Supplementary Figures for "Gut microbiota transmission prevents antibiotic-induced stochastic loss of colonization resistance"

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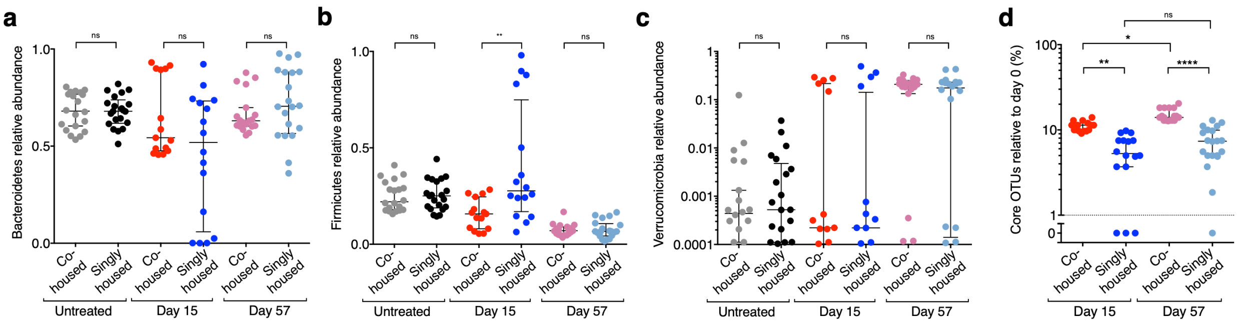
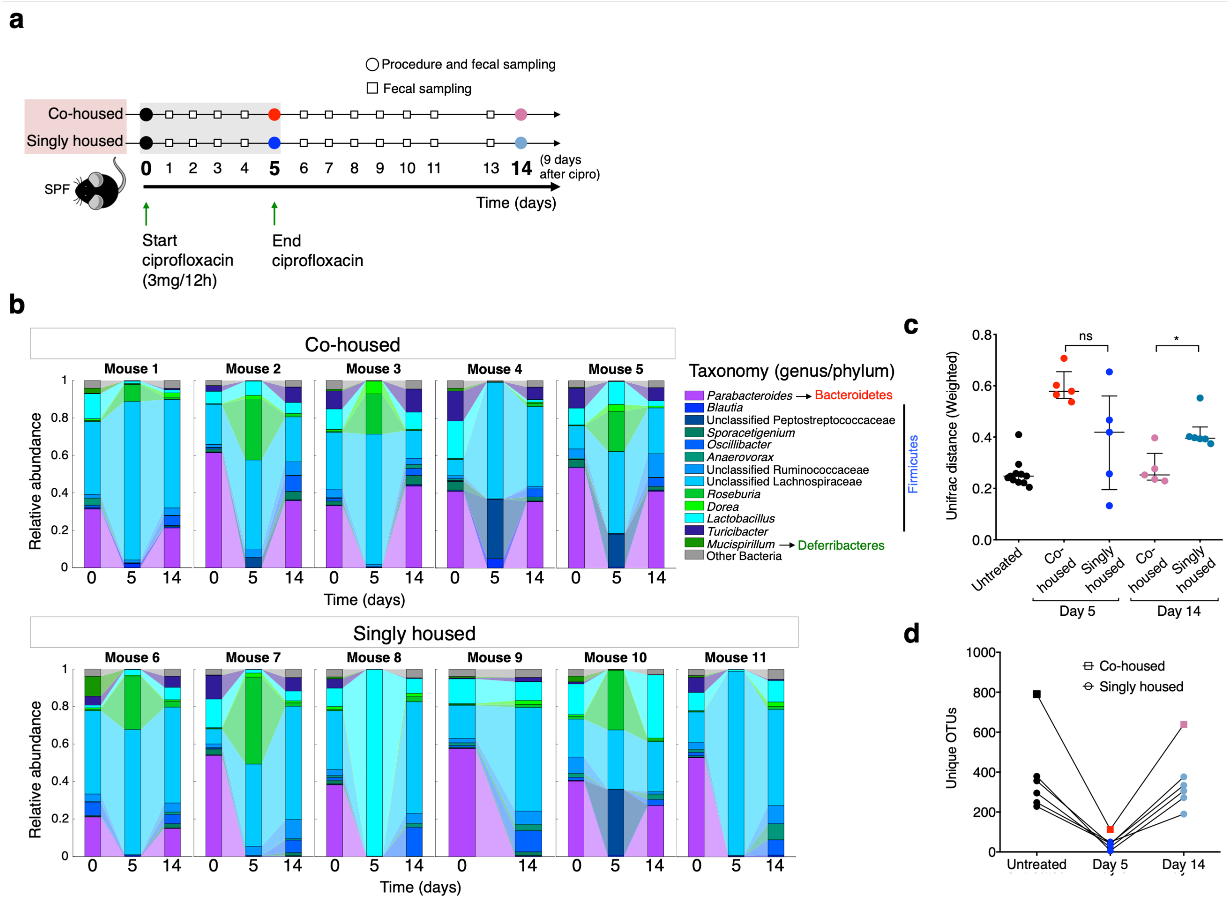
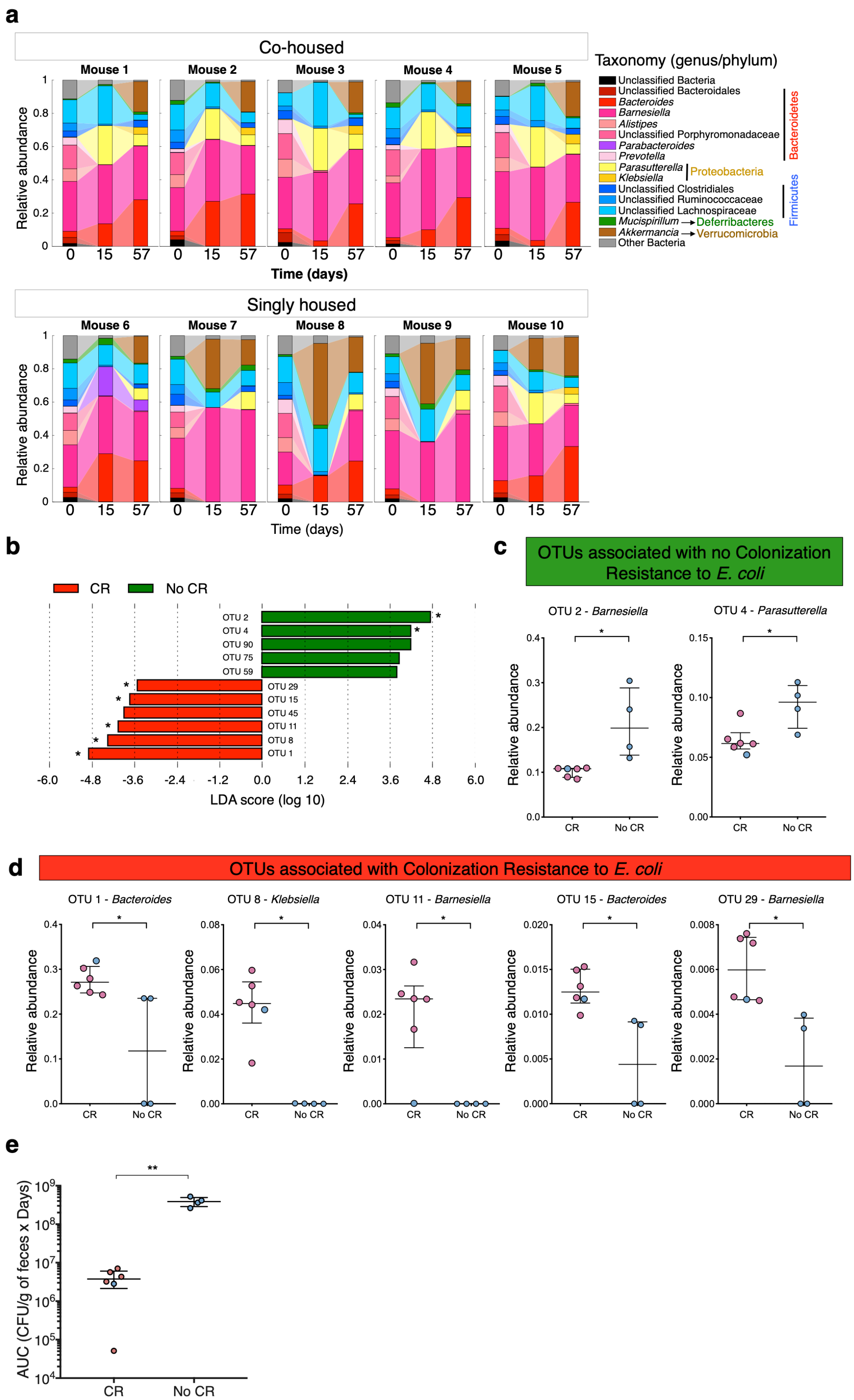


Supplementary Fig. 1| Streptomycin treatment leads to increased loss of core OTUs in singly housed mice compared to co-housed mice, but does not differentially affect Bacteroidetes, Firmicutes, or Verrucomicrobia relative abundance. a-c, Relative abundances of the **a**, Bacteroidetes, **b**, Firmicutes and **c**, Verrucomicrobia phyla in the co-housed and singly housed mice at indicated time points**.** Data shown are the median, and the error bars show the interquartile range of n=15-21 per group. **d**, Percentage of core OTUs (defined as OTUs present in every mouse of each group before treatment) present on days 15 and 57, relative to day 0. Data shown are the median, and the error bars show the interquartile range of n=15-21 per group. For **a-c** data were analyzed with a Mann-Whitney test (**: p<0.01; ns, not significant). For **d**, data were analyzed using the Kruskal-Wallis test with Dunn's correction test for multiple comparisons (*: q < 0.1, **: q < 0.05; ****: q < 0.001; ns, not significant).

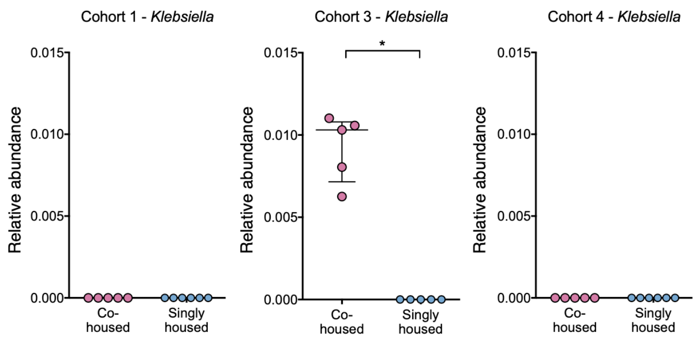


Supplementary Fig. 2| Ciprofloxacin treatment results in stochastic extinction of the Bacteroidetes phylum in singly housed mice. a, Experimental scheme for ciprofloxacin treatment. Three milligrams of ciprofloxacin was administered orally every 12 h to conventional Swiss Webster mice for 5 days. Microbiota composition was analyzed from fecal samples collect from day 0 (before antibiotics), day 5 (last day of antibiotic treatment), and day 14 (9 days after stopping antibiotic treatment). **b**, Fecal microbiota compositions of day 0, 5, and 14 of co-housed (Mouse 1-5) and singly housed (Mouse 6-11) mice. Each stacked bar represents the microbiota composition in the indicated mouse at the indicated time points. The colored segments represent the relative fraction of each genus-level taxon present at >3%. All other genera were combined in the “Other Bacteria” category. **c**, Phylogenetic dissimilarities on each day determined by the mean weighted

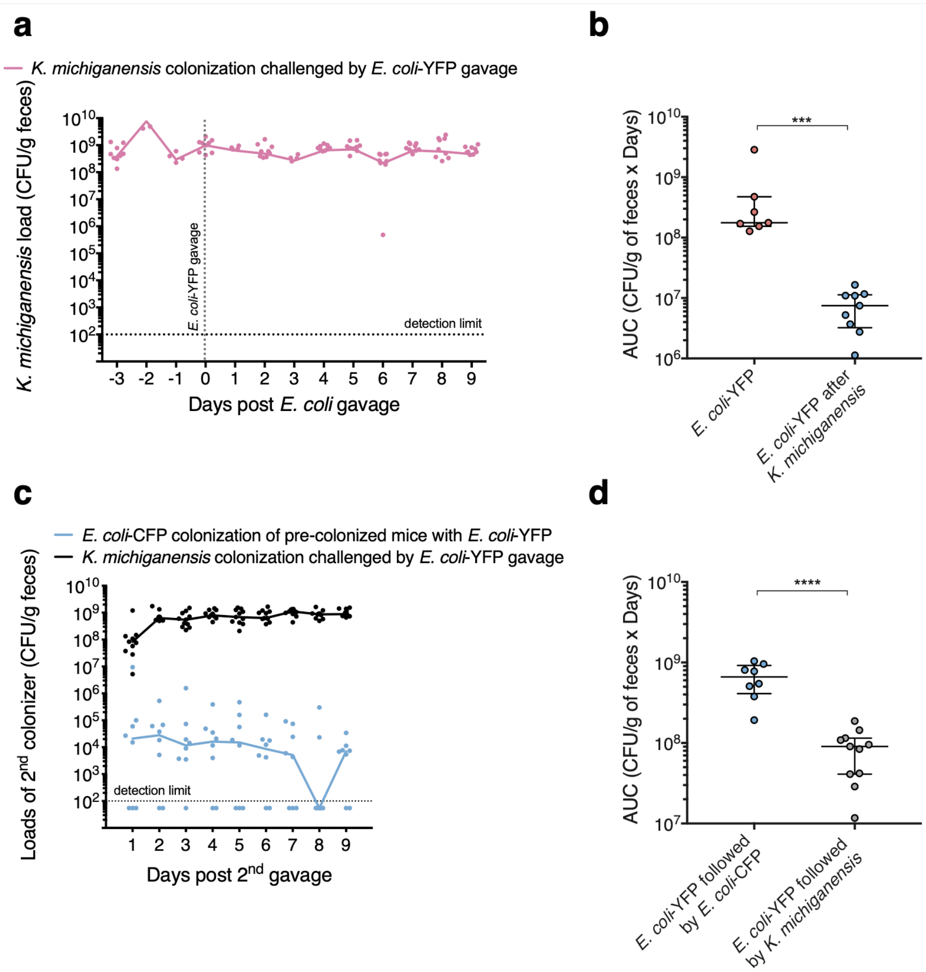
Unifrac distance of the bacterial communities of each mouse to each other mouse within the same group. Data shown are medians, and the error bars show interquartile range (*: $p<0.05$; ns, not significant, Mann-Whitney test, n=5-6 per group). **d**, Gamma diversity of gut microbiota of co-housed and singly housed mice at indicated time points (n=5-6 per group).


a
Co-housed
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Relative abundance
Time (days)
Singly housed
Mouse 6
Mouse 7
Mouse 8
Mouse 9
Mouse 10
Taxonomy (genus/phylum)
Unclassified Bacteria
Unclassified Bacteroidales
Bacteroides
Barnesiella
Alistipes
Unclassified Porphyromonadaceae
Parabacteroides
Prevotella
Parasutterella
Klebsiella
Unclassified Clostridiales
Unclassified Ruminococcaceae
Unclassified Lachnospiraceae
Mucispirillum
Akkermancia
Other Bacteria
Bacteroidetes
Proteobacteria
Firmicutes
Deferribacteres
Verrucomicrobia
b
CR
No CR
OTU 2
OTU 4
OTU 90
OTU 75
OTU 59
OTU 29
OTU 15
OTU 45
OTU 11
OTU 8
OTU 1
LDA score (log 10)
c
OTUs associated with no Colonization Resistance to E. coli
OTU 2 - Barnesiella
OTU 4 - Parasutterella
d
OTUs associated with Colonization Resistance to E. coli
OTU 1 - Bacteroides
OTU 8 - Klebsiella
OTU 11 - Barnesiella
OTU 15 - Bacteroides
OTU 29 - Barnesiella
e
AUC (CFU/g of feces x Days)
CR
No CR

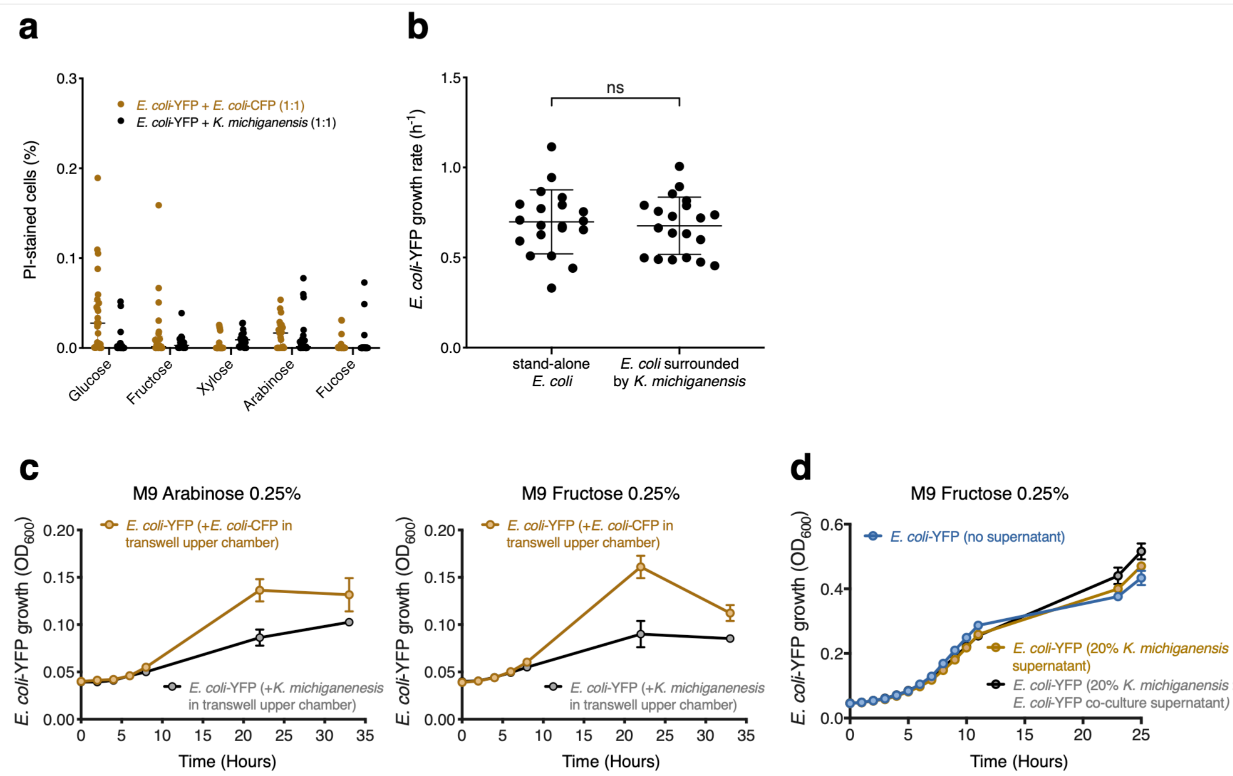
Supplementary Fig. 3| Effects of streptomycin treatment in microbiota composition and OTUs associated with colonization resistance to *E. coli* in cohort 2 (supplementary data for the experiment shown in Fig. 1 and 2). a, Fecal microbiota composition of untreated (day 0), streptomycin-treated (day 15), and post-streptomycin-treated (day 57) samples of co-housed and singly housed mice from cohort 2. Each stacked bar represents the microbiota composition in each mouse at the indicated time points. The colored segments represent the relative fraction of each genus-level bacterial taxon present at >3%. All other genera were combined in the "Other Bacteria" category. **b**, Histogram of linear discriminant analysis (LDA) scores >2.0 for differentially abundant OTUs on day 57 of cohort 2 from the experiment in Fig. 2c, d. (Red) OTUs enriched in mice with colonization resistance to *E. coli* (CR); (green) OTUs enriched in mice lacking colonization resistance (No CR). **c**, Relative abundances of significantly different individual OTUs that are associated with no colonization resistance against *E. coli*. **d**, Relative abundances of significantly different individual OTUs that are associated with colonization resistance against *E. coli*. **e**, Area Under the Curve (AUC) calculated from the dynamics of *E. coli*-YFP CFU/g feces during the cohort 2 experiment of each mouse with and without colonization resistance, showing that *Klebsiella*-associated mice had significantly lower loads of *E. coli* throughout the experiment. In **c-e**, medians and interquartile ranges are shown. Data shown in **c** and **d** were analyzed with the Wilcoxon test using a Benjamini-Hochberg correction (∗: $q<0.1$). Data in **e** were analyzed with the Mann-Whitney test (**: $p<0.01$).



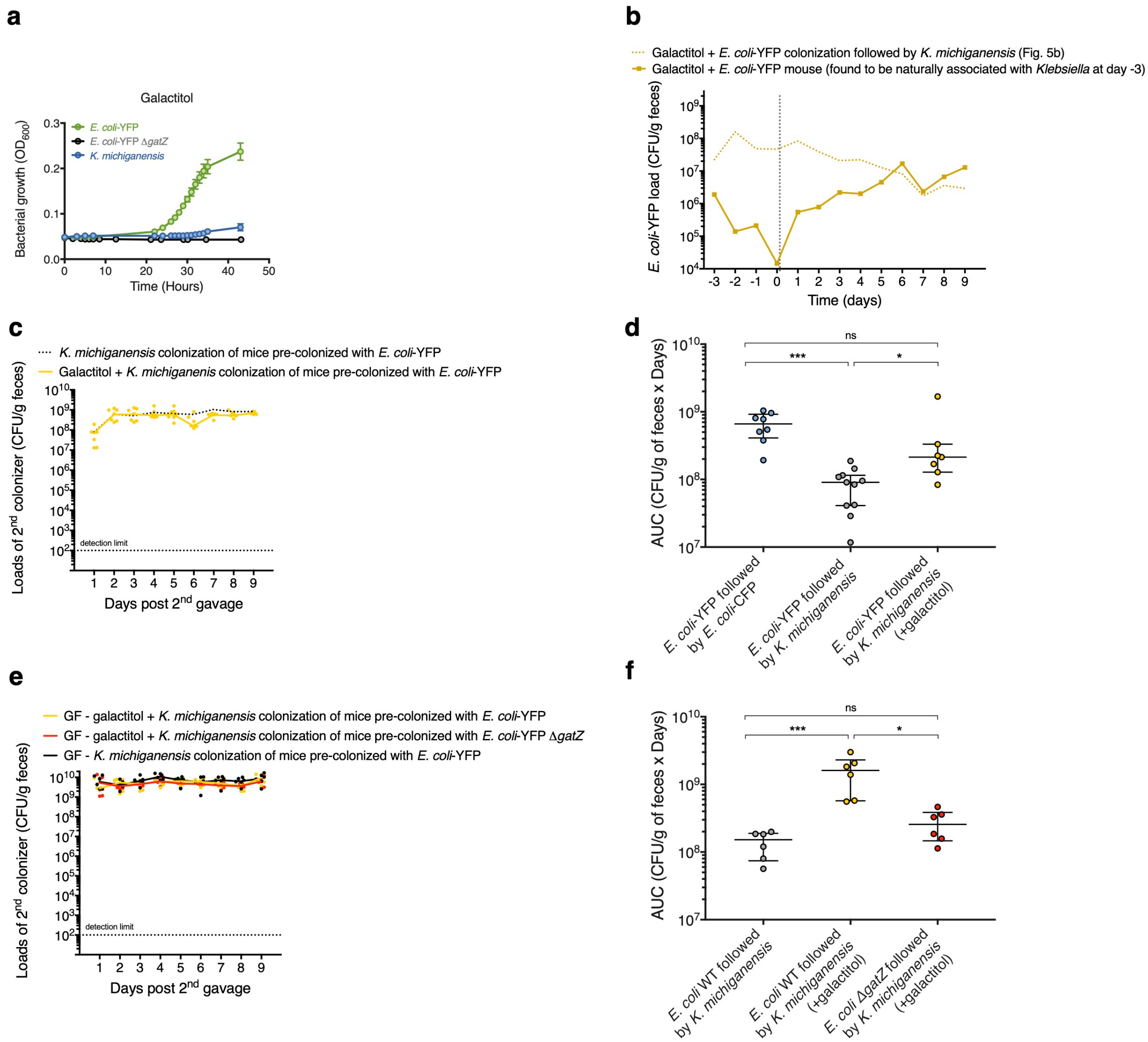
Supplementary Fig. 4| *Klebsiella* relative abundance in cohorts 1, 3, and 4. Relative abundances of *Klebsiella* in cohorts 1, 3, and 4 at day 57 of the experiment (*Klebsiella* abundance in cohort 2 is shown in Supplementary Fig. 3d). Medians are shown, and error bars represent the interquartile range. Data shown were analyzed with the Wilcoxon test using a Benjamini-Hochberg correction (∗: q<0.1).



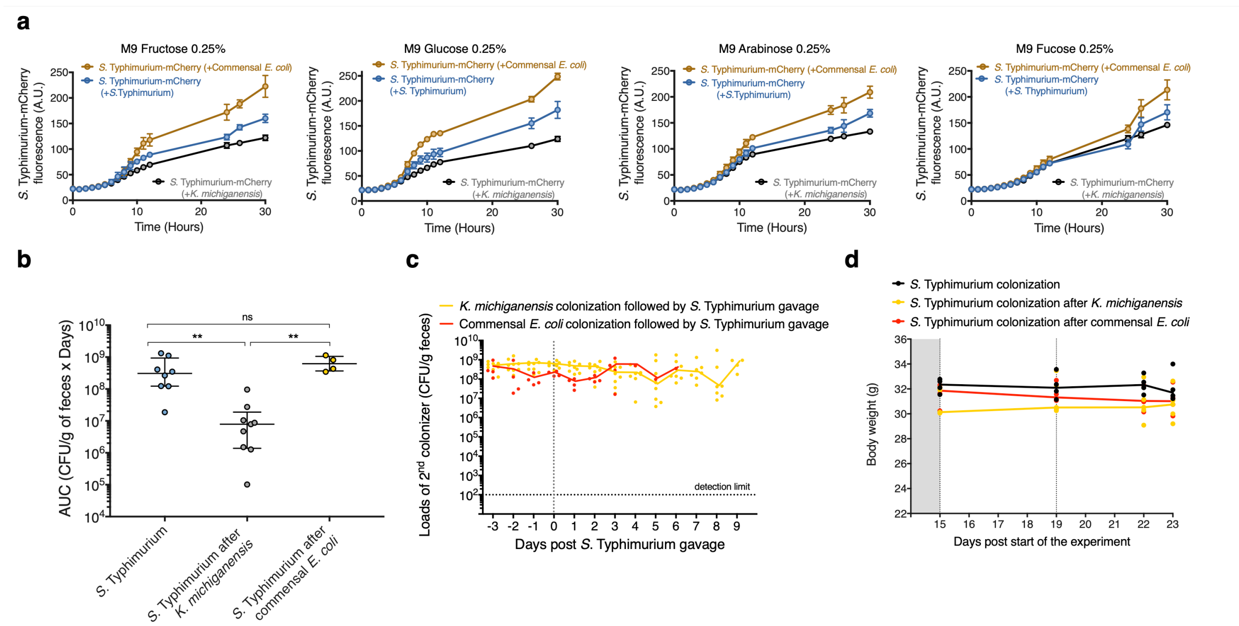
Supplementary Fig. 5| *K. michiganensis* and *E. coli*-CFP colonization loads from experiment in Fig. 3c-e. a, Loads of *K. michiganensis* (CFUs/g feces) before and after challenge with *E. coli*-YFP (*n*=9 across two independent experiments, Fig. 3d). **b**, Area Under the Curve (AUC) calculated from the dynamics of *E. coli*-YFP CFU/g feces of each mouse with and without *K. michiganensis.* Mice associated with *K. michiganensis* had significantly lower loads of *E. coli* throughout the experiment (*n*=9 across two independent experiments, Fig. 3d). **c**, *K. michiganensis* (n=11, across three independent experiments) and *E. coli*-CFP (n=8, across two independent experiments) loads (CFUs/g feces) in mice pre-colonized with *E. coli*-YFP (Fig. 3c, e). **d**, Area Under the Curve (AUC) calculated from the dynamics of *E. coli*-YFP CFU/g feces of each mouse after challenge with *K. michiganensis* or *E. coli*-CFP*.* Mice challenged with *K. michiganensis* had significantly lower loads of *E. coli* throughout the experiment (n=11, across three independent experiments) as compared to challenge with *E. coli*-CFP (n=8, across two independent experiments; Fig. 3e). In **a** and **c**, circles and lines represent values from individual mice and medians, respectively. In **b** and **d**, medians and interquartile ranges are shown. Data in **b** and **d** were analyzed with the Mann-Whitney test (***: $p<0.001$; ****: $p<0.0001$).



Supplementary Fig 6| No killing of *E. coli* or direct physical inhibition by *K. michiganensis* was observed *in vitro*. a, Co-cultures of *E. coli*-YFP with *E. coli*-CFP or *K. michiganensis* in minimal media containing 0.25% of the indicated carbon source exhibited similar percentages of PI-stained cells, suggesting the absence of active killing of *E. coli*. **b,** There was no statistically significant difference in the growth rates of *E. coli* microcolonies that were well separated from versus surrounded by *K. michiganensis* cells over >50% of the periphery in in minimal media with arabinose. **c**, Cell density of *E. coli*-YFP in the bottom chamber of a transwell incubated with *K. michiganensis* or *E. coli*-CFP in the upper chamber in minimal media with arabinose or fructose. Growth was monitored by OD_{600} measurements for 35 h (*n*=3 per condition). **d**, Cell density of *E. coli*-YFP in minimal medium with fructose with or without supplementation of 20% cell-free spent medium from overnight cultures of *K. michiganensis* or *E. coli*-YFP+*K. michiganensis* grown in minimal medium with fructose. Growth was monitored by OD_{600} measurements for 25 h (*n*=3 per condition). Data in **b** were analyzed with the Mann-Whitney test (ns, not significant). Data in **b**-**d** represent means and standard deviations.



Supplementary Fig 7| Galactitol can sustain *E. coli*-YFP growth but not *K. michiganensis in vitro*; *E. coli*-YFP colonization of a singly housed mouse in which *Klebsiella* spp. were not eliminated by streptomycin treatment, and loads of the 2nd colonizer from the experiment shown in Fig. 5. a, Galactitol growth capacity of *K. michiganensis, E. coli*-YFP, and *E. coli*-YFP Δ*gatZ* alone in minimal media containing 0.25% galactitol. Growth was monitored by OD_{600} measurements at the indicated times for 43 h (*n*=6 per condition). *K. michiganensis* and *E. coli*-YFP Δ*gatZ* were unable to grow on galactitol, in contrast to *E. coli*-YFP. **b,** *E. coli*-YFP loads (CFUs/g feces) decreased immediately after gavage (day -3) in a singly housed mouse found to have maintained *Klebsiella* spp. after streptomycin treatment (yellow solid line). This mouse was not included in the data displayed in Fig. 5b. Dashed yellow line shows the median CFUs/g feces of *E. coli*-YFP from Fig. 5b for comparison. **c**, Yellow line shows *K. michiganensis* loads (CFUs/g feces) in mice pre-colonized with *E. coli*-YFP and drinking water supplemented with 2% galactitol (*n*=7 across two independent experiments, Fig. 5a). Black dashed line show median CFUs/g feces of *K. michiganensis* from the experiment in Fig. 2c,e for comparison (Supplementary Fig. 5c). **d**, Area Under the Curve (AUC) calculated from the dynamics of *E. coli*-YFP CFUs/g feces of each mouse under galactitol after challenge with *K. michiganensis* (*n*=7 across two independent experiments, Fig. 5b) comparing with dynamics of *E. coli*-YFP CFUs/g feces of each mouse under non-supplemented water with and without *K. michiganensis* (data from Supplementary Fig. 5d) *.* Mice under galactitol treatment have significantly higher *E. coli* loads throughout the experiment when compared with mice in non-supplemented water, even in the presence of *K. michiganensis*. **e**, *K. michiganensis* loads (CFUs/g feces) in germ-free mice pre-colonized with *E. coli*-YFP or *E. coli*-YFP Δ*gatZ* with non-supplemented or supplemented drinking water with 2% galactitol (*n*=6 per condition across two independent experiments, Fig. 5c). **f**, Area Under the Curve (AUC) calculated from the dynamics of *E. coli*-YFP CFUs/g feces of each germ-free mouse under non-supplemented or supplemented drinking water with 2% galactitol after challenge with *K. michiganensis* (*n*=6 per group across two independent experiments, Fig. 5d)*.* Mice under galactitol treatment have significantly higher *E. coli*-YFP loads throughout the experiment when compared with mice in non-supplemented water or *E. coli*-YFP Δ*gatZ* in mice under galactitol treatment, even in the presence of *K. michiganensis*. Data in **a** represent means and standard deviations. In **c** and **e** circles and lines represent values from individual mice and medians, respectively. In **d** and **f**, median and interquartile range is shown. Data in **d** and **f** data were analyzed using Kruskal-Wallis test with Dunn's correction test for multiple comparison (*$q < 0.1$; ***$q < 0.01$; ns, not significant).



Supplementary Fig. 8| Competition for simple sugars of *K. michiganensis* and commensal *E. coli* with *S*. Typhimurium, and colonization loads of *K. michiganensis* and commensal *E. coli* from the experiment in Fig. 6. a, *S.* Typhimurium-mCherry growth capacity in co-cultures with *K. michiganensis*, commensal *E. coli* or an isogenic *S.* Typhimurium (with no fluorescent marker) in minimal media containing 0.25% of the indicated carbon source. *S.* Typhimurium-mCherry growth was monitored by mCherry fluorescence quantification (*n*=6 per condition). **b,** Area Under the Curve (AUC) calculated from the dynamics of *S*. Typhimurium CFUs/g feces of each mouse in the first 3 days after *S*. Typhimurium challenge in mice pre-colonized with *K. michiganensis* (*n*=9 across 2 independent experiments), as compared to mice not pre-colonized or pre-colonized with a commensal *E. coli* (*n*= 8 across 2 independent experiments and *n*=4 in 1 experiment, respectively; Fig. 6). Mice colonized with *K. michiganensis* had significantly lower *S*. Typhimurium loads as compared to mice not pre-colonized or pre-colonized with a commensal *E. coli*. **c**, Loads of *K. michiganensis* and commensal *E. coli* (CFUs/g feces) before and after challenge with *S.* Typhimurium (*n*=4-9, Fig. 6b). **d,** Mice body-weight is unaffected by *K. michiganensis* or commensal *E. coli* colonization (Day 19). Data in **a**, represent means and standard deviations. In **b**, median and interquartile range is shown. Data in **b** was analyzed using the Kruskal-Wallis test with Dunn's correction test for multiple comparisons (**: $q < 0.05$; ns, not significant). In **c,d** circles and line represent values from individual mice and medians, respectively.

Litter ID	**Number of mice per litter**	**Date of birth**	***Klebsiella***
1	9	09/01/18	-
2	8	27/01/18	-
3	8	18/02/18	-
4	8	23/02/18	+
5	9	26/02/18	-
6	8	11/03/18	-
7	8	19/03/18	+
8	7	19/03/18	+
9	7	30/03/18	-
10	9	16/04/18	-
11	13	20/04/18	-
12	11	28/04/18	-
13	6	18/05/18	-
14	12	25/05/18	+
15	8	30/05/18	-
16	6	04/06/18	-
17	7	17/06/18	-
18	8	26/06/18	-
19	8	10/07/18	-
20	8	10/08/18	-
21	6	27/08/18	-
22	6	06/09/18	+
23	10	15/09/18	+
24	5	09/10/18	+
25	8	21/10/18	-
26	9	02/11/18	-
27	11	21/12/18	-
28	9	17/01/19	-

Supplementary Table 1 – List of mice cohorts tested for *Klebsiella* presence. Seven out of twenty eight litters (25%) were *Klebsiella*-positive.