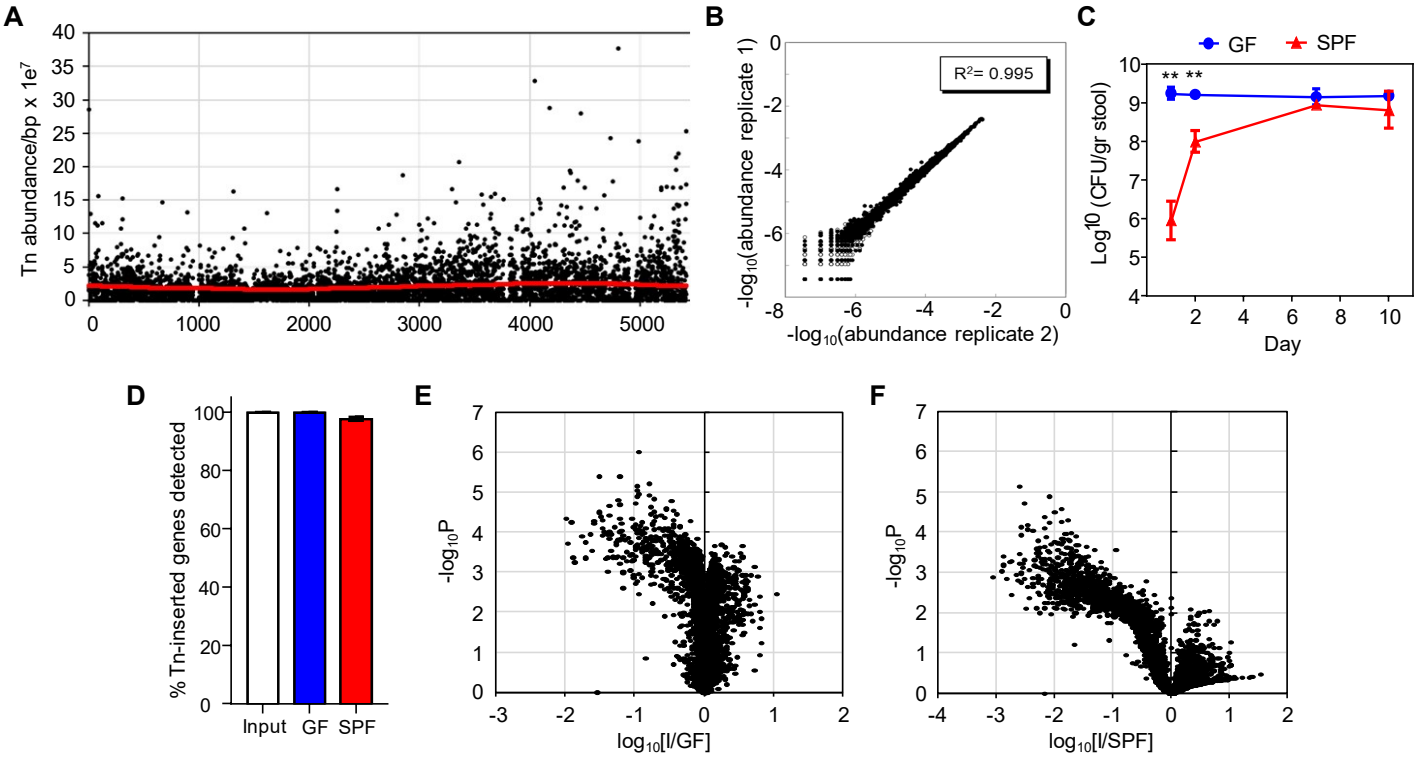

Figure. S1 Characterization of the *C. rodentium* mutant library *in vitro* and after ~~infection~~ in SPF and GF mice (related to Figure 1). (A) Genome-wide distribution and frequency of mutations in the library. Individual Tn densities per gene (dots) are shown. Red line, 3,000 gene moving average. (**B**) Correlation plot of relative Tn abundance per gene from two individual library replicates grown *in vitro*. R^2, coefficient of determination. (**C**) Total pathogen loads in feces of GF and SPF mice orally inoculated with the mutant library (input). (**D**) Percentage of mutated (Tn-inserted) genes detected by Illumina sequencing before (input) and two days after inoculation (outputs) in GF and SPF mice. Input=100%. (**E-F**) Volcano plots showing relative mutant abundances (Tn density ratios) and p-values (P) for individual genes two days post-infection in GF (E) and SPF mice (F) compared to the input. Data are means ± SD (C-D). **, p, 0.01 by Mann-Whitney U test (C).

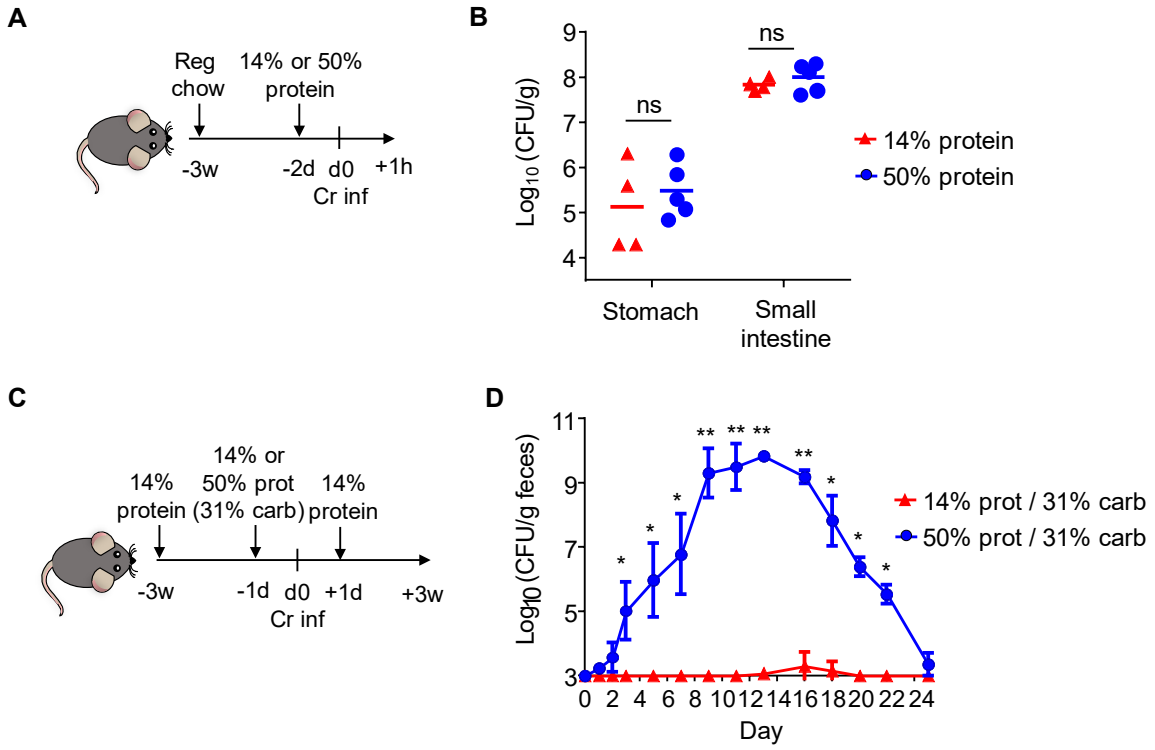

Figure S2. Increased pathogen colonization is not due to a buffering effect in the stomach or lower carbohydrate content in the 50% protein diet (related to Figure 4). (A) Mice were weaned on regular chow and then exposed to 14% or 50% protein diet for two days before oral inoculation with *C. rodentium*. Mice were euthanized 1 h after infection and pathogen loads were determined both in the tissue and luminal contents of stomach and small intestine. **(B)** *C. rodentium* loads in stomach and small intestine in mice treated as in (A). (**C**) Mice were weaned on 14% protein diet and then exposed to carbohydrate-normalized 14% or 50% protein diets one day before and after inoculation with 10^5 cfu of *C. rodentium*. (**D)** Pathogen loads in feces of mice treated as in (C). Representative results of two independent experiments with n= 4-5 per group are shown (B and D). Each symbol represents one mouse (B). *, $p<0.05$; **, $p<0.01$; ns, not significant by Mann-Whitney U test.

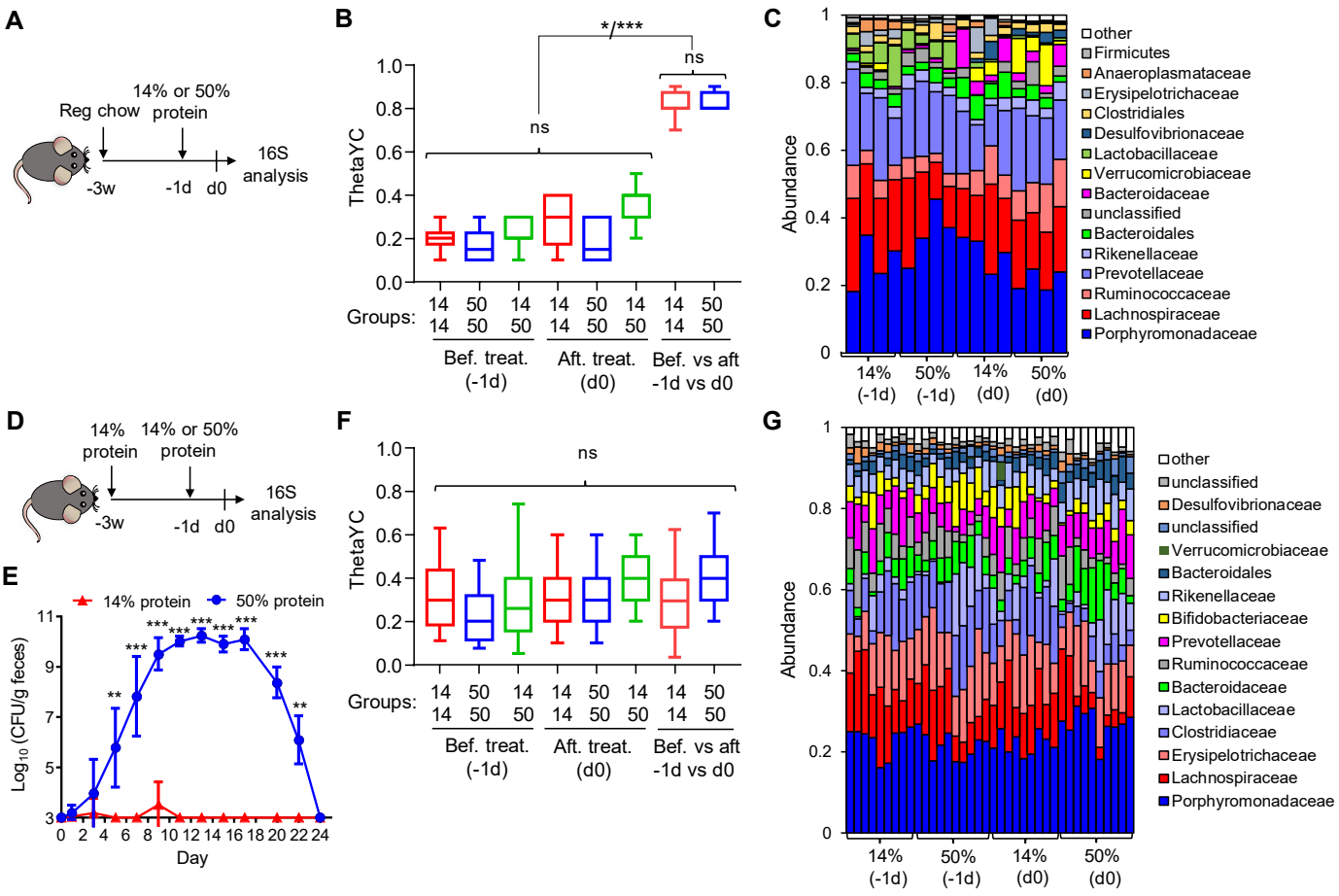

Figure S3. Analyses of fecal microbiota after short-term treatment with protein diets (related to Figure 4). (**A-G**) SPF mice were weaned and maintained for 3 weeks on regular chow (A-C) or 14 % protein diet (D-G), and then treated for one day with the indicated diets (A and D). Composition of the fecal microbiota was determined by 16S rDNA sequencing one day before and after treatment. **(B and F)** Yue/Clayton (ThetaYC) measurements of β-diversity dissimilarity of fecal microbiota from mice treated as in (A) and (D), respectively. **(C and G)** Relative abundance of dominant bacterial families in fecal microbiota of mice treated as in (A) and (D), respectively. (**E**) Pathogen loads in feces of mice treated as in (D) and inoculated with 10^5 cfu of *C. rodentium*. Representative results of two independent experiments with n= 4 (B) or n= 9-10 (F) per group are shown. Error bars indicate min to max (B and F) or SD (E) values. Each bar represents an individual mouse (C and G). *, $p<0.05$; **, $p<0.01$; ***, 0.001; ns, not significant by Kruskal-Wallis test (B and F) or unpaired two-tailed T- test (E).

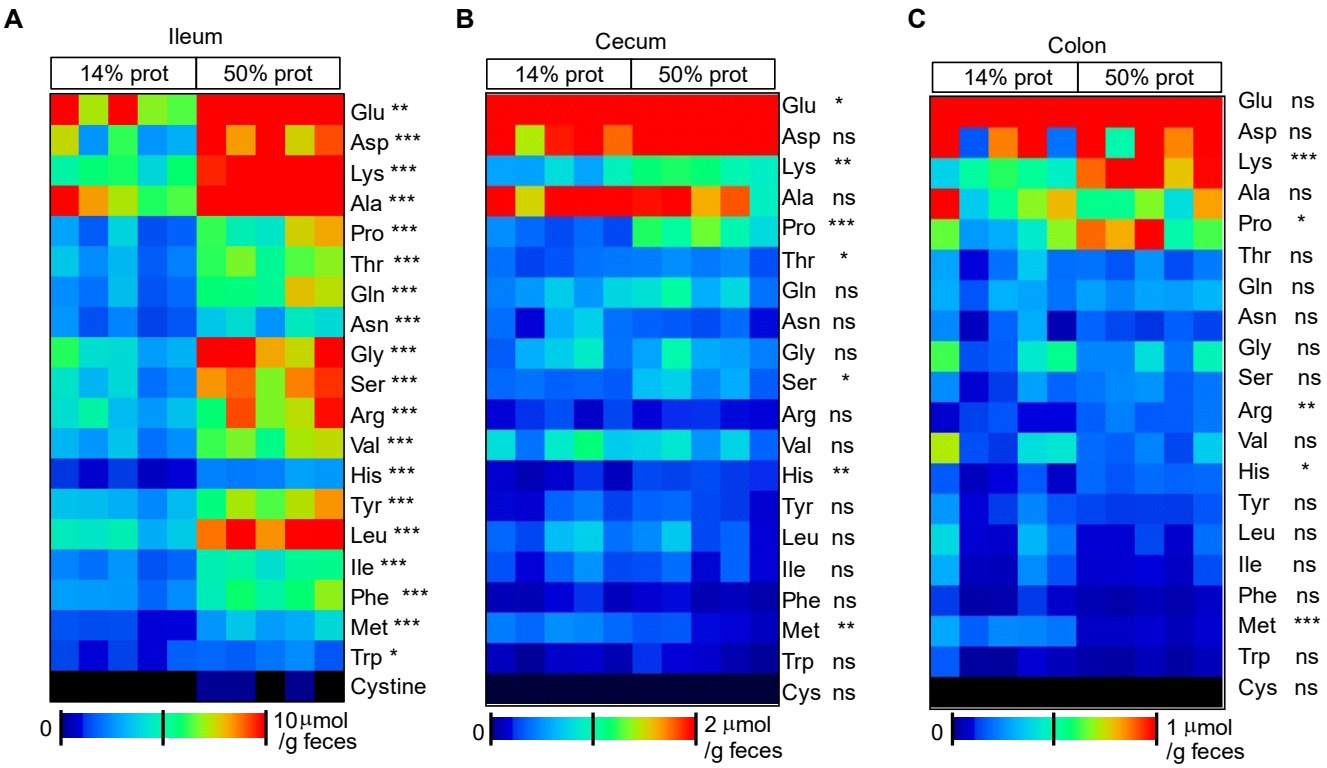

Figure. S4. Short exposure to high protein diet increases AA levels ~~primally~~ in the small intestine (related to Figure 4). (**A-C**) Heat-map showing luminal AA concentrations in the small intestine cecum and colon of SPF mice fed with 14% or 50% protein diet for two days. Amino acid levels were determined by CE-TOFMS. Representative results of two independent experiments with n= 5 per group are shown. Each column represents one mouse. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ns, not significant by unpaired two-tailed T- test comparing 14% vs 50% treatments. Scales are shown below each panel.

Table S2. Primers used in this study (related to Figures 1 to 3)

Name	Sequence 5'-3'	Use	Reference
BioSamA	Bio-TEG-CAAGCAGAAGACGGCATACGAAGACC	Tn-Seq library preparation	Goodman *et al.,* 2011
M12_top	CTGTCCGTTCCGACTACCCTCCCGAC		
M12_bot	GTCGGGAGGGTAGTCGGAACGGACAG		
LIB_PCR_5	CAAGCAGAAGACGGCATACGAAGACCGGGGACTTATCATCCAACCTGT		
LIB_PCR_3	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCT		
LIB_AdaptT_DD	TTCCCTACACGACGCTCTTCCGATCT**AACC**NN	Input barcodings	Goodman *et al.,* 2011
LIB_AdaptB_DD	**GGTT**AGATCGGAAGAGCGTCGTGTAGGGAA		
LIB_AdaptT_EE	TTCCCTACACGACGCTCTTCCGATCT**TTGG**NN		
LIB_AdaptT_EE	**CCAA**AGATCGGAAGAGCGTCGTGTAGGGAA		
LIB_AdaptT_O	TTCCCTACACGACGCTCTTCCGATCT**GCTA**NN	SPF barcodings	Goodman *et al.,* 2011
LIB_AdaptB_O	**TAGC**AGATCGGAAGAGCGTCGTGTAGGGAA		
LIB_AdaptT_P	TTCCCTACACGACGCTCTTCCGATCT**GGAA**NN		
LIB_AdaptB_P	**TTCC**AGATCGGAAGAGCGTCGTGTAGGGAA		
LIB_AdaptT_Q	TTCCCTACACGACGCTCTTCCGATCT**GGGG**NN		
LIB_AdaptB_Q	**CCCC**AGATCGGAAGAGCGTCGTGTAGGGAA		
LIB_AdaptT_R	TTCCCTACACGACGCTCTTCCGATCT**GTAC**NN		
LIB_AdaptB_R	**GTAC**AGATCGGAAGAGCGTCGTGTAGGGAA		
LIB_AdaptT_A	TTCCCTACACGACGCTCTTCCGATCT**TTTT**NN	SPF barcodings	Goodman *et al.,* 2011
LIB_AdaptB_A	**AAAA**AGATCGGAAGAGCGTCGTGTAGGGAA		
LIB_AdaptT_B	TTCCCTACACGACGCTCTTCCGATCT**ACCA**NN		
LIB_AdaptB_B	**TGGT**AGATCGGAAGAGCGTCGTGTAGGGAA		
LIB_AdaptT_C	TTCCCTACACGACGCTCTTCCGATCT**ACGT**NN		
LIB_AdaptB_C	**ACGT**AGATCGGAAGAGCGTCGTGTAGGGAA		
LIB_AdaptT_D	TTCCCTACACGACGCTCTTCCGATCT**AGGA**NN		
LIB_AdaptB_D	**TCCT**AGATCGGAAGAGCGTCGTGTAGGGAA		
LIB_AdaptT_V	TTCCCTACACGACGCTCTTCCGATCT**TCAG**NN		
LIB_AdaptB_V	**CTGA**AGATCGGAAGAGCGTCGTGTAGGGAA		
LIB_AdaptT_W	TTCCCTACACGACGCTCTTCCGATCT**TCGA**NN		
LIB_AdaptB_W	**TCGA**AGATCGGAAGAGCGTCGTGTAGGGAA		
LIB_AdaptT_Y	TTCCCTACACGACGCTCTTCCGATCT**TTAA**NN		
LIB_AdaptB_Y	**TTAA**AGATCGGAAGAGCGTCGTGTAGGGAA		
LIB_AdaptT_Z	TTCCCTACACGACGCTCTTCCGATCT**AAAA**NN		
LIB_AdaptB_Z	**TTTT**AGATCGGAAGAGCGTCGTGTAGGGAA		
D-Arg-Fw	*GTCTACCAAGCAGGGTTTTATTTTATGACGACGATTCTCAAGCATCTT*CATATGAATATCCTCCTTAG	Deletion of *argG*	This paper
D-Arg-Rv	*CGCCATCAGGCTTCGCTTGTATCGTTACTTCGCTTTGTTTTCCAGATT*TGTAGGCTGGAGCTGCTTCG		
D-ThrC-Fw	*GGCGCACGAGTACTGGGATAATTAATGAAACTCTACAATCTTAAAGAT*CATATGAATATCCTCCTTAG	Deletion of *thrC*	This paper
D-ThrC-Rv	*CAGCGCCATCAGGCAGTCAGGCGCTTATCTCCGGCTCATCATCAACTT*TGTAGGCTGGAGCTGCTTCG		

D-HisI-Fw	*AATGCAGGGCGTGGAGATCAGGATATGTTAACAGAGCAAC AGCGCCGC*CATATGAATATCCTCCTTAG	Deletion of *hisI*	This paper
D-HisI-Rv	*GCCCGGTTTTTTTACGCCCGAAGCTTACTGATGACGTCTAC GCAGGTT*TGTAGGCTGGAGCTGCTTCG		
D-TrpA-Fw	*TCTGAAAGCGCGAGGGGAAATCTGATGGAACGCTACGAAA CGCTGTTT*CATATGAATATCCTCCTTAG	Deletion of *trpA*	This paper
D-TrpA-Rv	*AGCGTGTCTGGCGGTCTGTGGGCGCTAACGACGGGTGGC GGCTTTCAT*TGTAGGCTGGAGCTGCTTCG		
D-IlvA-Fw	*TAAATCGAAACTGGGAGGCTGATGATGGCCGAATCGCAAC CTCTGTCA*CATATGAATATCCTCCTTAG	Deletion of *ilvA*	This paper
D-IlvA-Rv	*GCCTTATCCGGCCTACAGTCGTGATCAGGCGCTTTTCTGA AAGTCCTG*TGTAGGCTGGAGCTGCTTCG		
qArgG-Fw	GAATACGGTATCGGATAATC	qRT-PCR of *argG*	This paper
qArgG-Rv	GGTCAACAAACCAGACC		
qHisA-Fw	GGATGTGCGTATTGATGAGAAC	qRT-PCR of *hisA*	This paper
qHisA-Rv	ACCAACTGGAAGATAGGTGGCA		
qIlvA-Fw	ACCCGTCTTAATGAACTG	qRT-PCR of *ilvA*	This paper
qIlvA-Rv	CGCTTTTCTGAAAGTCCTG		
qTrpC-Fw	AGACATTTGCGACAAAGTCAC	qRT-PCR of *trpC*	This paper
qTrpC-Rv	ATCCACGAACTGGTAATCACG		
qKan-Fw4	TTCTGGATTCATCGACTGTGG	qRT-PCR of Kan^R cassette	This paper
qKan-Rv4	GATACCGTAAAGCACGAGGAAG		

Bold, unique barcodes; *Italicized*, homologous sequences to target gene.

Table S3. Diets used in this study (related to Figure 4)

Diet	Nutrient	% by weight	% kcal from
Regular chow[a] (5LOD)	Protein	23	26.5
	Carbohydrate	48.5	58
	Fat	5	13.5
14% protein[b] (TD. 00168)	Protein	14	15.5
	Carbohydrate	66.5	71.5
	Fat	5.5	13
50% protein[b] (TD. 94266)	Protein	50	53.5
	Carbohydrate	31.5	33.5
	Fat	5.5	13
14% protein, 32% carbohydrate[b] (TD.190300)	Protein	14	15.5
	Carbohydrate	31.5	34
	Fat	21	51

[a] Complex carbohydrate-based standard rodent diet. [b] Simple sugar-based special diets.