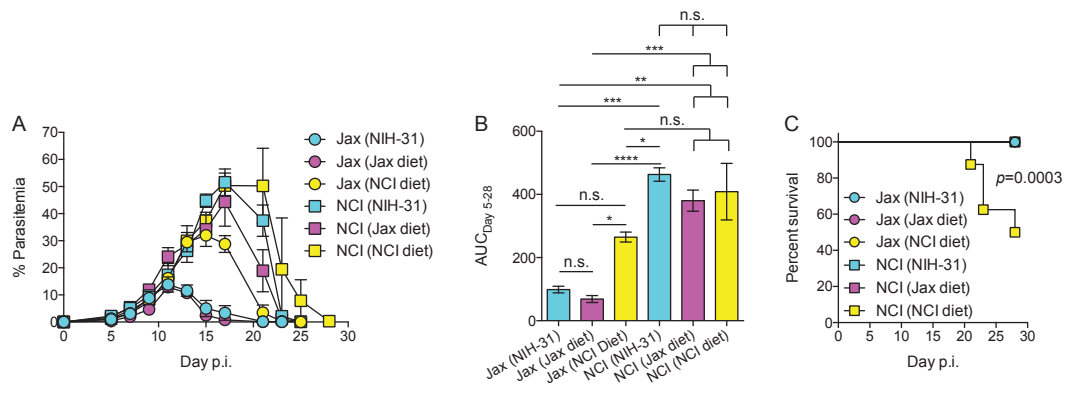


Fig. S5. Inversion of Jax and NCI in-house diets minimally effects severity of *P. yoelii* infection. C57BL/6 mice were placed on one of three different rodent chows; NIH-31, Jax in-house diet, or NCI in-house diet, for one-week prior to infection through resolution of infection. Mice were infected with 1×10^5 *P. yoelii* parasitized red blood cells. (A) Fraction of red blood cells infected with *P. yoelii* (% parasitemia). (B) Area under the parasitemia curve (AUC) analysis. (A-B) Data (mean $\pm$ S.E.) are from 3-4 mice/group and representative of two experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, n.s. = not significant. (C) Survival of mice following infection (n=7-8 mice/group) from two experiments. Survival curves were analyzed by Log-rank (Mantel-Cox) test.

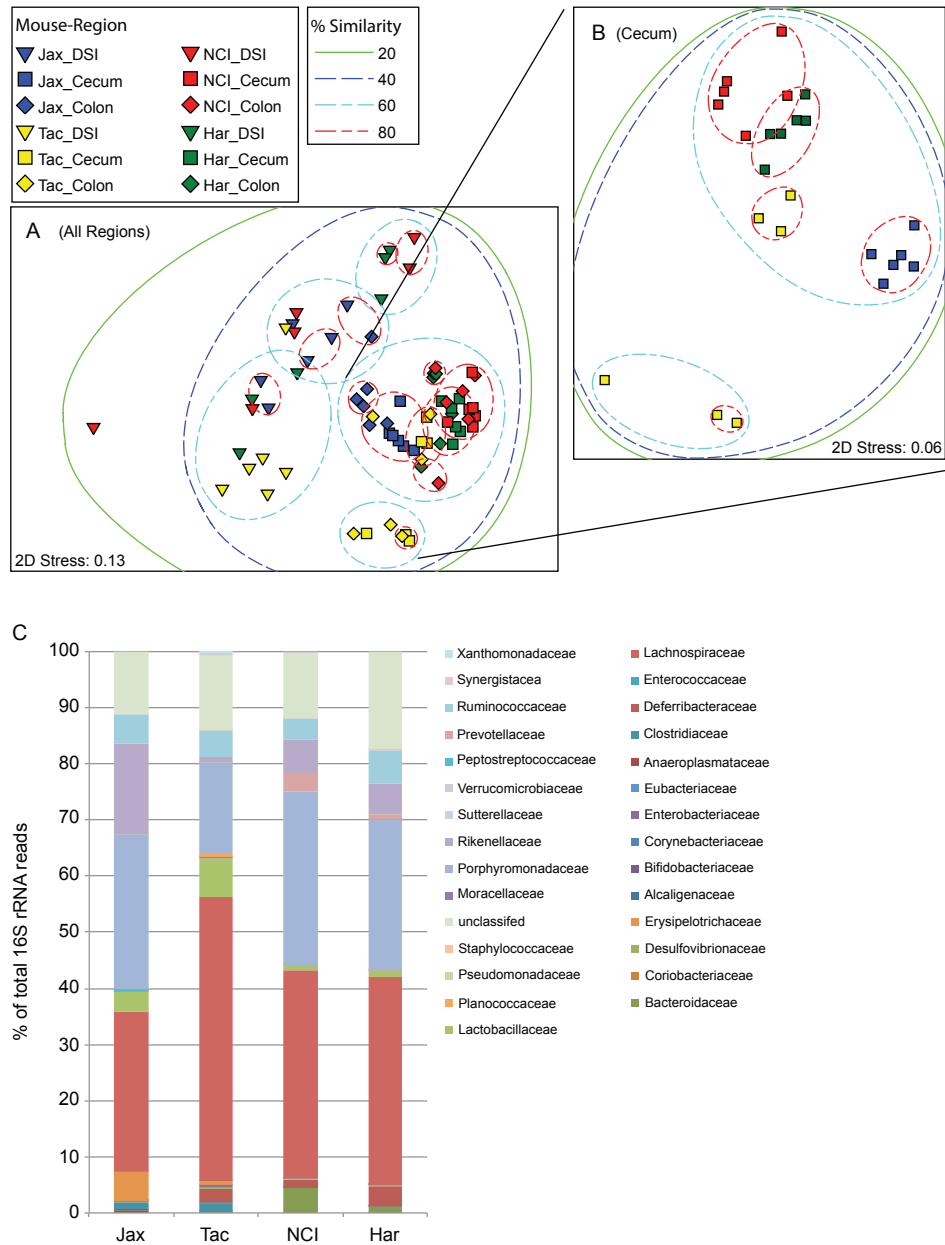


Fig. S6. Susceptibility to malaria correlates with differences in the gut microbial community structure. Bacterial population analysis was performed using nonmetric multidimensional scaling analysis. The closer together two points are the more similar the libraries are to one another. Libraries with similarities greater than 20%, 40%, 60% or 80% are encircled with green, blue, teal or red dotted lines, respectively. Triangle=distal portion of the small intestine (DSI); Square=cecum; Diamond=colon. (A) Analysis of all three regions of the digestive tract. (B) Analysis of only the cecum bacterial populations. (C) Percentage of 16S rRNA gene reads of bacterial families comprising at least 0.01% of reads from at least 1 sample. Abundances of 16S reads at the Family level were first normalized to proportional abundances and then the average proportional abundance (n=6) for each family was calculated for mice from each of the four vendors.

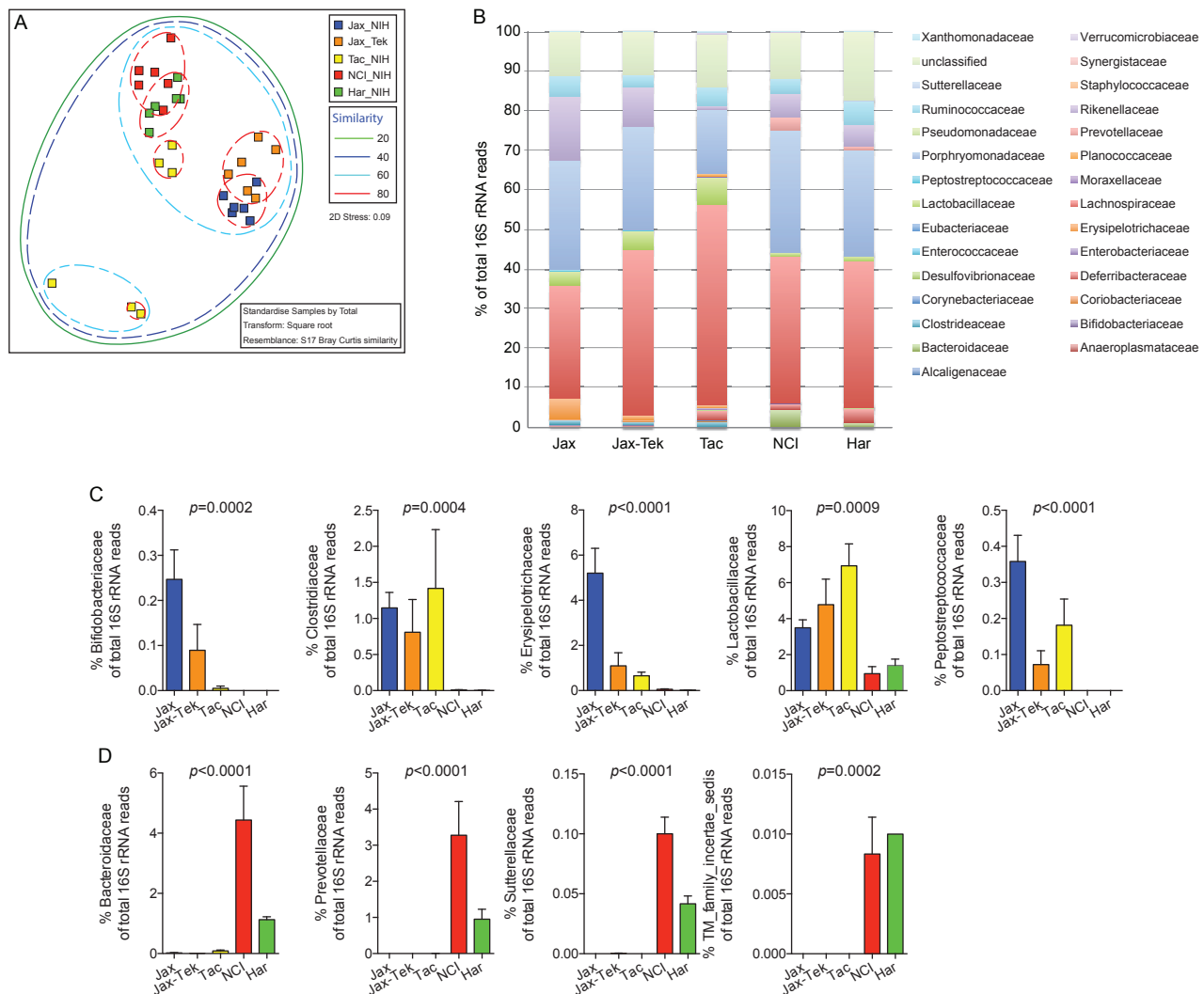


Fig. S7. Jackson mice fed Teklad 22/5 diet exhibit defined changes in cecal bacterial populations. All mice were fed NIH-31, except one group of Jackson mice that received Teklad 22/5 (Jax-Tek). (A) Cecal bacterial population analysis was performed using NMDS. The closer together two points are the more similar the libraries are to one another. Libraries with similarities greater than 20%, 40%, 60% or 80% are encircled with green, blue, teal or red dotted lines, respectively. Analysis of cecal bacterial populations. Each symbol represents an individual mouse. (B) Percentage of 16S rRNA gene reads of bacterial families comprising at least 0.01% of reads from at least 1 sample. Abundances of 16S reads at the Family level were first normalized to proportional abundances and then the average proportional abundance ($n=6$) for each family was calculated for mice from each of the groups. (C) Bacterial families that were identified as being significantly enriched in Jackson or Taconic mice. (D) Bacterial families identified as being significantly enriched in NCI or Harlan mice. (C-D) Data (mean $\pm$ S.E.) are from 6 mice per group and extracted from analysis in panel B. Data were analyzed by Kruskal-Wallis test.

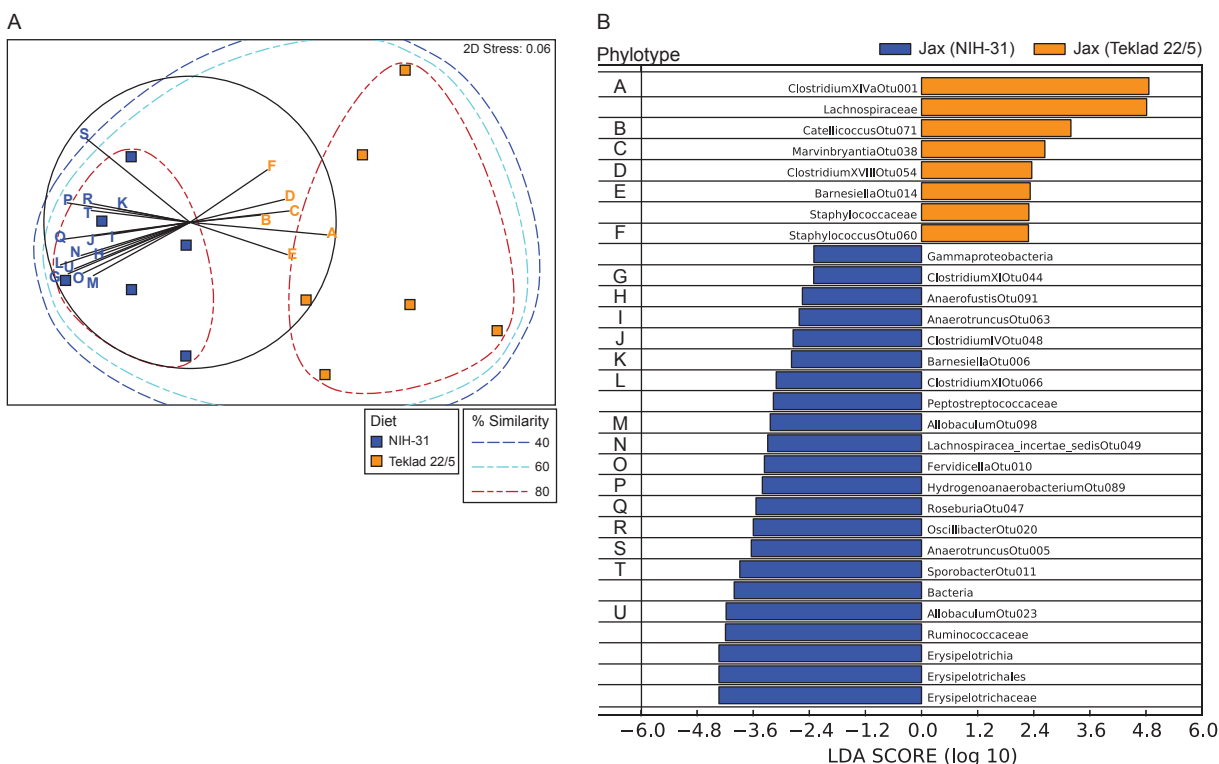


Fig. S8. Distinct bacterial community structure in Jackson mice fed separate diets. C57BL/6 mice from Jackson Laboratory (Jax) were fed two separate diets (NIH-31 (n=6) or Teklad 22/5 (n=6)) for one to three weeks prior to removal of gut contents and analysis of gut bacterial community. (A) NMDS plot displaying the similarity between bacterial populations within the cecum of mice fed the different diets. Each symbol represents a single mouse. The closer together two points are in the figure, the more similar the libraries are to one another. Libraries with similarities greater than 40%, 60% or 80% are encircled with blue, teal or red dashed lines, respectively. Selected vectors (lettered A-U), overlaid on the figure, were taken from a PCA plot made from all phylotype abundances in this study after identification by LefSe analysis as having statistically significant differential abundances in mice fed different diets. (B) LefSe output highlighting differentially abundant phylotypes between Jax mice fed the two separate diets. Some of these phylotypes are included in (A) as vectors.

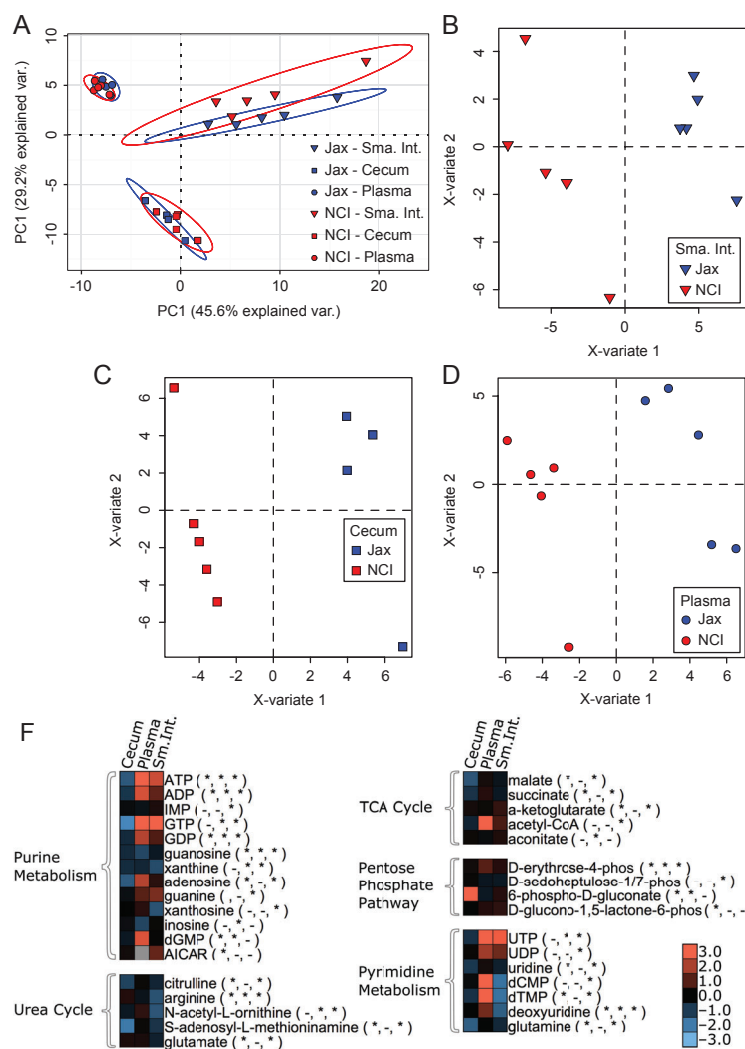
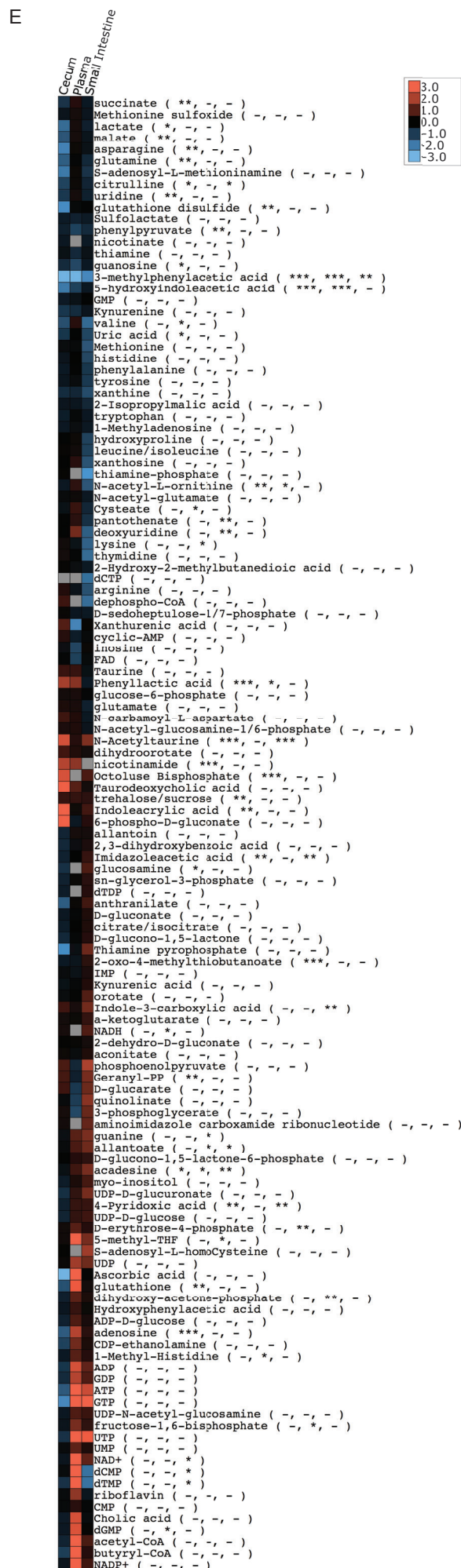


Fig. S9. Changes in the gut microbial community structure correlate with changes in the gut and plasma metabolome in resistant and susceptible mice. (A) Principal component analysis of metabolite profiles for all sample types. Ellipses size indicates 95% normal probability. Triangle=small intestine, square=cecum, circle=plasma. Each symbol represents an individual mouse. (B-D) Partial least squares discriminant analysis within individual samples types: small intestine, cecum, and plasma, respectively. Separation between points indicates relative amount of variability. (E) Color intensity indicates magnitude of the fold change with red being an increase and blue being a decrease in metabolite concentrations of Jax with respect to NCI mice. Asterisks in parentheses next to metabolite names indicate significance for the change for the corresponding row and column. *** $p \leq 0.01$, ** $p \leq 0.05$, * $p \leq 0.10$, (-) not significant. (F) Metabolites grouped according to metabolic pathways that were major drivers in the differentiation of resistant and susceptible mice using PLS-DA. Data are expressed as a ratio of Jax/NCI with red shades representing metabolites higher in Jax mice and blue shades representing metabolites higher in NCI mice. (*) = Metabolite that was a driving factor in differentiating resistant and susceptible mice, respective to column order. (-) = Metabolite that was not a driving factor in variation for the respective column.



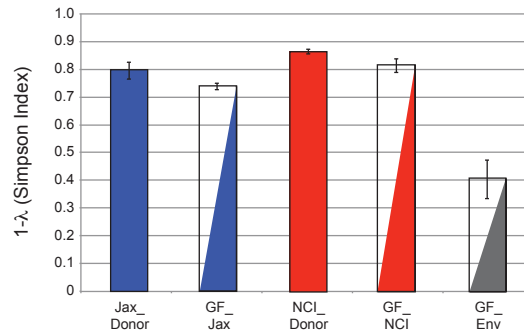


Fig. S10. Germ-free mice colonized with cecal content have similar bacterial diversity as donor sample. Germ-free mice were colonized with cecal contents from Jax or NCI mice. The $1-\lambda$ (Simpson Index) was calculated for 5 different mouse types (Jax-Donors (n=4), GF-Jax recipients post-cecal transplant (n=5), NCI Donors (n=3), GF-NCI recipients post-cecal transplant (n=5) and GF mice receiving no transplant (n=5)). Data are mean $\pm$ SD. Communities with values closer to 1 are considered to be more diverse.

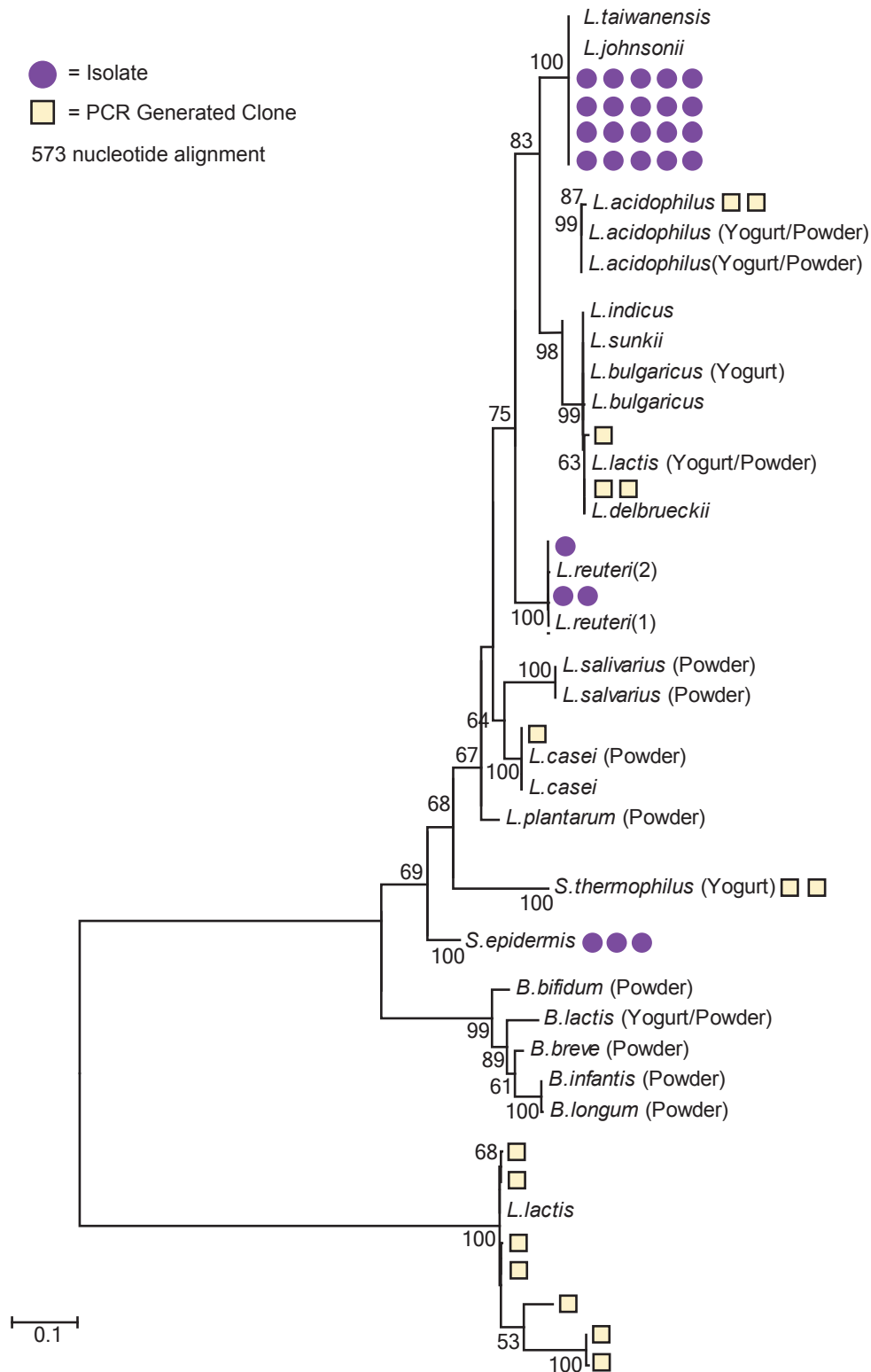


Fig. S12. Phylogenetic tree of bacteria isolated from stool samples and lab-cultured yogurt. A maximum likelihood tree was constructed from an alignment of 16S rRNA gene sequences approximately 573 base pairs long. Sequences used to construct the tree were obtained from bacterial isolates (cultured from Jackson and NCI mouse fecal pellets) and from PCR amplicons retrieved from DNA extracted from lab-cultured yogurt containing probiotic powder. Isolate sequences = purple circles, clone sequences (yogurt or powder) = tan squares. Distance bar represents 0.1 substitutions per base.

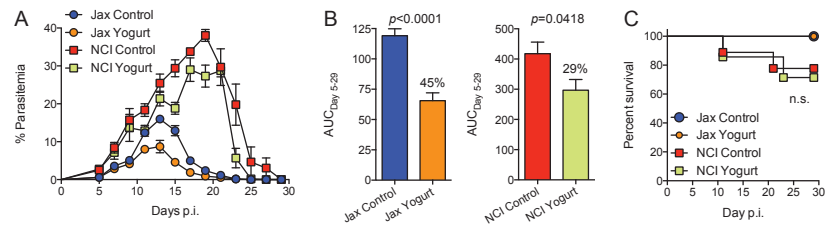


Fig. S13. Treatment of gut microbiota intact mice with yogurt has a modest effect on parasite burden. Mice were treated 5-6 times/week with yogurt for 3 weeks prior to *P. yoelii* infection and 3 times per week following infection. (A) Percent parasitemia following *P. yoelii* infection. (B) AUC analysis. Data were analyzed by unpaired two-tailed *t* test. (C) Survival of mice following infection. Survival curves were analyzed by Log-rank (Mantel-Cox) test. (A-C) Data (mean±S.E.) from 7-9 mice/group are cumulative data from two experiments.

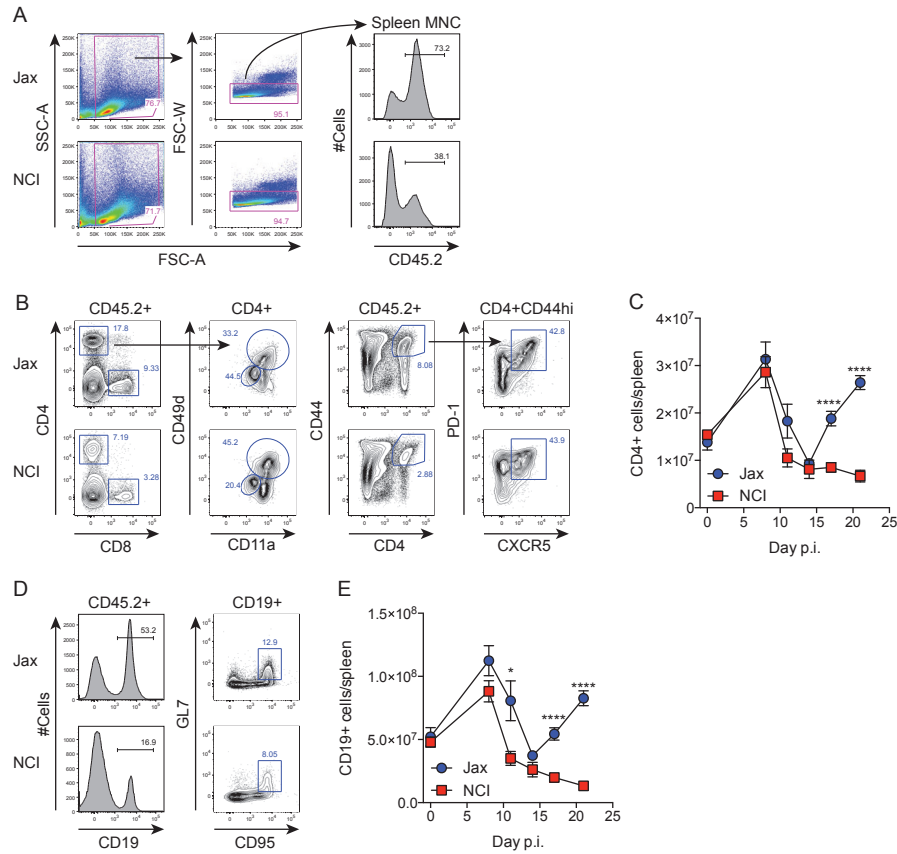


Fig. S14. Gating strategy for T cell and B cell populations in Jax and NCI mice infected with *P. yoelii*. Jax and NCI mice were infected with *P. yoelii*. Representative contour plots and histograms are from day 21-post infection. (A) Representative contour plots and histograms showing gating strategy of CD45.2+ cells. (B) Representative contour plots showing gating strategy for T cell subsets: CD4+ T cells, *P. yoelii*-specific CD4+ T cells (CD4+CD49d^{hi}CD11a^{hi}), and T follicular helper (Tfh) cells (CD4+CD44^{hi}CXCR5+PD-1+). (C) Total number of CD4+ cells per spleen. (D) Representative contour plots and histograms showing gating strategy of B cell subsets: CD19+ B cells and GC B cells (CD19+GL7+CD95+). (E) Total number of CD19+ cells per spleen. Data (mean±S.E.) are cumulative results (n=5-10 mice/data point) from three experiments. Data were analyzed by unpaired two-tailed *t* test. * *p*<0.05, **** *p*<0.0001.

Supplementary Table 1. Sequencing and diversity metrics from Jax, Har, NCI, and Tac mice. Total number of sequences from all samples = 8,665,599. S = species richness; N = number of sequences; d = Margalef species richness; J' = Pielou's evenness; H' = Shannon index; 1/λ = Inverse Simpson.

Sample ID	Mouse	Region	Resistant?	S	N	d	J'	H'(log10)	1/λ
H_4612	Har	DSI	N	85	103,055	7.28	0.25	0.48	0.52
H_4613	Har	Cecum	N	85	191,572	6.91	0.51	0.99	0.82
H_4614	Har	Colon	N	88	246,215	7.01	0.44	0.85	0.69
H_4622	Har	DSI	N	95	26,498	9.23	0.47	0.93	0.79
H_4623	Har	Cecum	N	77	144,414	6.40	0.51	0.97	0.78
H_4624	Har	Colon	N	123	140,969	10.29	0.49	1.03	0.77
H_4632	Har	DSI	N	94	22,480	9.28	0.49	0.96	0.82
H_4633	Har	Cecum	N	85	310,706	6.64	0.54	1.05	0.84
H_4634	Har	Colon	N	82	63,897	7.32	0.60	1.14	0.89
H_4712	Har	DSI	N	56	1,534	7.50	0.58	1.01	0.81
H_4713	Har	Cecum	N	89	299,579	6.98	0.47	0.92	0.79
H_4714	Har	Colon	N	80	188,189	6.50	0.48	0.91	0.79
H_4722	Har	DSI	N	63	44,509	5.79	0.27	0.49	0.50
H_4723	Har	Cecum	N	82	188,386	6.67	0.48	0.91	0.78
H_4724	Har	Colon	N	88	335,328	6.84	0.46	0.90	0.78
H_4732	Har	DSI	N	72	41,215	6.68	0.28	0.52	0.46
H_4733	Har	Cecum	N	74	168,137	6.07	0.51	0.96	0.81
H_4734	Har	Colon	N	84	215,138	6.76	0.50	0.97	0.80
J_4612	Jax	DSI	Y	58	10,220	6.17	0.50	0.89	0.76
J_4613	Jax	Cecum	Y	83	212,544	6.68	0.50	0.96	0.82
J_4614	Jax	Colon	Y	109	144,423	9.09	0.45	0.92	0.76
J_4622	Jax	DSI	Y	69	6,396	7.76	0.53	0.97	0.78
J_4623	Jax	Cecum	Y	74	290,135	5.80	0.50	0.94	0.81
J_4624	Jax	Colon	Y	87	171,168	7.14	0.53	1.03	0.85
J_4632	Jax	DSI	Y	78	32,429	7.41	0.32	0.61	0.53
J_4633	Jax	Cecum	Y	73	163,210	6.00	0.54	1.00	0.84
J_4634	Jax	Colon	Y	74	38,128	6.92	0.44	0.82	0.68
J_4712	Jax	DSI	Y	66	141,766	5.48	0.45	0.82	0.72
J_4713	Jax	Cecum	Y	98	147,576	8.15	0.51	1.02	0.84
J_4714	Jax	Colon	Y	91	97,286	7.84	0.50	0.98	0.79
J_4722	Jax	DSI	Y	77	17,265	7.79	0.46	0.87	0.73
J_4723	Jax	Cecum	Y	73	128,780	6.12	0.45	0.84	0.75
J_4724	Jax	Colon	Y	81	149,433	6.71	0.50	0.96	0.81
J_4732	Jax	DSI	Y	69	17,351	6.97	0.24	0.44	0.35
J_4733	Jax	Cecum	Y	71	176,751	5.79	0.53	0.98	0.81
J_4734	Jax	Colon	Y	70	83,812	6.09	0.27	0.49	0.40
N_4612	NCI	DSI	N	78	5,862	8.87	0.57	1.08	0.81
N_4613	NCI	Cecum	N	81	153,710	6.70	0.48	0.91	0.75

N_4614	NCI	Colon	N	73	60,882	6.54	0.53	0.99	0.83
N_4622	NCI	DSI	N	129	32,346	12.33	0.57	1.20	0.90
N_4623	NCI	Cecum	N	92	196,467	7.47	0.51	1.01	0.83
N_4624	NCI	Colon	N	88	155,236	7.28	0.53	1.02	0.84
N_4632	NCI	DSI	N	80	6,446	9.01	0.47	0.90	0.72
N_4633	NCI	Cecum	N	82	160,507	6.76	0.50	0.96	0.78
N_4634	NCI	Colon	N	85	170,630	6.97	0.47	0.91	0.79
N_4712	NCI	DSI	N	83	140,322	6.92	0.33	0.63	0.70
N_4713	NCI	Cecum	N	87	134,246	7.28	0.46	0.89	0.77
N_4714	NCI	Colon	N	149	148,733	12.43	0.39	0.84	0.61
N_4722	NCI	DSI	N	67	7,778	7.37	0.34	0.63	0.51
N_4723	NCI	Cecum	N	78	196,757	6.32	0.53	1.01	0.84
N_4724	NCI	Colon	N	76	219,072	6.10	0.57	1.07	0.87
N_4732	NCI	DSI	N	75	54,099	6.79	0.37	0.69	0.73
N_4733	NCI	Cecum	N	76	346,990	5.88	0.52	0.97	0.83
N_4734	NCI	Colon	N	77	170,635	6.31	0.48	0.90	0.75
T_4612	Tac	DSI	Y	72	10,268	7.69	0.41	0.77	0.62
T_4613	Tac	Cecum	Y	82	129,923	6.88	0.50	0.95	0.79
T_4614	Tac	Colon	Y	89	119,367	7.53	0.46	0.90	0.73
T_4622	Tac	DSI	Y	71	3,309	8.64	0.62	1.15	0.84
T_4623	Tac	Cecum	Y	80	160,273	6.59	0.49	0.94	0.77
T_4624	Tac	Colon	Y	77	137,109	6.43	0.52	0.98	0.82
T_4632	Tac	DSI	Y	68	1,852	8.90	0.76	1.38	0.93
T_4633	Tac	Cecum	Y	85	176,059	6.95	0.49	0.94	0.80
T_4634	Tac	Colon	Y	44	8,525	4.75	0.56	0.92	0.78
T_4712	Tac	DSI	Y	92	5,302	10.61	0.71	1.39	0.93
T_4713	Tac	Cecum	Y	140	165,425	11.57	0.43	0.93	0.71
T_4714	Tac	Colon	Y	81	147,027	6.72	0.46	0.88	0.76
T_4722	Tac	DSI	Y	57	2,676	7.10	0.60	1.05	0.80
T_4723	Tac	Cecum	Y	83	129,267	6.97	0.46	0.89	0.72
T_4724	Tac	Colon	Y	88	90,087	7.63	0.55	1.06	0.87
T_4732	Tac	DSI	Y	50	1,385	6.77	0.70	1.18	0.86
T_4733	Tac	Cecum	Y	68	130,609	5.69	0.49	0.90	0.74
T_4734	Tac	Colon	Y	90	125,924	7.58	0.46	0.90	0.74

Supplementary Table 2. Variability in each sample type for PLS-DA. For each sample type, the intra condition distance between the extreme values in each dimension are listed. Comparison is also shown for the distance between the average of the points for each condition in each dimension. Additionally, the p values are shown for the inter conditional comparison. Results show a high level of significance between the Jax and NCI samples in the variate 1 dimension.

Small Intestine				
Jax Variability		NCI Variability		
Variate 1	Variate 2	Variate 1	Variate 2	
3.84	5.23	6.90	10.86	
Jax – NCI Variability				
	Variate 1	Y		
Avg. Distance	10.03	1.72		
p-value	0.000269	0.411425		
Cecum				
Jax Variability		NCI Variability		
Variate 1	Variate 2	Variate 1	Variate 2	
3.03	12.35	2.32	11.47	
Jax – NCI Variability				
	Variate 1	Variate 2		
Avg. Distance	9.12	1.76		
p value	0.000145	0.629464		
Plasma				
Jax Variability		NCI Variability		
Variate 1	Variate 2	Variate 1	Variate 2	
4.93	9.08	3.34	11.71	
Jax – NCI Variability				
	Variate 1	Variate 2		
Avg. Distance	8.23	2.36		
p value	0.000104	0.432392		

Supplementary Table 3: Average small intestine metabolite ion counts and relative standard deviations (RSD) for Jax (n=5) and NCI (n=5) mice.

Metabolite	Jax		NCI	
	Average	RSD (%)	Average	RSD (%)
lactate	1.12E+07	33.2	1.82E+07	43.1
succinate	4.01E+06	22.6	5.72E+06	16.7
valine	1.02E+05	72.0	3.22E+05	47.7
nicotinamide	N.D.	N.D.	4.39E+03	N.D.
nicotinate	1.43E+05	83.0	1.51E+05	59.7
Taurine	2.32E+06	37.0	3.05E+06	39.2
hydroxyproline	7.51E+05	41.3	1.63E+06	26.4
leucine/isoleucine	7.75E+05	44.8	1.66E+06	31.3
asparagine	6.12E+05	42.4	6.67E+05	84.4
malate	3.44E+06	34.3	4.46E+06	37.6
anthranilate	1.05E+05	61.0	7.04E+04	47.8
Imidazoleacetic acid	1.43E+05	22.2	1.08E+05	53.7
α -ketoglutarate	3.46E+08	56.0	2.18E+08	43.5
glutamine	1.09E+08	106.2	2.07E+08	79.6
lysine	3.10E+07	48.0	9.79E+07	45.1
glutamate	4.94E+09	43.4	7.34E+09	31.7
2-oxo-4-methylthiobutanoate	1.27E+06	69.1	1.02E+06	50.7
2-Hydroxy-2-methylbutanedioic acid	1.58E+08	31.7	2.23E+08	31.4
methionine	1.77E+08	36.8	4.47E+08	45.0
3-methylphenylacetic acid	4.06E+06	44.7	2.18E+07	60.5
guanine	4.10E+06	49.4	1.44E+06	56.7
xanthine	1.83E+08	31.5	4.28E+08	46.4
Hydroxyphenylacetic acid	1.61E+06	14.6	1.61E+06	22.8
2,3-dihydroxybenzoic acid	6.52E+07	46.5	6.37E+07	75.5
histidine	3.83E+07	50.3	8.95E+07	39.5
orotate	2.28E+07	94.4	1.31E+07	114.1
dihydroorotate	2.80E+06	74.7	2.85E+06	83.9
allantoin	8.37E+07	46.2	7.90E+07	48.5
Indole-3-carboxylic acid	3.09E+06	22.6	1.19E+06	42.0
phenylpyruvate	2.40E+07	66.8	4.17E+07	39.2
methionine sulfoxide	2.90E+06	27.9	4.17E+06	41.5
phenylalanine	8.78E+08	16.8	1.72E+09	26.0
Phenyllactic acid	1.09E+07	81.1	1.73E+07	39.5
quinolinate	8.94E+07	110.6	3.48E+07	54.3
N-Acetyltaurine	4.74E+08	25.8	1.75E+08	22.0
phosphoenolpyruvate	4.22E+06	116.7	1.20E+06	41.5
uric acid	2.79E+08	20.5	6.48E+08	41.2
cysteate	3.12E+06	38.3	5.83E+06	52.9
1-Methyl-Histidine	5.14E+05	69.6	4.27E+05	54.7
Sulfolactate	5.32E+07	29.3	8.83E+07	67.5
dihydroxy-acetone-phosphate	1.52E+08	93.8	1.18E+08	57.8
sn-glycerol-3-phosphate	3.80E+09	41.0	2.74E+09	21.3
aconitate	1.52E+08	53.2	1.35E+08	28.7
N-acetyl-L-ornithine	7.59E+06	32.6	2.01E+07	34.6
arginine	7.78E+07	47.5	2.04E+08	39.3
citrulline	8.51E+06	37.7	1.56E+07	35.0
ascorbic acid	4.94E+09	60.1	5.38E+09	47.9
N-carbamoyl-L-aspartate	1.16E+07	66.5	1.89E+07	73.5

allantoate	9.06E+06	63.4	4.25E+06	62.4
2-Isopropylmalic acid	3.92E+06	32.1	7.89E+06	80.7
glucono-1,5-lactone	5.72E+08	56.8	4.60E+08	47.5
glucosamine	8.14E+05	73.6	5.28E+05	113.1
myo-inositol	1.18E+09	63.1	8.00E+08	49.8
tyrosine	6.65E+08	22.9	1.33E+09	41.8
4-Pyridoxic acid	4.11E+08	27.4	2.98E+08	44.7
3-phosphoglycerate	4.79E+07	104.9	2.09E+07	59.5
indoleacrylic acid	3.00E+04	89.4	2.55E+04	44.1
kynurenic acid	3.75E+06	38.4	3.06E+06	44.3
<i>N</i> -acetyl-glutamate	2.88E+08	57.6	3.97E+08	25.0
5-hydroxyindoleacetic acid	8.00E+07	28.0	1.31E+08	57.8
citrate/isocitrate	2.37E+09	62.5	1.73E+09	52.8
2-dehydro-D-gluconate	4.88E+08	40.0	3.96E+08	42.2
D-gluconate	1.51E+09	81.8	1.24E+09	37.3
D-erythrose-4-phosphate	2.22E+05	48.8	1.70E+05	29.5
Tryptophan	5.54E+08	21.6	7.72E+08	26.3
xanthurenic acid	4.23E+06	24.4	4.34E+06	51.6
Kynurenine	5.47E+05	40.7	7.39E+05	20.3
D-glucarate	2.04E+08	107.9	9.91E+07	59.1
pantothenate	4.01E+08	37.4	7.13E+08	28.6
deoxyuridine	4.82E+06	79.8	1.30E+07	74.7
Thymidine	2.56E+06	113.0	8.05E+06	70.5
Uridine	2.26E+07	33.7	3.47E+07	17.5
D-glucono-1,5-lactone-6-phosphate	1.06E+07	39.1	8.49E+06	40.3
Acadesine	1.50E+06	42.3	7.16E+05	78.2
glucose-6-phosphate	1.49E+08	74.3	1.62E+08	25.1
Thiamine	8.16E+06	46.2	1.06E+07	24.2
Adenosine	3.85E+06	27.9	2.71E+06	35.6
Inosine	3.85E+08	51.7	3.58E+08	30.8
6-phospho-D-gluconate	4.34E+07	63.7	3.31E+07	32.6
1-methyladenosine	5.30E+06	55.5	8.70E+06	66.0
guanosine	2.51E+07	34.5	3.16E+07	58.6
xanthosine	2.83E+06	51.7	7.52E+06	65.3
D-sedoheptulose-1/7-phosphate	8.44E+06	34.0	1.21E+07	14.1
<i>N</i> -acetyl-glucosamine-1/6-phosphate	7.25E+07	44.6	9.33E+07	31.8
dCMP	7.57E+06	108.0	3.06E+07	53.9
glutathione	1.39E+09	38.6	1.21E+09	33.2
Geranyl-PP	8.73E+07	89.7	4.08E+07	76.4
dTMP	1.40E+07	97.9	5.39E+07	51.6
CMP	7.11E+07	62.0	7.53E+07	38.4
UMP	5.08E+08	43.9	3.81E+08	46.7
cyclic-AMP	5.33E+06	73.6	6.60E+06	39.1
AICAR	1.59E+07	138.0	7.27E+06	71.3
fructose-1-6-bisphosphate	7.71E+07	103.1	4.48E+07	97.5
trehalose/sucrose	5.61E+07	22.8	4.55E+07	61.7
thiamine-phosphate	6.50E+06	177.7	2.78E+07	113.1
dGMP	2.45E+09	52.9	2.21E+09	47.6
IMP	1.13E+08	43.0	8.29E+07	67.4
<i>S</i> -adenosyl-L-methioninamine	1.78E+06	93.5	4.25E+06	84.5
GMP	2.57E+08	77.5	2.49E+08	55.9
riboflavin	8.67E+06	23.3	1.18E+07	33.5
<i>S</i> -adenosyl-L-homocysteine	1.21E+07	122.5	3.99E+06	114.8
octoluse bisphosphate	5.01E+06	61.3	3.11E+06	72.1

dTDP	3.22E+05	48.4	2.37E+05	57.3
UDP	4.83E+06	70.7	2.13E+06	49.1
Cholic acid	4.67E+10	95.8	5.50E+10	52.7
Thiamine pyrophosphate	4.69E+06	143.0	2.55E+06	75.8
ADP	3.88E+08	72.6	1.64E+08	71.7
GDP	1.96E+07	89.7	8.97E+06	89.4
CDP-ethanolamine	1.30E+07	62.7	1.09E+07	40.3
5-methyl-THF	4.72E+06	87.5	2.02E+06	40.0
dCTP	1.51E+05	12.3	2.21E+05	185.0
UTP	1.28E+07	89.6	2.03E+06	151.3
taurodeoxycholic acid	7.36E+10	15.2	6.05E+10	39.2
ATP	1.16E+08	87.5	2.15E+07	107.4
GTP	7.16E+06	79.0	6.73E+05	126.0
UDP-D-glucose	2.67E+08	49.8	1.66E+08	45.7
UDP-D-glucuronate	1.34E+08	64.2	5.55E+07	48.5
ADP-D-glucose	2.08E+07	69.0	1.80E+07	36.9
UDP-N-acetyl-glucosamine	5.12E+08	44.0	3.42E+08	52.9
glutathione disulfide	6.76E+07	50.1	8.28E+07	99.5
NAD ⁺	5.17E+07	39.8	2.68E+07	62.5
NADH	1.54E+07	54.1	8.20E+06	56.3
dephospho-CoA	1.15E+07	56.7	3.72E+07	61.9
NADP ⁺	1.03E+07	74.8	6.33E+06	49.5
FAD	8.96E+06	50.2	1.04E+07	36.2
acetyl-CoA	9.78E+06	52.2	6.30E+06	66.3
butyryl-CoA	3.72E+06	108.5	3.30E+06	133.6

Supplementary Table 4: Average cecum metabolite ion counts and relative standard deviations (RSD) for Jax (n=4) and NCI (n=5) mice.

Metabolite	Jax		NCI	
	Average	RSD (%)	Average	RSD (%)
lactate	5.88E+05	166.1	1.60E+06	59.4
succinate	2.44E+06	29.4	3.46E+06	29.6
valine	8.80E+03	35.1	1.12E+04	44.7
nicotinamide	9.65E+05	45.6	1.70E+05	47.2
nicotinate	6.21E+05	19.3	6.73E+05	53.8
Taurine	1.22E+06	48.8	5.98E+05	40.2
hydroxyproline	4.02E+04	45.3	3.31E+04	30.8
leucine/isoleucine	4.16E+04	51.5	3.07E+04	51.3
asparagine	7.73E+04	79.6	2.83E+05	64.0
malate	1.87E+06	35.9	3.78E+06	37.6
anthranilate	1.58E+06	128.8	3.62E+06	98.1
Imidazoleacetic acid	7.05E+05	21.2	8.54E+05	22.2
α -ketoglutarate	1.28E+09	38.7	9.21E+08	50.8
glutamine	4.56E+07	24.9	1.16E+08	46.4
lysine	8.64E+06	37.8	5.88E+06	45.1
glutamate	1.09E+10	28.1	7.20E+09	25.1
2-oxo-4-methylthiobutanoate	7.00E+05	33.5	7.43E+05	22.0
2-hydroxy-2-methylbutanedioic acid	1.68E+09	19.4	1.33E+09	14.1
methionine	5.04E+07	29.2	4.51E+07	25.9
3-methylphenylacetic acid	3.60E+07	45.0	6.00E+09	35.4
guanine	1.08E+07	32.5	9.73E+06	35.0
xanthine	1.63E+08	34.6	2.07E+08	56.8
hydroxyphenylacetic acid	1.49E+07	48.8	1.50E+07	26.9
2,3-dihydroxybenzoic acid	2.99E+08	40.6	3.87E+08	57.6
histidine	3.85E+06	50.9	3.95E+06	71.0
orotate	5.31E+07	41.2	5.09E+07	56.3
dihydroorotate	5.35E+06	44.4	2.64E+06	72.4
allantoin	1.93E+06	87.1	2.59E+06	41.6
indole-3-carboxylic acid	1.51E+07	16.9	7.40E+06	45.7
phenylpyruvate	2.09E+06	10.4	2.52E+06	27.0
methionine sulfoxide	1.93E+07	72.0	1.92E+07	26.9
phenylalanine	5.28E+07	54.2	5.40E+07	43.6
phenyllactic acid	1.14E+10	29.1	2.37E+09	43.4
quinolate	5.08E+07	46.3	4.57E+07	30.6
N-Acetyltaurine	1.53E+09	24.2	2.30E+08	69.7
phosphoenolpyruvate	4.24E+07	94.6	2.31E+07	63.3
uric acid	2.53E+07	53.8	3.75E+07	37.3
cysteate	7.38E+07	39.1	7.81E+07	38.1
1-methyl-histidine	4.90E+05	66.1	4.97E+05	61.5
sulfolactate	1.21E+08	32.4	1.31E+08	42.1
dihydroxy-acetone-phosphate	4.24E+07	50.1	5.63E+07	49.8
sn-glycerol-3-phosphate	5.90E+08	33.8	7.63E+08	44.7
aconitate	9.50E+07	85.0	7.01E+07	56.0
N-acetyl-L-ornithine	5.22E+06	33.9	5.47E+06	24.3
arginine	9.06E+06	29.4	5.32E+06	65.1
citrulline	9.84E+06	74.0	1.54E+07	57.7
ascorbic acid	6.55E+06	23.3	3.64E+07	168.3
N-carbamoyl-L-aspartate	3.36E+07	42.1	1.68E+07	52.0

allantoate	2.21E+05	100.0	2.50E+05	66.9
2-Isopropylmalic acid	2.04E+08	69.0	2.01E+08	72.3
glucono-1,5-lactone	3.20E+08	28.2	4.09E+08	44.2
glucosamine	1.35E+06	87.3	1.59E+06	40.7
myo-inositol	1.35E+09	37.2	1.49E+09	38.0
tyrosine	7.94E+07	31.6	8.28E+07	22.8
4-pyridoxic acid	1.29E+09	13.6	1.55E+09	19.1
3-phosphoglycerate	1.32E+08	116.6	1.14E+08	44.3
indoleacrylic acid	6.97E+05	49.6	3.51E+04	68.7
kynurenic acid	1.38E+07	60.1	1.42E+07	70.5
<i>N</i> -acetyl-glutamate	1.58E+09	30.7	1.54E+09	24.6
5-hydroxyindoleacetic acid	8.76E+08	33.3	2.66E+09	25.2
citrate/isocitrate	4.46E+08	21.4	5.46E+08	90.3
2-dehydro-D-gluconate	6.62E+07	23.5	5.30E+07	36.3
D-gluconate	1.05E+08	53.4	1.30E+08	50.8
D-erythrose-4-phosphate	8.66E+05	39.5	6.65E+05	25.5
tryptophan	4.33E+07	41.7	5.00E+07	41.2
xanthurenic acid	1.14E+07	71.1	5.07E+06	49.5
kynurenine	2.52E+04	107.7	4.10E+04	84.9
D-glucarate	2.78E+06	125.1	1.76E+06	120.4
pantothenate	4.25E+08	43.5	3.35E+08	30.6
deoxyuridine	4.12E+06	41.0	3.25E+06	52.3
thymidine	4.01E+06	49.1	2.51E+06	52.9
uridine	2.63E+06	42.3	3.90E+06	43.6
D-glucono-1,5-lactone-6-phosphate	4.94E+07	20.9	4.10E+07	15.9
acadesine	4.55E+06	33.8	4.19E+06	19.7
glucose-6-phosphate	3.96E+08	46.9	3.09E+08	54.2
thiamine	8.28E+06	66.0	7.20E+06	34.0
adenosine	1.57E+07	37.5	3.36E+07	31.3
inosine	2.33E+08	25.8	2.21E+08	49.6
6-phospho- D-gluconate	7.61E+05	147.6	1.11E+05	74.4
1-methyladenosine	7.74E+06	51.7	8.61E+06	52.6
guanosine	2.52E+07	14.3	3.47E+07	32.6
xanthosine	8.31E+06	61.7	6.16E+06	61.9
D-sedoheptulose-1/7-phosphate	5.73E+07	54.0	4.90E+07	45.2
<i>N</i> -acetyl-glucosamine-1/6-phosphate	4.60E+08	32.6	3.17E+08	13.9
dCMP	1.54E+06	40.8	1.52E+06	70.1
glutathione	1.12E+08	52.1	2.47E+08	34.6
geranyl-PP	5.23E+07	41.1	2.25E+07	29.0
dTMP	1.35E+07	46.6	2.12E+07	38.6
CMP	2.51E+07	26.4	2.33E+07	22.2
UMP	1.43E+08	34.1	1.40E+08	23.4
cyclic-AMP	2.82E+05	59.2	1.73E+05	95.6
AICAR	1.59E+06	9.1	1.10E+06	24.9
fructose-1-6-bisphosphate	1.32E+07	94.5	1.53E+07	73.1
trehalose/sucrose	4.72E+07	39.9	2.31E+07	49.0
thiamine-phosphate	6.54E+05	37.6	5.43E+05	85.1
dGMP	4.88E+08	42.8	5.82E+08	27.7
IMP	2.69E+07	47.1	2.58E+07	23.6
S-adenosyl-L-methioninamine	3.92E+05	138.2	9.79E+05	100.7
GMP	2.73E+07	48.6	3.25E+07	32.1
riboflavin	1.40E+06	31.3	1.29E+06	24.7
S-adenosyl-L-homocysteine	1.35E+06	34.1	1.02E+06	39.2
octoluse bisphosphate	1.41E+06	39.8	1.91E+05	54.6

dTDP	4.88E+06	79.7	6.85E+06	85.5
UDP	9.10E+05	71.1	7.80E+05	74.5
cholic acid	2.44E+09	49.4	2.91E+09	56.6
thiamine pyrophosphate	5.60E+04	135.8	1.72E+05	110.0
ADP	3.52E+07	72.7	6.28E+07	68.9
GDP	1.20E+06	140.5	2.10E+06	89.5
CDP-ethanolamine	4.18E+05	50.1	7.92E+05	56.8
5-methyl-THF	1.07E+06	91.8	5.97E+05	46.4
dCTP	N.D.	N.D.	N.D.	N.D.
UTP	1.95E+06	132.2	3.67E+06	128.5
taurodeoxycholic acid	3.16E+09	77.6	3.64E+08	33.7
ATP	4.59E+06	111.7	1.23E+07	90.4
GTP	1.00E+05	N.D.	2.23E+05	97.0
UDP-D-glucose	6.35E+07	35.8	8.77E+07	68.5
UDP-D-glucuronate	5.17E+06	48.1	8.12E+06	86.0
ADP-D-glucose	4.30E+06	41.1	5.86E+06	58.9
UDP-N-acetyl-glucosamine	2.00E+08	31.3	2.39E+08	52.5
glutathione disulfide	2.37E+06	75.1	1.00E+07	55.2
NAD ⁺	5.65E+07	18.1	6.10E+07	70.6
NADH	2.10E+06	68.1	1.47E+06	86.2
dephospho-CoA	1.54E+07	97.6	9.03E+06	33.9
NADP ⁺	9.81E+06	47.1	1.16E+07	74.8
FAD	3.31E+07	25.5	3.06E+07	53.8
acetyl-CoA	5.55E+07	48.3	7.84E+07	98.7
butyryl-CoA	3.00E+07	52.1	5.82E+07	135.0

Supplementary Table 5: Average plasma metabolite ion counts and relative standard deviations (RSD) for Jax (n=5) and NCI (n=5) mice.

Metabolite	Jax		NCI	
	Average	RSD (%)	Average	RSD (%)
lactate	4.14E+06	16.2	3.43E+06	40.5
succinate	6.30E+05	42.4	4.53E+05	31.6
valine	6.67E+04	25.1	4.71E+04	33.8
nicotinamide	6.91E+03	22.1	4.36E+03	N.D.
nicotinate	N.D.	N.D.	N.D.	N.D.
taurine	3.66E+05	48.7	2.31E+05	73.2
hydroxyproline	9.15E+04	30.6	8.26E+04	22.2
leucine/isoleucine	9.92E+04	29.5	8.62E+04	17.0
asparagine	1.10E+05	32.8	9.79E+04	39.5
malate	9.03E+05	57.4	7.42E+05	28.4
anthranilate	3.00E+04	66.8	2.73E+04	27.8
imidazoleacetic acid	5.41E+03	14.6	5.53E+03	6.0
α -ketoglutarate	6.83E+08	46.1	6.14E+08	20.7
glutamine	6.38E+08	13.5	6.74E+08	12.8
lysine	3.56E+06	32.6	4.40E+06	40.4
glutamate	2.26E+08	22.1	1.82E+08	43.4
2-oxo-4-methylthiobutanoate	1.21E+05	68.7	1.55E+05	27.7
2-hydroxy-2-methylbutanedioic acid	1.24E+08	28.3	1.44E+08	53.9
methionine	1.16E+08	26.9	1.28E+08	19.1
3-methylphenylacetic acid	1.74E+06	84.7	3.46E+07	23.3
guanine	2.04E+04	90.5	9.86E+03	52.1
xanthine	5.63E+07	97.6	8.89E+07	39.5
hydroxyphenylacetic acid	5.21E+06	43.2	3.29E+06	21.5
2,3-dihydroxybenzoic acid	6.49E+07	35.3	7.53E+07	35.5
histidine	2.54E+06	24.0	2.55E+06	26.7
orotate	1.33E+07	40.6	1.38E+07	33.6
dihydroorotate	5.11E+05	34.4	3.85E+05	29.8
allantoin	1.24E+08	21.4	1.03E+08	21.3
indole-3-carboxylic acid	5.50E+05	39.6	4.45E+05	25.9
phenylpyruvate	2.13E+06	48.0	4.53E+06	65.6
methionine sulfoxide	1.11E+07	134.3	9.10E+06	45.1
phenylalanine	2.03E+08	22.9	2.09E+08	13.9
phenyllactic acid	1.48E+07	58.8	4.90E+06	35.0
quinolinate	7.89E+05	67.0	1.60E+06	82.4
<i>N</i> -acetyltaurine	4.69E+07	32.3	3.41E+07	29.9
phosphoenolpyruvate	5.96E+06	132.3	9.50E+06	175.9
uric acid	1.92E+08	23.7	1.97E+08	21.8
cysteate	1.84E+05	30.9	1.15E+05	30.6
1-methyl-histidine	1.08E+05	42.1	6.62E+04	42.5
sulfolactate	2.24E+06	36.9	3.35E+06	53.2
dihydroxy-acetone-phosphate	1.62E+07	33.9	7.62E+06	57.8
sn-glycerol-3-phosphate	1.64E+08	29.2	1.51E+08	32.7
aconitate	1.78E+08	28.5	2.03E+08	34.8
<i>N</i> -acetyl-L-ornithine	4.09E+06	27.0	2.76E+06	15.0
arginine	2.56E+06	36.1	3.22E+06	45.1
citrulline	6.72E+07	24.1	6.06E+07	33.1
ascorbic acid	4.81E+06	134.9	9.80E+04	55.5
<i>N</i> -carbamoyl-L-aspartate	5.98E+05	62.1	4.41E+05	13.4

allantoate	2.81E+05	46.1	1.51E+05	25.4
2-isopropylmalic acid	5.97E+06	51.5	7.82E+06	76.9
glucono-1,5-lactone	1.54E+06	35.9	1.65E+06	37.1
glucosamine	3.02E+04	N.D.	N.D.	N.D.
myo-inositol	4.86E+07	9.8	3.85E+07	38.0
tyrosine	1.96E+08	30.0	2.23E+08	14.2
4-pyridoxic acid	5.91E+06	48.7	3.75E+06	19.9
3-phosphoglycerate	4.18E+07	54.2	9.50E+07	133.5
indoleacrylic acid	7.75E+05	47.8	6.79E+05	34.9
kynurenic acid	3.54E+05	42.3	3.82E+05	47.7
N-acetyl-glutamate	1.20E+07	34.2	1.06E+07	28.7
5-hydroxyindoleacetic acid	1.08E+06	44.0	2.31E+06	12.1
citrate/isocitrate	8.59E+09	27.2	8.66E+09	25.8
2-dehydro-D-gluconate	1.98E+07	25.5	1.86E+07	19.6
D-gluconate	1.54E+08	19.4	1.64E+08	18.9
D-erythrose-4-phosphate	4.01E+06	31.7	1.96E+06	50.4
tryptophan	1.74E+08	33.4	1.91E+08	14.9
xanthurenic acid	2.41E+05	60.1	1.08E+06	160.5
kynurenine	5.00E+05	40.6	8.34E+05	51.6
D-glucarate	4.34E+06	62.4	8.36E+06	112.4
pantothenate	2.87E+08	20.8	1.98E+08	13.7
deoxyuridine	5.99E+06	34.0	2.34E+06	36.7
thymidine	1.17E+06	88.6	1.23E+06	33.3
uridine	9.53E+06	26.5	7.90E+06	7.3
D-glucono-1,5-lactone-6-phosphate	1.59E+04	61.1	1.46E+04	37.4
acadesine	3.80E+04	39.2	2.24E+04	14.8
glucose-6-phosphate	2.02E+07	28.6	1.63E+07	45.4
thiamine	1.30E+05	50.1	2.12E+05	62.6
adenosine	7.65E+03	50.9	3.51E+03	N.D.
inosine	6.87E+06	106.4	1.53E+07	56.7
6-phospho- D-gluconate	1.72E+06	85.1	2.33E+06	62.1
1-methyladenosine	4.73E+05	33.8	5.47E+05	20.7
guanosine	2.64E+05	128.1	6.02E+05	61.0
xanthosine	1.19E+05	86.2	8.36E+04	90.0
D-sedoheptulose-1/7-phosphate	1.61E+06	90.1	2.11E+06	162.7
N-acetyl-glucosamine-1/6-phosphate	7.37E+06	27.8	5.61E+06	34.4
dCMP	4.08E+05	163.7	1.92E+04	106.8
glutathione	1.73E+07	173.8	2.49E+06	110.8
geranyl-PP	6.31E+04	78.9	9.28E+04	147.2
dTMP	7.99E+05	123.3	7.03E+03	39.0
CMP	2.98E+06	32.2	2.09E+06	80.0
UMP	6.54E+06	86.5	3.02E+06	162.4
cyclic-AMP	5.31E+04	36.7	7.49E+04	63.0
AICAR	5.32E+03	N.D.	N.D.	N.D.
fructose-1-6-bisphosphate	3.44E+07	58.8	1.30E+07	54.1
trehalose/sucrose	1.13E+06	63.9	7.31E+05	43.3
thiamine-phosphate	N.D.	N.D.	N.D.	N.D.
dGMP	4.16E+07	80.2	7.03E+06	172.1
IMP	4.84E+06	120.2	5.97E+06	201.2
S-adenosyl-L-methioninamine	4.38E+07	30.5	3.97E+07	34.5
GMP	6.15E+06	59.4	7.59E+06	165.9
riboflavin	8.96E+03	9.3	5.60E+03	N.D.
S-adenosyl-L-homoCysteine	2.12E+03	5.9	N.D.	N.D.

octoluse Bisphosphate	N.D.	N.D.	5.09E+03	15.1
dTDP	1.23E+05	111.3	N.D.	N.D.
UDP	2.73E+04	112.7	1.48E+04	26.0
Cholic acid	8.82E+07	130.8	1.57E+07	71.2
thiamine pyrophosphate	1.30E+06	100.1	1.54E+06	78.9
ADP	3.77E+07	100.8	6.70E+06	70.7
GDP	3.16E+06	135.6	7.24E+05	141.8
CDP-ethanolamine	1.84E+05	84.5	7.31E+04	122.4
5-methyl-THF	4.43E+04	57.4	9.40E+03	N.D.
dCTP	N.D.	N.D.	N.D.	N.D.
UTP	5.72E+05	127.6	9.94E+03	17.7
taurodeoxycholic acid	2.04E+07	70.4	1.00E+07	62.8
ATP	1.85E+07	116.3	1.70E+06	79.7
GTP	2.15E+06	177.7	9.97E+04	79.7
UDP-D-glucose	1.31E+06	111.5	8.52E+05	164.0
UDP-D-glucuronate	1.41E+05	138.4	1.15E+05	174.6
ADP-D-glucose	1.49E+05	85.2	7.88E+04	132.1
UDP-N-acetyl-glucosamine	1.56E+06	52.7	8.15E+05	106.9
glutathione disulfide	5.14E+07	50.0	5.66E+07	56.2
NAD ⁺	2.71E+05	157.1	3.24E+04	58.0
NADH	1.03E+04	13.6	N.D.	N.D.
dephospho-CoA	9.94E+04	92.2	N.D.	N.D.
NADP ⁺	7.06E+05	113.1	1.63E+05	N.D.
FAD	1.18E+06	42.2	2.48E+06	68.8
acetyl-CoA	1.87E+07	159.6	9.90E+03	N.D.
butyryl-CoA	3.56E+07	173.1	7.96E+04	N.D.