Supplemental Information for "Recovery of the gut microbiota after antibiotics depends on host diet and environmental reservoirs"

Authors

Katharine Michelle Ng[1], Andrés Aranda-Diaz[1], Carolina Tropini[2], Matthew Ryan Frankel[1], William Winter Van Treuren[2], Colleen O'Laughlin[1], Bryan Douglas Merrill[2], Feiqiao Brian Yu[3], Kali Meredith Pruss[2], Rita Almeida Oliveira[4], Steven Kyle Higginbottom[2], Norma F. Neff[3], Michael Andrew Fischbach[1,3], Karina Bivar Xavier[4], Justin Laine Sonnenburg[2,3], Kerwyn Casey Huang[1,2,3,*]

Affiliations

[1]Department of Bioengineering, Stanford University School of Medicine, Stanford, CA 94305, USA

[2]Department of Microbiology & Immunology, Stanford University School of Medicine, Stanford, CA 94305, USA

[3]Chan Zuckerberg Biohub, San Francisco, CA 94158

[4]Instituto Gulbenkian de Ciência, Oeiras, Portugal

*Corresponding author: kchuang@stanford.edu

Lead author: Kerwyn Casey Huang (kchuang@stanford.edu)

Methods

Supplemental Figure Legends

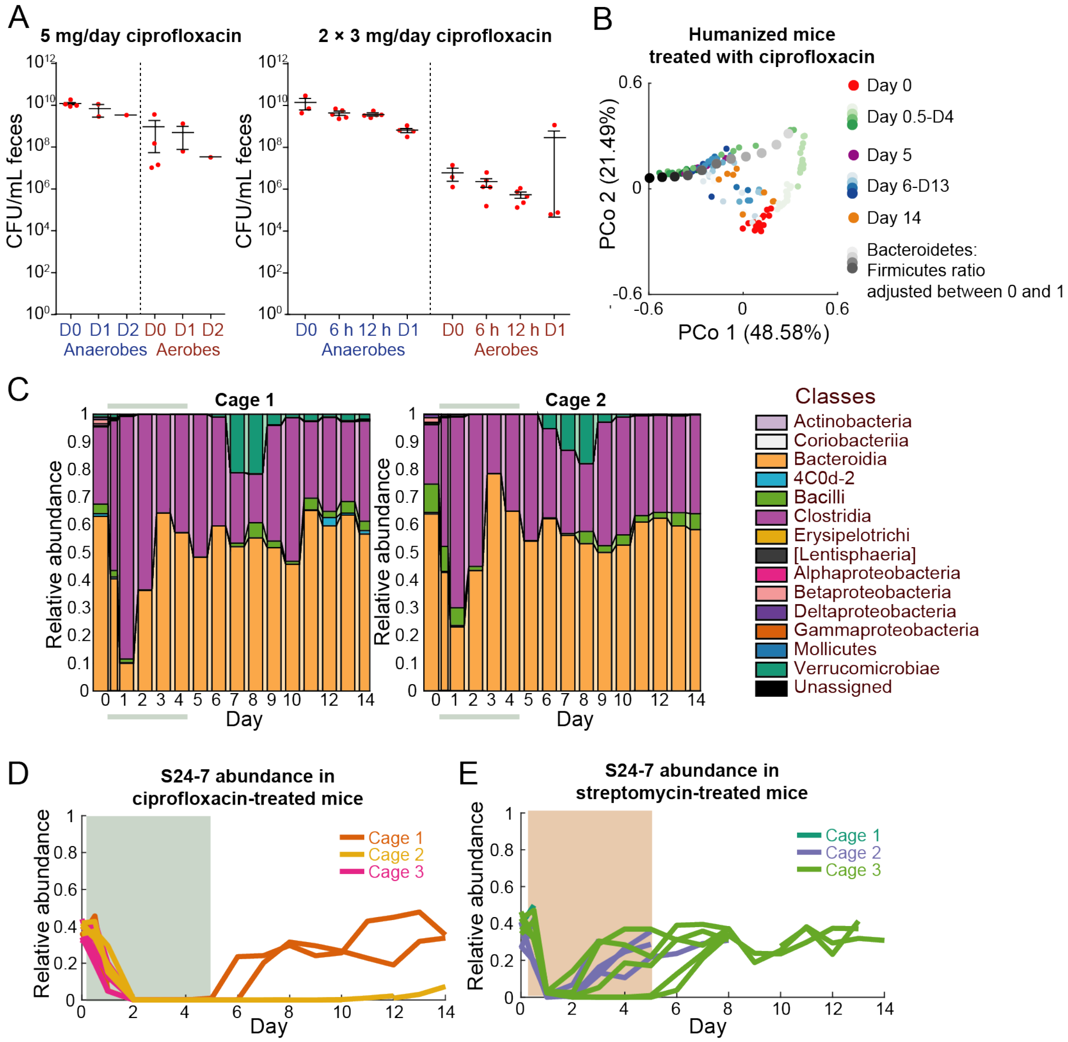

Figure S1: Analyses of streptomycin- and ciprofloxacin-treated mice, related to Figure 1.

A) Culturable bacterial densities in feces of mice treated with 5 mg ciprofloxacin/day or 3 mg twice daily revealed a larger effect with twice daily gavaging.

B) Computational adjustment of the Bacteroidetes:Firmicutes ratio through global rescaling of the fractional abundances of each phylum resulted in movement along principal coordinate 1.

C) Class-level community composition in feces in two cages of ciprofloxacin-treated humanized mice demonstrates insufficiency of class-level analyses to detect cage-specific differences in composition.

D,E) Relative abundance of S24-7 during (D) ciprofloxacin and (E) streptomycin treatment of humanized mice.

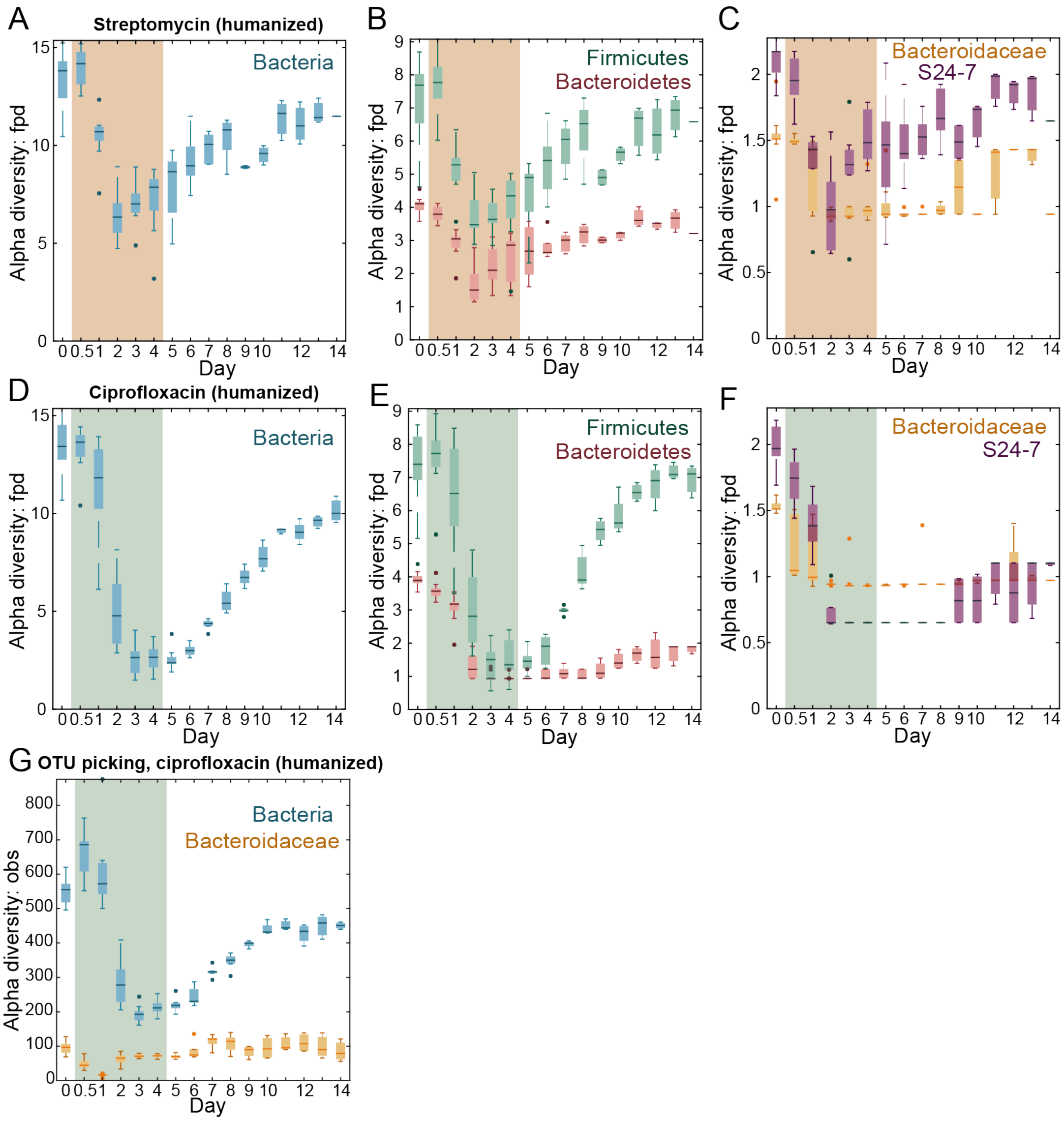

Figure S2: Diversity analyses at different taxonomic levels for streptomycin- and ciprofloxacin-treated humanized mice, related to Figure 2.

A,D) Alpha diversity (Faith's PD) of all bacteria in (A) streptomycin-treated humanized mice and (D) ciprofloxacin-treated humanized mice. Antibiotic treatment period marked in colored area.

B,E) Alpha diversity (Faith's PD) of phyla Firmicutes (green) and Bacteroidetes (pink) in (B) streptomycin-treated humanized mice and (E) ciprofloxacin-treated humanized mice. Antibiotic treatment period marked in colored area.

C,F) Alpha diversity (Faith's PD) of families S24-7 (purple) and Bacteroidaceae (yellow) in (C) streptomycin-treated humanized mice and (F) ciprofloxacin-treated humanized mice. Antibiotic treatment period marked in colored area.

G) Alpha diversity (observed ASVs) of ciprofloxacin-treated humanized mice calculated using *de novo* OTU-picking instead of DADA2.

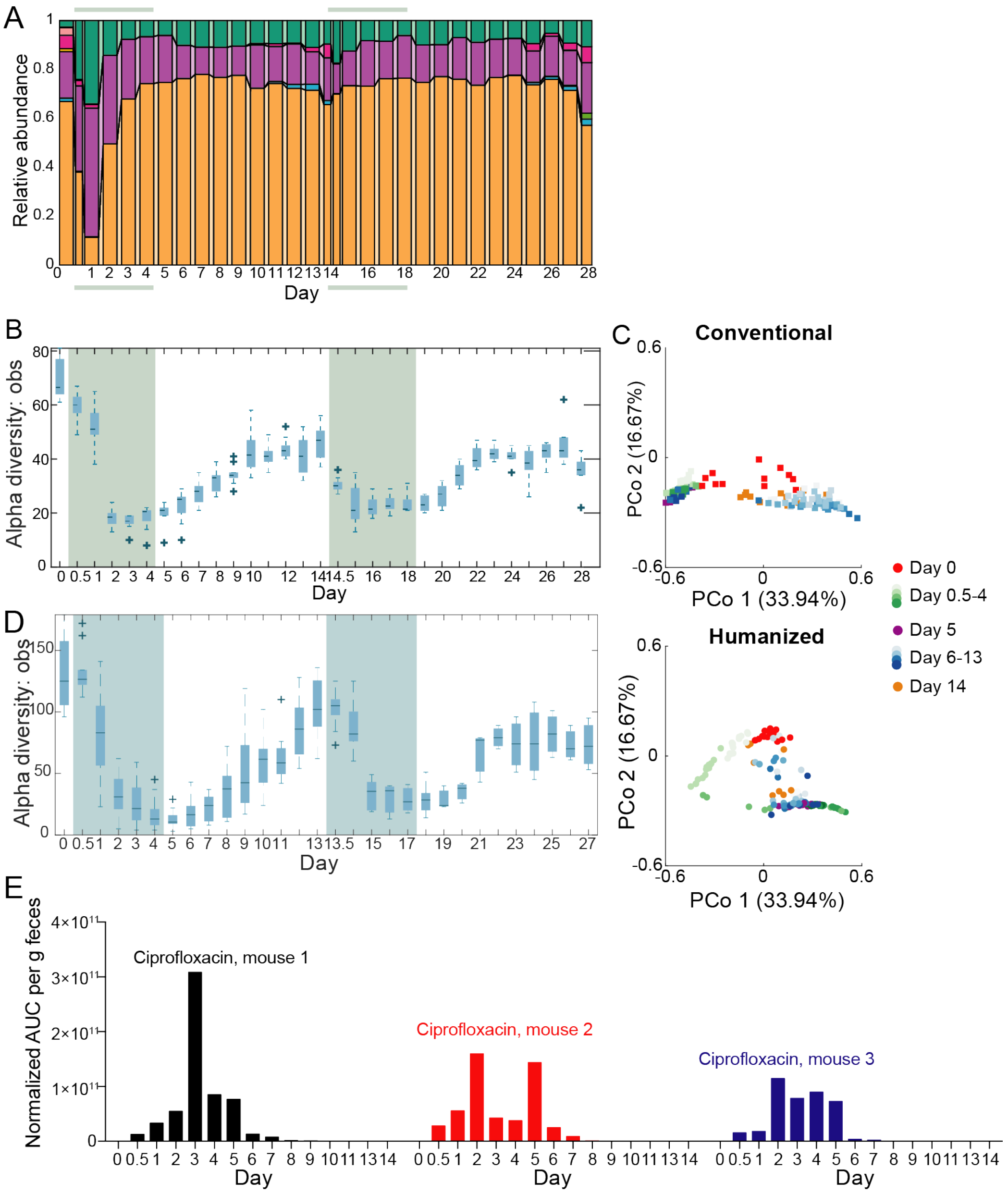

Figure S3: Comparisons of humanized and conventional mice during double treatment with ciprofloxacin, and mass spectrometry analyses of ciprofloxacin-treated mice, related to Figure 3.

A) Class-level community composition in feces of humanized mice further demonstrated the robustness of humanized mice to the second treatment.

B,D) Alpha diversity (observed ASVs) of all bacteria in (B) humanized and (D) conventional mice. The diversity decreased less in the humanized population during the second treatment than during the first.

C) Principal coordinates analyses (PCoA) of community composition in conventional and humanized mice during ciprofloxacin treatment reveal a conserved trajectory. Analyses used weighted UniFrac distances (Methods).

E) Normalized concentrations of ciprofloxacin in feces of conventional mice treated with ciprofloxacin.

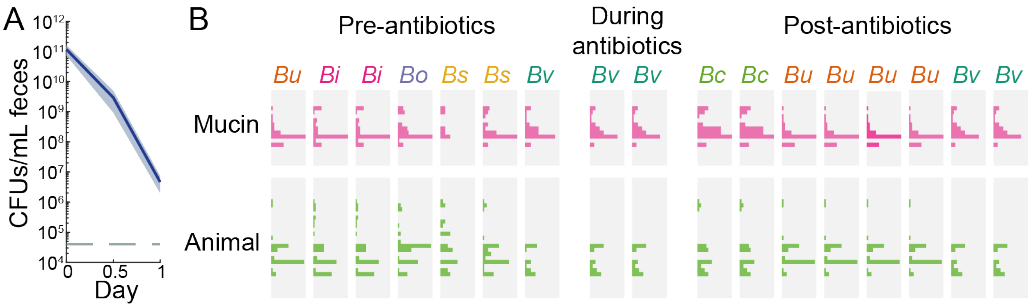

Figure S4: Culturable loads decrease during streptomycin treatment in gnotobiotic mice monocolonized with *Bacteroides thetaiotaomicron*, and glycoside hydrolase abundances vary across *Bacteroides* spp., related to Figures 1, 3, and 4.

A) Culturable anaerobic fecal densities in ex-germ-free mice (*n* = 10) colonized with *B. thetaiotaomicron* for 2 weeks prior to treatment with 20 mg streptomycin on day 0 and day 1. Bacterial loads collapsed as in humanized mice (Fig. 1B). Error bars: standard error of the mean.

B) Presence of carbohydrate active enzymes in *Bacteroides* spp. isolated from humanized mice. Mucin enzymes were over-represented in *Bc* post-antibiotics. *Bu*: *B. uniformis*; *Bi*: *B. intestinalis*; *Bo*: *B. ovatus*; *Bs*: *B. salyersiae*; *Bv*: *B. vulgatus*; *Bc*: *B. caccae*.

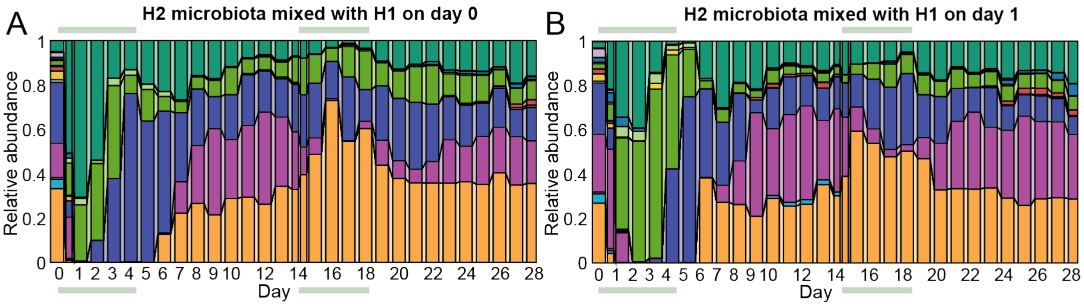

Figure S5: Analyses of microbiota dynamics and trajectories during cross-housing and cohousing experiments, related to Figure 6.

A,B) Family-level community composition in feces of H2 mice mixed with H1 mice on (A) day 0 and (B) day 1.

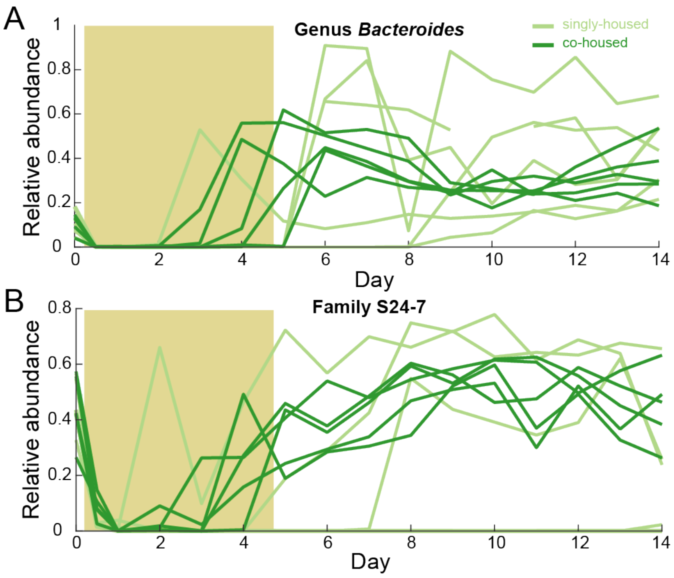

Figure S6: Trajectories of Bacteroidetes taxonomic classes in singly housed and co-housed conventionalized mice, related to Figure 7.

A,B) Relative abundance of the (A) *Bacteroides* genus and (B) S24-7 family in singly- and co-housed conventional mice. Antibiotic treatment period is denoted by colored rectangle. Recovery of both families in more heterogeneous in singly housed mice.

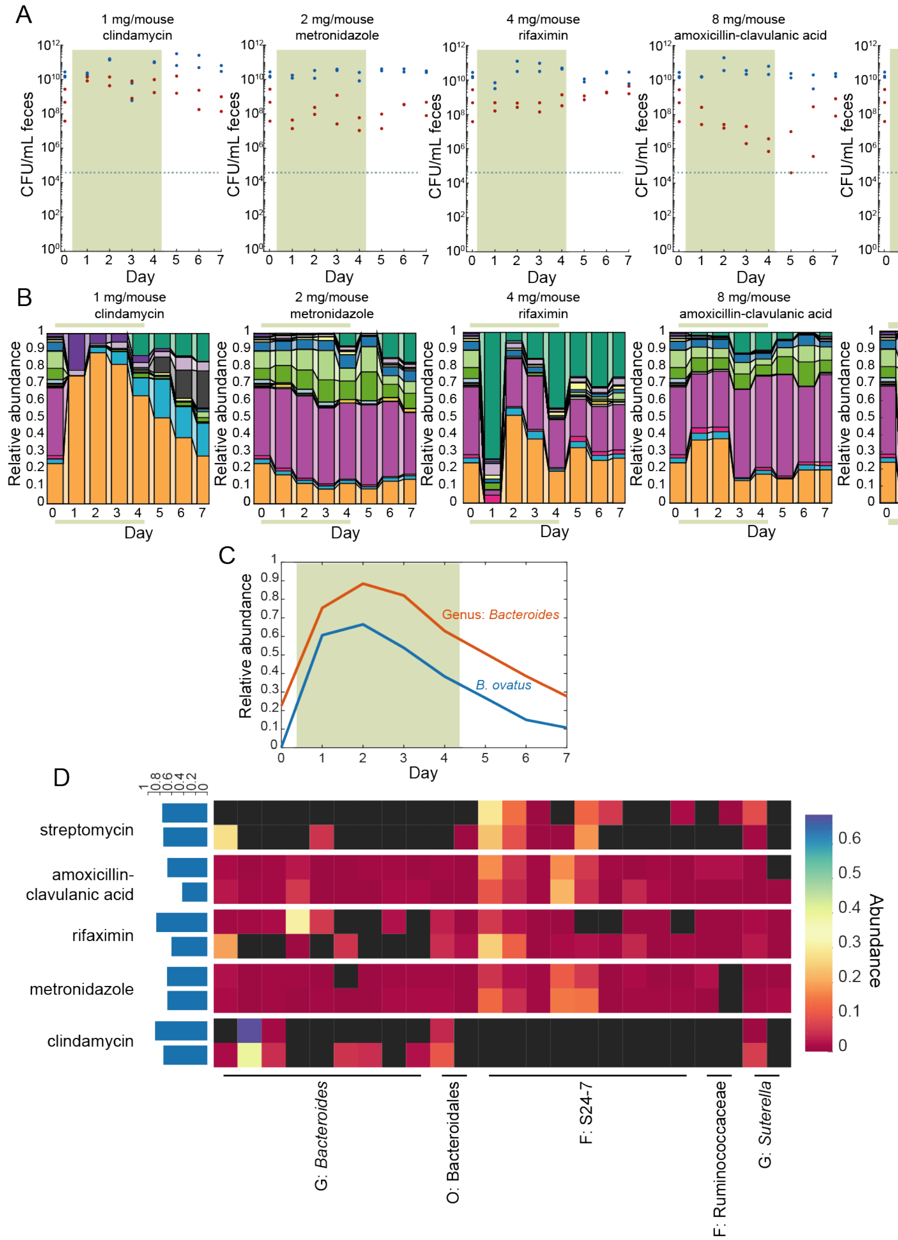

Figure S7: Response of humanized mice to a wide range of antibiotics, related to Figure 5.

A) Culturable anaerobic (blue) and aerobic (red) fecal densities in humanized mice gavaged with antibiotics for 5 days.

B) Family-level community composition in feces of mice in (A).

C) Relative abundance of a *B. ovatus* ASV and the genus *Bacteroides* in clindamycin-treated mice reveals domination of *B. ovatus*.

D) A conserved core microbiota of 24 species is observed in the 10 non-ciprofloxacin treated mice across antibiotics, largely composed of *Bacteroides* and S24-7 species.

Source	**Species**	**Mouse**	**Day**	**MIC cipro**	**MIC strep**	**ResFinder**
Humanized	*Bacteroides vulgatus*	1	0	8	>512	none
Humanized	*Bacteroides vulgatus*	1	4	512	>512	*ermB*, *cfxA*, *tetQ*
Humanized	*Bacteroides vulgatus*	1	12	512	>512	*ermB*, *cfxA*, *tetQ*
Humanized	*Bacteroides caccae*	1	12	64	>512	*tetQ*, *ermF*
Humanized	*Bacteroides vulgatus*	1	16	512	>512	*cfxA*, *tetQ*
Humanized	*Bacteroides salyersiae*	2	0	4	>512	none
Humanized	*Bacteroides stercoris*	2	0	8	>512	*tetQ*
Humanized	*Bacteroides xylanisolvens*	2	0	8	N.D.	*tetQ*
Humanized	*Bacteroides intestinalis*	2	0	2	>512	none
Humanized	*Bacteroides intestinalis*	2	0	4	>512	none
Humanized	*Bacteroides uniformis*	2	0	16	N.D.	*tetQ*
Humanized	*Bacteroides uniformis*	2	10	64	>512	*tetQ*
Humanized	*Bacteroides uniformis*	2	10	64	16	*tetQ*
Humanized	*Bacteroides vulgatus*	2	16	>512	>512	*ermB*, *cfxA*, *tetQ*
Humanized	*Bacteroides caccae*	2	22	64	>512	*tetQ*, *ermF*
Humanized	*Bacteroides uniformis*	2	22	64	>512	*tetQ*
Humanized	*Bacteroides uniformis*	2	22	64	N.D.	*tetQ*
Conventional	*Bacteroides vulgatus*	1	0	4	>512	none
Conventional	*Bacteroides vulgatus*	1	0	8	>512	none
Conventional	*Bacteroides acidifaciens*	1	0	2	N.D.	none
Conventional	*Bacteroides uniformis*	1	0	4	1	*tetQ*
Conventional	*Bacteroides vulgatus*	1	11	4	>512	none
Conventional	*Bacteroides acidifaciens*	1	11	4	1	none
Conventional	*Bacteroides uniformis*	1	11	4	N.D.	*tetQ*
Humanized H2	*Bacteroides* sp. D1	E1.1	11	8	16	*cfxA*, *tetQ*
Humanized H2	*Bacteroides* sp. D1	E1.1	11	4	N.D.	*cfxA*, *tetQ*
Humanized H2	*Bacteroides salyersiae*	E1.1	11	4	>512	none
HMP	*Bacteroides cellulosilyticus* CL02T12C19			8	N.D.	
HMP	*Bacteroides salyersiae* CL02T12C01			32	>512	
HMP	*Bacteroides stercoris* CC31F			4	512	
HMP	*Bacteroides vulgatus* CL09T03C04			8	>512	
Type strain	*Bacteroides theta* VPI5482			4	256	
Type strain	*Bacteroides fragilis* NCTC9343			4	512	
Type strain	*Bacteroides vulgatus* ATCC8482			8	>512	
Type strain	*Bacteroides distasonis* ATCC8503			8	>512	
Type strain	*Bacteroides caccae* ATCC43185			32	N.D.	
Type strain	*Bacteroides ovatus* ATCC8483			8	512	
Type strain	*Bacteroides uniformis* ATCC8492			4	256	
Type strain	*Bacteroides intestinalis* DSM 17393			4	256	

Table S1: Ciprofloxacin and streptomycin MICs for representative *Bacteroides* isolates and type strains, related to Figures 1, 3, and 6. Columns 3 and 4 contain the mouse number and day of fecal sample from which the isolate was obtained, where day 0 is when antibiotic treatment commenced. N.D.: not determined. Table also includes any antibiotic resistance genes detected using ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/).