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Endogenous Enterobacteriaceae underlie variation in susceptibility to *Salmonella* infection

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Lack of reproducibility is a prominent problem in biomedical research. An important source of variation in animal experiments is the microbiome, but little is known about specific changes in the microbiota composition that cause phenotypic differences. Here, we show that genetically similar laboratory mice obtained from four different commercial vendors exhibited marked phenotypic variation in their susceptibility to *Salmonella* infection. Faecal microbiota transplant into germ-free mice replicated donor susceptibility, revealing that variability was due to changes in the gut microbiota composition. Co-housing of mice only partially transferred protection against *Salmonella* infection, suggesting that minority species within the gut microbiota might confer this trait. Consistent with this idea, we identified endogenous Enterobacteriaceae, a low-abundance taxon, as a keystone species responsible for variation in the susceptibility to *Salmonella* infection. Protection conferred by endogenous Enterobacteriaceae could be modelled by inoculating mice with probiotic *Escherichia coli*, which conferred resistance by using its aerobic metabolism to compete with *Salmonella* for resources. We conclude that a mechanistic understanding of phenotypic variation can accelerate development of strategies for enhancing the reproducibility of animal experiments.

A recent survey suggests that the majority of researchers have tried and failed to reproduce their own experiments or experiments from other scientists[1]. In animal experiments, this lack of reproducibility can be due to an unappreciated variation in the microbiota[2,3]. It has been suggested that this lack of reproducibility in animal experiments could be addressed by co-housing animals or by comparing the gut microbiota of animals exhibiting differing phenotypes[3]. However, neither of these approaches is effective in addressing phenotypic variation caused by keystone species present at low abundance in the gut microbiota[2]. Instead, a return to a reductionist approach seems necessary to pinpoint how specific changes in the microbiota composition contribute to phenotypic variation[4]. Specific pathogens or pathobionts, including *Helicobacter hepaticus*, segmented filamentous bacteria or enteric viruses, can contribute to phenotypic variation by inducing immune responses through microbe–host interactions[3]. For example, colonization with segmented filamentous bacteria induces differentiation of interleukin-22-producing T cells to confer resistance to *Citrobacter rodentium* infection[5]. Infection of laboratory mice with *Salmonella enterica* serovar Typhimurium is a commonly used model in bacterial pathogenesis research[6]. Induction of innate immune responses by viruses or the gut microbiota can increase resistance of mice to *S.* Typhimurium infection[7,8]. A disruption of the gut microbiota by antibiotic treatment increases susceptibility to *S.* Typhimurium infection[9], suggesting that the resident microbial community is required for protection. However, it is not known whether more subtle changes in the microbiota composition, such as those observed between mice obtained from different vendors, could alter susceptibility to infection.

Results

To determine whether genetically similar strains of mice obtained from different commercial sources varied in their susceptibility to *S.* Typhimurium infection, C57BL/6 mice were obtained from Charles River Laboratories (C57BL/6NCrl), Harlan (C57BL/6NHsd), Taconic Farms (C57BL/6NTac) and Jackson Laboratories (C57BL/6J). Whereas all mice inoculated intragastrically with high doses of *S.* Typhimurium developed lethal morbidity (Fig. 1a,b), a low-dose challenge (10^5 colony-forming units (c.f.u.) per mouse) caused greater lethal morbidity in C57BL/6J mice than in mice from other vendors (Fig. 1c). Susceptibility to lethal *S.* Typhimurium infection correlated with intestinal carriage, which developed in all mice challenged with high doses of *S.* Typhimurium (Fig. 1d,e), but after a low-dose challenge (10^5 c.f.u. per mouse) most C57BL/6J mice became colonized whereas most mice obtained elsewhere did not (Fig. 1f). To investigate whether these differences in susceptibility varied with time, we repeated the experiment one year later, after Harlan had been integrated into Envigo. Differences in susceptibility to lethal *S.* Typhimurium infection and development of intestinal carriage were similar to those observed one year earlier (Supplementary Figs. 1 and 2), suggesting that variability was linked to differences among vendors.

Combining data from both experiments suggested that the dose at which intestinal carriage with *S.* Typhimurium developed in 50% of animals (ID_{50}) was approximately 100-fold higher in mice belonging to the C57BL/6N substrain than in C57BL/6J mice (Fig. 1g). Therefore, we hypothesized that genetic differences between substrains of C57BL/6 mice were responsible for phenotypic variation. The C57BL/6J substrain diverged from the C57BL/6N substrain at the National Institutes of Health (NIH) in 1951. This strain

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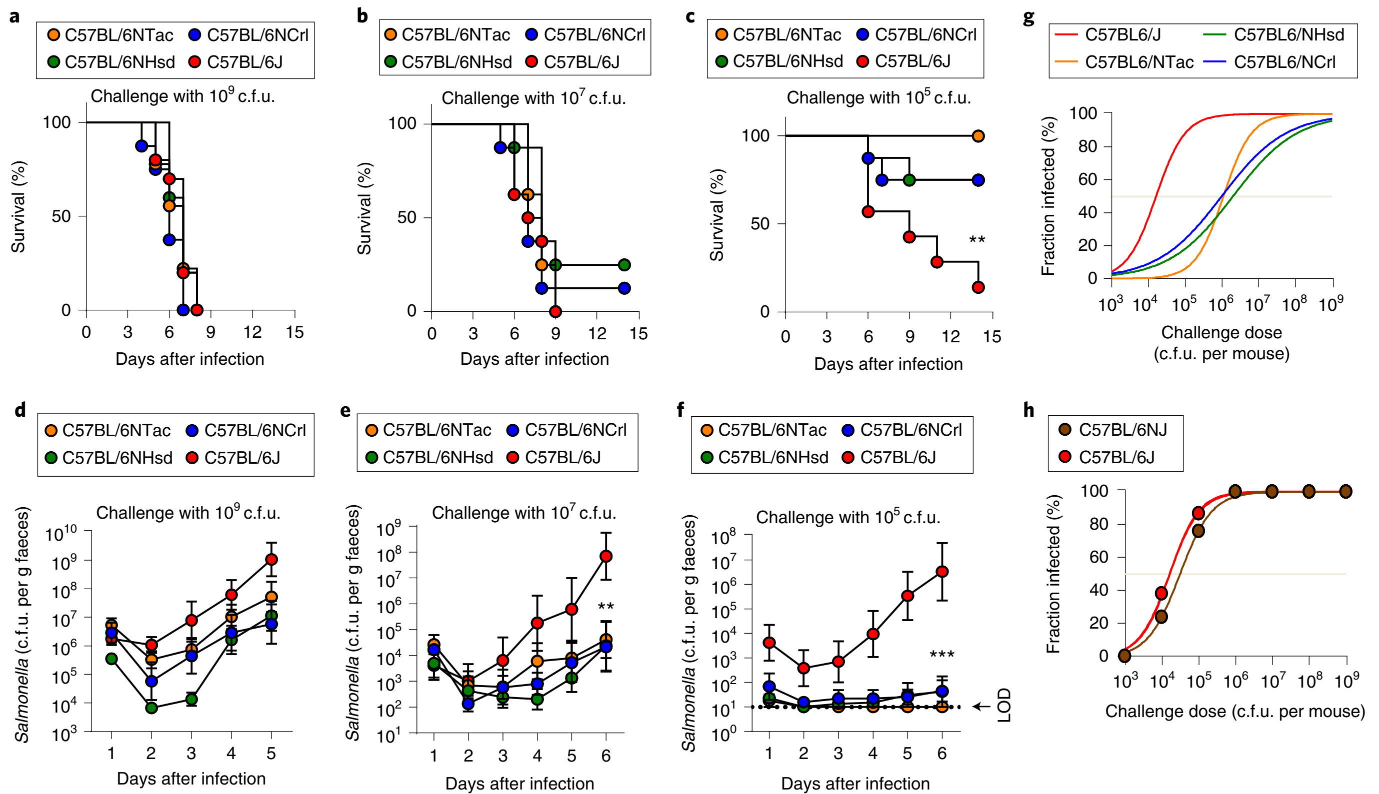

Fig. 1 | Phenotypic variation in the susceptibility to *Salmonella* infection is observed in C57BL/6 mice from different vendors. a–g, Mice from Charles River Laboratories (C57BL/6NCrl), Harlan (C57BL/6NHsd), Taconic Farms (C57BL/6NTac) or Jackson Laboratories (C57BL/6J) were challenged with *S.* Typhimurium ($n \geq 7$). Lethal morbidity (**a–c**) and *S.* Typhimurium shedding with faeces (**d–f**) were monitored at the indicated time points after challenge with 10^9 c.f.u. (**a**,**d**), 10^7 c.f.u. (**b**,**e**) or 10^5 c.f.u. (**c**,**f**) per mouse. **g**, Fraction of mice developing intestinal carriage at different *S.* Typhimurium challenge doses. **d–f**, Dots represent geometric mean ± s.e.m. **h**, Two C57BL/6 substrains (C57BL/6J and C57BL/6NJ) from Jackson Laboratories were challenged with different *S.* Typhimurium doses and the fraction of animals developing intestinal carriage determined ($n = 3$–8 per dose). $^{**}P \leq 0.01$; $^{***}P \leq 0.001$. LOD, limit of detection.

was subsequently passed to Charles River Laboratories in 1974 (C57BL/6NCrl), to Harlan in 1983 (C57BL/6NHsd) and to Taconic Farms in 1991 (C57BL/6NTac)[10,11]. Genetic drift produced a range of phenotypic differences between the C57BL/6J and C57BL/6N substrains[12]. To test our hypothesis, we obtained a C57BL/6N substrain that was established at Jackson Laboratories in 1997 from frozen embryos provided by the NIH (C57BL/6NJ)[12]. Notably, C57BL/6NJ and C57BL/6J mice were equally susceptible to *S.* Typhimurium infection (Fig. 1h and Supplementary Fig. 3), which did not support the idea that genetic differences between substrains of C57BL/6 mice were responsible for variation in the *S.* Typhimurium disease resistance phenotype.

Differences in *S.* Typhimurium susceptibility are caused by gut microbiota variation. To determine whether the gut microbiota was an environmental factor causing phenotypic variation, faecal microbiota from C57BL/6NCrl mice, C57BL/6NHsd mice, C57BL/6NTac mice or C57BL/6J mice was transferred to germ-free Swiss Webster mice. After allowing the Swiss Webster recipients to adjust to the faecal microbiota transplant, they were given different doses of *S.* Typhimurium by oral gavage. Swiss Webster recipients of faecal microbiota transplants phenocopied the respective C57BL/6 donor mice in their susceptibility to intestinal *S.* Typhimurium carriage (Fig. 2a–d and Supplementary Fig. 4), suggesting that vendor-linked variability in the gut microbiota was a dominant source of phenotypic variation. Furthermore, a low-dose challenge (10^5 c.f.u. per mouse) caused greater lethal morbidity in Swiss Webster mice with a faecal microbiota transplant from C57BL/6J mice than in Swiss Webster mice with faecal microbiota transplants from other vendors (Fig. 2e).

Clostridia are a dominant taxon within the gut microbiota, and the obligate anaerobic bacteria forming this class are important for colonization resistance against *S.* Typhimurium[13,14]. To test the hypothesis that differences in the abundance of *Clostridia* might explain phenotypic variation, we performed 16S ribosomal RNA gene sequencing (microbiota profiling). Principal coordinates analysis revealed differences in the microbiota composition of mice obtained from different vendors, and faecal microbial communities from Swiss Webster mice receiving a faecal microbiota transplant resembled those of the respective C57BL/6 donor mice (Fig. 2f). However, microbiota profiling did not support the idea that increased resistance to *S.* Typhimurium infection was associated with an increased abundance of *Clostridia* (Fig. 1g and Supplementary Fig. 5). Linear discriminant analysis also did not identify any biomarkers of protection that had a consistently higher relative abundance in resistant mice (C57BL/6NCrl, C57BL/6NHsd or C57BL/6NTac) compared with susceptible mice (C57BL/6J) (Supplementary Fig. 6).

Commensal Enterobacteriaceae are biomarkers for protection against *Salmonella*. To test whether bacterial taxa conferring protection in C57BL/6NCrl mice could be transferred to susceptible C57BL/6J mice, we co-housed these mice for 14 d. Principal coordinates analysis of bacterial communities suggested that co-housing changed the faecal microbiota composition of C57BL/6J mice (Fig. 3a), but increased resistance to *S.* Typhimurium challenge in

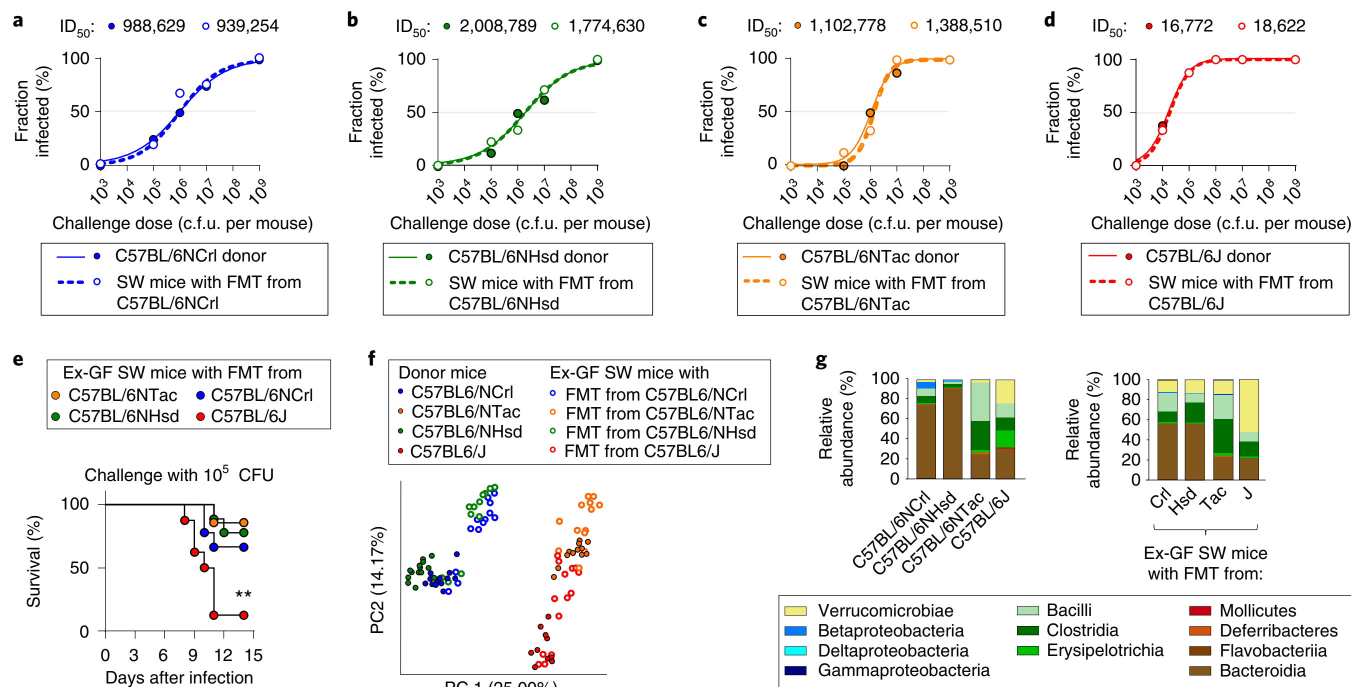

Fig. 2 | The gut microbiota is a driver of phenotypic variation. a–d, Solid lines and closed circles: mice from different vendors were challenged with *S.* Typhimurium ($n=3$–11 per dose). Closed circles indicate the fraction of animals developing intestinal carriage at different *S.* Typhimurium challenge doses. Dashed lines and open circles: germ-free Swiss Webster (SW) mice received a faecal microbiota transplant (FMT) from C57BL/6NCrl mice (**a**), C57BL/6NHsd mice (**b**), C57BL/6NTac mice (**c**) or C57BL/6J mice (**d**) and were subsequently challenged with the indicated *S.* Typhimurium challenge doses ($n=3$–11 per dose). Open circles indicate the fraction of animals developing intestinal carriage at different *S.* Typhimurium challenge doses. The *S.* Typhimurium ID_{50} (in c.f.u. per mouse) for each group of donor mice (C57BL/6NCrl, C57BL/6NHsd, C57BL/6NTac or C57BL/6J) and each corresponding group of ex-germ-free Swiss Webster (ex-GF SW) recipient mice is indicated above each graph. **e**, Germ-free Swiss Webster mice received faecal microbiota transplants from the indicated donor mice and were subsequently challenged with *S.* Typhimurium (*n* is shown in **f**). Lethal morbidity was monitored at the indicated time points after challenging ex-germ-free Swiss Webster mice with 10^5 c.f.u. per mouse. **f**, Principal coordinates analysis of faecal bacterial communities from the indicated animal groups. Each dot represents data from one animal ($n=9$–15 per group). **g**, 16S profiling of bacterial communities from the indicated animal groups at the class level. Each bar represents the average relative abundance of animals shown in Supplementary Fig. 5. $^{**}P\leq0.01$.

only a fraction of C57BL/6J mice (Fig. 3b). A possible explanation for this observation was that conventional mice might impose a bottleneck on transfer of minority species within the faecal microbiota that might be responsible for phenotypic variation. To identify potential biomarkers associated with phenotypic variation after co-housing, we performed linear discriminant analysis of the microbiota composition from C57BL/6J mice before co-housing and from the same animals after 14 d of co-housing. This analysis suggested that increased resistance to *S.* Typhimurium infection was associated with an increased abundance of Deferribacteraceae (phylum Deferribacteres), candidate phylum Saccharibacteria and Enterobacteriaceae (phylum Proteobacteria) (Fig. 3c). The Enterobacteriaceae were of particular interest, because inoculation with probiotic *Escherichia coli* (family Enterobacteriaceae) strains can reduce the severity of *S.* Typhimurium infection in animal models[15,16].

We therefore attempted to culture Enterobacteriaceae from the co-housing experiment on MacConkey agar, which revealed that faeces of C57BL/6NCrl mice contained culturable bacteria, but these minority species were absent from C57BL/6J mice. Curiously, culturable bacteria had not been transferred during co-housing experiments to those C57BL/6J mice that remained susceptible to *S.* Typhimurium challenge (Fig. 3d). By contrast, C57BL/6J mice that acquired *S.* Typhimurium resistance during co-housing had also acquired bacteria that formed lactose-positive and lactose-negative colonies (Fig. 3d and Supplementary Fig. 7a), which persisted after separating mice again (Supplementary Fig. 7b). We recovered 10^5–10^7 c.f.u. per g faeces on MacConkey agar from the faeces of C57BL/6NCrl, C57BL/6NTac or C57BL/6NHsd mice, which was below the limit of detection by conventional microbiota profiling (Supplementary Fig. 8a). By contrast, we detected no increase in the number of bacteria culturable on *Lactobacillus*-selective De Man, Rogosa and Sharpe (MRS) agar in C57BL/6NCrl, C57BL/6NTac or C57BL/6NHsd mice compared with C57BL/6J mice (Supplementary Fig. 7c). Notably, bacteria culturable on MacConkey agar had been efficiently transferred to germ-free Swiss Webster mice through faecal microbiota transplant (Supplementary Fig. 8b). Collectively, these data suggested that the presence of bacteria culturable on MacConkey agar was associated with protection against *S.* Typhimurium infection.

Commensal Enterobacteriaceae are keystone species responsible for phenotypic variation. To determine whether bacteria culturable on MacConkey agar caused phenotypic variation, C57BL/6J mice were inoculated with mixtures of bacteria that were culturable on MacConkey agar; the bacteria were selected on the basis of differences in morphology of colonies isolated from resistant mice. C57BL/6J recipient mice shed bacteria culturable on MacConkey agar (Fig. 3e) in quantities similar to those observed in the respective donor mice (Supplementary Fig. 8a). Remarkably, transfer of bacteria caused C57BL/6J recipient mice to become resistant to subsequent *S.* Typhimurium challenge, as indicated by

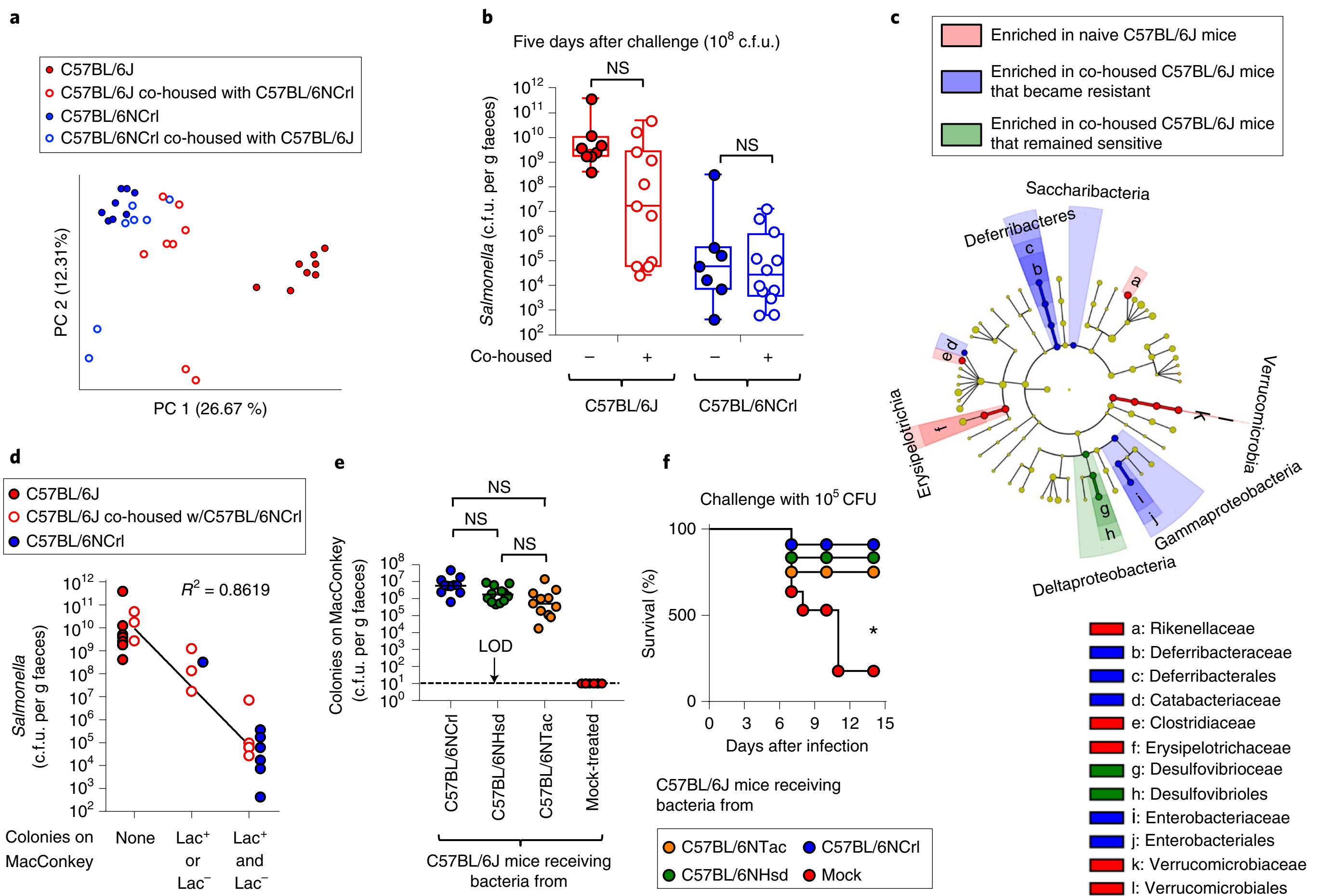

Fig. 3 | Enterobacteriaceae are biomarkers of phenotypic variation. a, C57BL/6J mice were housed normally or co-housed with C57BL/6NCrl mice, and faecal bacterial communities were analysed by principal coordinates analysis ($n=7$ or 8). **b**, C57BL/6J mice were housed normally or co-housed with C57BL/6NCrl mice and subsequently challenged with *S.* Typhimurium. *S.* Typhimurium shedding with faeces was quantified 5 d after challenge. In box plots, whiskers represent minimum to maximum points and the box extends from the 25th to 75th percentile with the median indicated by a line. **c**, The cladogram shows differences in taxa composition between C57BL/6J mice without co-housing and after 14 d of co-housing with C57BL/6NCrl mice. **d**, The presence of Enterobacteriaceae in mice from the co-housing experiment shown in **a** was determined by spreading dilutions of faecal homogenates on MacConkey agar plates before *S.* Typhimurium challenge. *S.* Typhimurium shedding with faeces was quantified 5 d after challenge. **e**,**f**, C57BL/6J mice were mock-treated or received Enterobacteriaceae from the indicated donor mice. **e**, The presence of Enterobacteriaceae in faeces of mice before *S.* Typhimurium challenge was determined by spreading dilutions on MacConkey agar plates ($n=\geq11$). The graph shows variation between animals (dots) and the geometric mean (line). **f**, Mice were challenged with *S.* Typhimurium. Lethal morbidity was monitored over time after challenge with 10^5 c.f.u. per mouse. **a**,**b**,**d**,**e**, Each dot represents data from one animal; the number of dots thus indicate the number of replicates (n). $^*P\leq0.05$. NS, not significant.

reduced lethal morbidity (Fig. 3f), reduced pathogen burden in the faeces (Supplementary Figs. 7d–g and 8c) and a reduced ID_{50} (Supplementary Fig. 8d). Next, we determined the identity of bacteria culturable on MacConkey agar by sequencing their 16S ribosomal RNA genes. This analysis revealed that all bacteria isolated on MacConkey agar were members of the family Enterobacteriaceae. Lactose-negative enteric bacteria included *Proteus vulgaris* and *Proteus mirabilis*, whereas lactose-positive colonies represented *Klebsiella oxytoca*, *Enterobacter cloacae* and *E. coli* isolates (Supplementary Fig. 8e). Next, C57BL/6J mice were colonized with individual Enterobacteriaceae isolates. With the exception of *K. oxytoca* isolate Tac148, all isolates colonized C57BL/6J recipient mice well (Supplementary Fig. 8f) and tended to confer protection against subsequent *S.* Typhimurium challenge, although this only reached statistical significance for *E. coli* Crl141 (Supplementary Fig. 8g). Collectively, these data supported the idea that commensal Enterobacteriaceae are keystone species responsible for phenotypic variation.

***E. coli* uses its aerobic metabolism to confer niche protection against *Salmonella*.** To investigate the mechanism by which endogenous Enterobacteriaceae conferred protection, homogenized faecal pellets were inoculated with *S.* Typhimurium and incubated under aerobic conditions. This revealed that pathogen growth was slowed in faecal homogenates of resistant mice (C57BL/6NCrl, C57BL/6NHsd or C57BL/6NTac) compared with susceptible mice (C57BL/6J) (Supplementary Fig. 9a). Inhibition of *S.* Typhimurium growth by faecal homogenates from C57BL/6NCrl and C57BL/6NTac mice was not observed under anaerobic conditions (Supplementary Fig. 9a). Growth inhibition was not due to the presence of inhibitory metabolites, because *S.* Typhimurium grew equally well in sterile-filtered faecal homogenates from resistant or susceptible mice (Supplementary Fig. 9c). Homogenized faecal pellets from susceptible C57BL/6J mice spiked with *P. vulgaris*, *P. mirabilis*, *E. cloacae*, *K. oxytoca* or *E. coli* isolates from resistant mice suppressed *S.* Typhimurium growth (Supplementary Fig. 9d), suggesting that pathogen growth inhibition was due to the presence

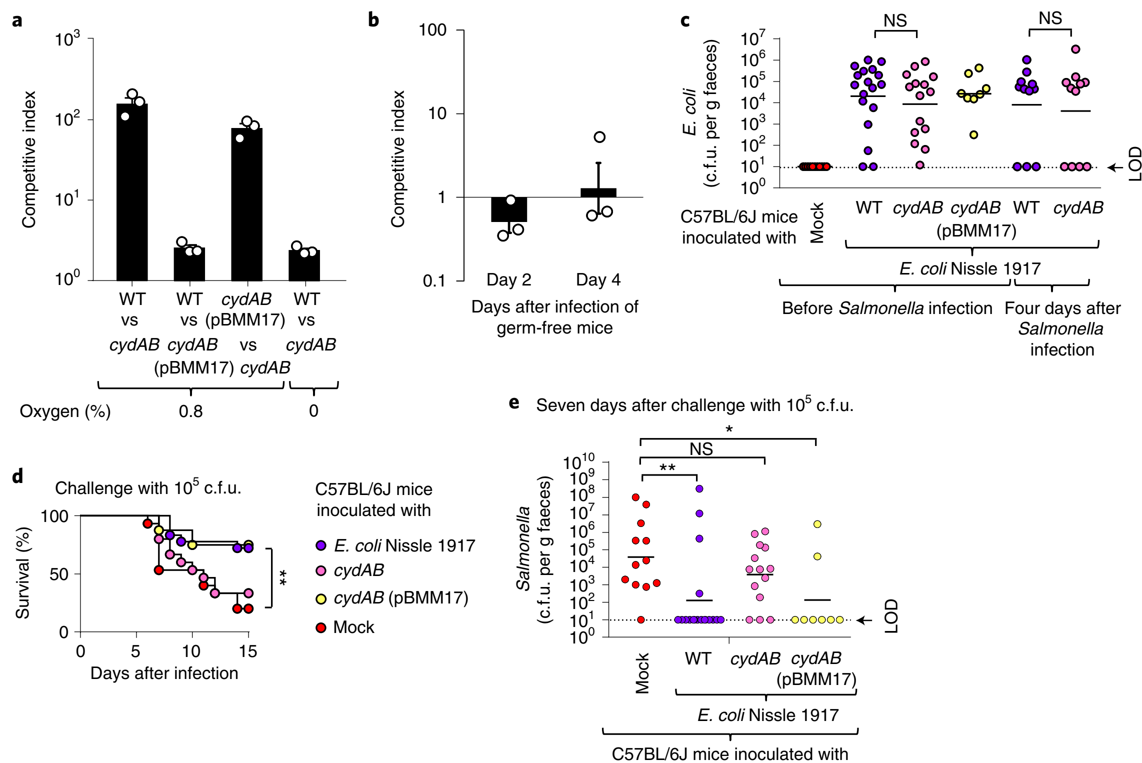

Fig. 4 | *E. coli* requires an aerobic metabolism to confer colonization resistance. a, Minimal medium containing 0.1% glucose was inoculated with a 1:1 ratio of the indicated bacterial strains and the competitive index was determined after 24 h incubation at the indicated oxygen concentrations. **b**, Germ-free mice ($n = 3$) were inoculated with a 1:1 mixture of wild-type E. coli Nissle 1917 (WT) and an isogenic *cydAB* mutant, and the competitive index was determined in the faeces at the indicated time points. **a**,**b**, Bars represent geometric mean ± s.e.m. **c**–**e**, C57BL/6J mice were mock-treated or were inoculated with 10^9 c.f.u. per mouse of *E. coli* Nissle 1917, a *cydAB* mutant or a *cydAB* mutant complemented with a plasmid carrying the cloned *cydAB* genes (pBMM17). Mice were challenged with *S.* Typhimurium, and *E. coli* colonization (**c**), lethal morbidity (**d**) and *S.* Typhimurium shedding with the faeces (**c**) were determined. **c**, Before challenge (corresponding to 5 d after *E. coli* inoculation) or 4 d after *Salmonella* challenge, the presence of *E. coli* in faeces was determined. **c**,**e**, Lines represent the geometric mean. Each dot represents data from one mouse, and thus indicates the number (n) of replicates. **d**, The number of mice (n) is shown in **e**. *cydAB* (pBMM17), *E. coli* Nissle 1917 *cydAB* mutant complemented with plasmid pBMM17. $^*P \leq 0.05$; $^{**}P \leq 0.01$.

of Enterobacteriaceae. Interestingly, inhibition of pathogen growth by Enterobacteriaceae was no longer observed for most isolates when the experiment was repeated under anaerobic conditions (Supplementary Fig. 9e).

The large intestine is considered a largely anaerobic environment, but the bioavailability of oxygen is increased during conditions of intestinal inflammation[17], which is associated with an expansion of facultative anaerobic Enterobacteriaceae. For instance, *E. coli* can use aerobic respiration to expand in the large intestine of mice with chemically induced colitis[18]. Interestingly, oxygen is also a critical resource for *S.* Typhimurium, which uses its virulence factors to trigger intestinal inflammation to drive a pathogen expansion in the lumen of the large intestine using aerobic respiration[13]. We thus hypothesized that Enterobacteriaceae confer protection against *S.* Typhimurium by using their aerobic metabolism to compete with the pathogen for critical resources in vivo. To test this idea, we modelled colonization resistance conferred by Enterobacteriaceae using *E. coli* Nissle 1917. Inoculation of C57BL/6J mice with *E. coli* Nissle 1917 conferred protection against *S.* Typhimurium challenge (Supplementary Fig. 8g). We then generated *E. coli* Nissle 1917 mutants deficient for aerobic respiration under microaerophilic conditions by inactivating cytochrome *bd* oxidase encoded by the *cydAB* genes. The resulting *cydAB* mutant was deficient for growth under microaerophilic conditions, which could be complemented by introducing the cloned *cydAB* genes on a plasmid (pBMM17) (Fig. 4a). When germ-free mice were inoculated with a 1:1 mixture of wild-type and *cydAB* mutant *E. coli* Nissle 1917, similar numbers of both strains were recovered from the faeces, suggesting that genetic ablation of cytochrome *bd* oxidase did not cause a general fitness defect in vivo (Fig. 4b). Compared with the *cydAB* mutant, the wild-type *E. coli* Nissle 1917 competed more successfully with *S.* Typhimurium during in vitro growth under microaerophilic conditions (0.8% oxygen) in sterile-filtered faecal homogenates from susceptible mice (C57BL/6J) (Supplementary Fig. 8f). Wild-type or *cydAB* mutant *E. coli* Nissle 1917 colonized C57BL/6J mice at similar levels (Fig. 4c). Challenge with *S.* Typhimurium revealed that genetic ablation of the ability to respire oxygen lowered the ability of *E. coli* Nissle 1917 to protect against lethal morbidity (Fig. 4d) and intestinal colonization by the pathogen (Fig. 4e), without reducing the numbers of *E. coli* during challenge (Fig. 4c). Collectively, these results supported the idea that commensal Enterobacteriaceae use their aerobic metabolism to compete with *S.* Typhimurium for resources that are critical for pathogen expansion in the intestinal lumen.

Discussion

During homoeostasis, physiologic hypoxia of the colonic epithelium limits the amount of oxygen emanating from the mucosal surface, thereby restricting growth of facultative anaerobic bacteria[19]. *S.* Typhimurium uses its virulence factors to induce intestinal inflammation, which is accompanied by migration of neutrophils into the intestinal lumen, thereby depleting butyrate-producing *Clostridia* from the gut microbiota and increasing epithelial oxygenation[13]. Oxygen emanating from the inflamed mucosa drives

an expansion of *S.* Typhimurium through consumption of non-fermentable microbiota-derived carbon sources[20,21]. Whereas microbiota-derived short-chain fatty acids can inhibit growth of Enterobacteriaceae[22], *S.* Typhimurium-induced inflammation triggers a luminal release of lactate by host cells[23], which neutralizes growth inhibition by short-chain fatty acids[24].

Commensal Enterobacteriaceae, such as *E. coli*, have been implicated in contributing to resistance against *S.* Typhimurium infection[15,16,25]. Metagenomic analysis of a defined microbial community that failed to confer colonization resistance against *S.* Typhimurium revealed an underrepresentation of pathways involved in microbial respiration compared with microbiota of conventional mice[25]. The addition of facultative anaerobic bacterial species that encode respiratory pathways, including *E. coli*, *Streptococcus danieliae* and *Staphylococcus xylosus*, correlated with a marked increase in colonization resistance conferred by the defined bacterial community against *S.* Typhimurium[25]. Here we used bacterial genetics to establish a causal connection between aerobic respiration and the ability of *E. coli* to confer colonization resistance against *S.* Typhimurium. Our results suggest that competition arises, because commensal Enterobacteriaceae have an aerobic metabolism that enables them to consume non-fermentable carbon sources. Genetic ablation of cytochrome *bd* oxidase synthesis prevents *E. coli* from consuming any of these non-fermentable carbon sources by aerobic respiration under microaerobic conditions and demonstrated that aerobic respiration is required for *E. coli* to confer colonization resistance. The scarcity of respiratory electron acceptors is an important factor limiting the abundance of Enterobacteriaceae during gut homoeostasis[17], which explains why members of this taxon were difficult to detect by microbiota profiling in treatment-naive mice (Supplementary Fig. 7). However, a disruption of gut homoeostasis by intestinal inflammation increases oxygen availability in the large intestine[21], and other resources, such as iron, can become limiting during the consequent Enterobacteriaceae expansion[26]. As a result, *E. coli* Nissle 1917 also competes with *S.* Typhimurium for siderophore-bound iron during gut inflammation[27]. The emerging picture is that commensal Enterobacteriaceae are important keystone species that contribute to colonization resistance against *S.* Typhimurium by competing for critical resources, such as non-fermentable carbon sources and iron. Our work shows that these keystone species are an important source of phenotypic variation in laboratory mice.

Methods

Experimental model and subjects. *Mouse lines.* Female 5-to-7-week-old C57BL/6NCrl, C57BL/6NHsd and C57BL/6NTac (microbiological status equivalent; specific-pathogen-free) were purchased from The Charles River Laboratories, Envigo (formerly Harlan) and Taconic Farms, respectively. C57BL/6J (stock no. 000664) and C57BL/6NJ (stock no. 005034) were purchased from The Jackson Laboratories. Gnotobiotic Swiss Webster mice (Tac:SW) were initially purchased from Taconic Farms and bred at the UC Davis Genome and Biomedical Sciences Facility, maintained by investigators. All animal experiments were approved by the Institution of Animal Care and Use Committee at University of California, Davis. The exact number of mice used in each group is indicated on each graph, in the figure legend or in Supplementary Table 1.

Bacterial strains. All strains used in this study are listed in Supplementary Table 2. *S.* Typhimurium and *E. coli* Nissle 1917 were cultured in Luria–Bertani (LB) broth (10 g l^{-1} tryptone, 5 g l^{-1} yeast extract and 10 g l^{-1} NaCl) or LB plates (LB broth, 15 g l^{-1} agar) and incubated at 37 °C. When needed, nalidixic acid (50 mg ml^{-1}), carbenicillin (100 mg ml^{-1}), kanamycin (100 mg ml^{-1}) or chloramphenicol (15 mg ml^{-1}) were added to LB broth and LB agar plates. Mouse commensal *Proteus*, *Klebsiella*, *Enterobacter* and *Escherichia* were grown aerobically in LB broth or on MacConkey agar plates (10 g l^{-1} pancreatic digest of gelatin, 3 g l^{-1} peptone, 10 g l^{-1} lactose, 1.5 g l^{-1} bile salts, 5 g l^{-1} sodium chloride, 13.5 g l^{-1} agar, 30 mg l^{-1} neutral red and 1 mg l^{-1} crystal violet) at 37 °C. All plasmids and primers used in this study are listed in Supplementary Table 2.

Experimental procedure. Specific−pathogen-free mouse husbandry. Before transportation, mice at Charles River, Envigo, Taconic and Jackson were fed (according to online rodent model information sheets) Purina 5L79 rodent chow, Teklad Global Rodent Diet 2018S, NIH #31 M Rodent Diet and LabDiet 5K52 formulation (6% fat), respectively. On arrival, mice from each cohort were randomly assigned into individually ventilated cages on one rack at a housing density of three to four mice per cage and allowed to acclimatize in our vivarium for at least a week undisturbed. Feed was switched to irradiated Teklad Global 18% protein rodent diet 2918 (Envigo) and no breeding was performed. Ethanol (70%) was used to disinfect surfaces and gloves between groups. Clean (but not sterile) paper towels were utilized for faecal sample collection. None of the experiments performed in this study involved treatment or pretreatment of mice with antibiotics.

Preparation of faecal transplants. Freshly voided pooled faeces (approximately 200 mg per cage) were collected from C57BL/6NCrl, C57BL/6NHsd, C57BL/6NTac and C57BL/6J mice. Samples were maintained on ice and processed within 1 h. An anaerobic chamber was not used. Faeces were diluted at 1:50 (approximately 10 ml) in sterile PBS solution (pH,7.4) and homogenized by vortexing for 5 min at room temperature. Tubes were briefly centrifuged (500*g* for 1 min) to pellet large particles. Aliquots of 500 µl were mixed with 500 µl glycerol (60% w/v) and immediately stored at −80 °C.

S. Typhimurium infection in C57BL/6 mice. Mice were infected upon reaching eight-to-twelve weeks of age. To generate contamination-free *S.* Typhimurium culture, LB broth supplemented with appropriate antibiotics in sterile flasks was aseptically inoculated from single colonies and incubated with shaking (200 r.p.m. at 37 °C) for 16 to 20 h. Bacteria were then collected by centrifugation (15 min, 4000*g*, 4 °C), and adjusted to a density of 1×10^{10} c.f.u. per ml in sterile LB. To generate lower density inocula, cultures were serially diluted tenfold. Sterile LB broth (0.1 ml) containing *S.* Typhimurium at the indicated densities was inoculated by oral gavage (between 06:00 and 12:00). Primary experimental outcomes assessed were: animal activity, weight loss, lethal morbidity and pathogen burdens. Weights were recorded daily and faeces were collected at the indicated time points (between 1 d and 7 d after infection). Mice were euthanized at the indicated time points or when they became moribund. Mice that were euthanized early due to health concerns were excluded from analysis, except in experiments determining lethal morbidity after challenge. Intestinal contents (approximately 15 mg per mouse) were homogenized in 1 ml PBS. Samples were then serially diluted in PBS and plated on selective agar to distinguish pathogen c.f.u. per gram. Challenged mice were considered infected when a positive stool culture was obtained more than 3 d after infection.

Germ-free mouse husbandry. Mice were housed in flexible-front glove-box isolators (Park Bioservices). Food (irradiated 2920X Teklad diet by Envigo) and autoclaved-water access was ad libitum. Bedding changes were provided weekly. The room was maintained on a 12 h light:dark cycle. Principles of the established 'out-of-the-isolator' gnotobiotic husbandry system[28] were utilized and validated in our facility to preserve gnotobiotic status for up to two weeks by standard quality control (negative aerobic and anaerobic culture in brain–heart infusion broth and 16S rRNA quantitative real-time PCR comparable to a no-template control). Littermates of both sexes were removed from the isolator at six weeks of age and aseptically transferred to a biosafety cabinet and placed into autoclaved individually ventilated cages. Groups of two to four males or females were randomly assigned to experimental groups and housed together to facilitate social interactions and establishment of their faecal microbiota transplants. Animals were maintained in a biosafety cabinet for at least 5 d undisturbed after receiving faecal transplants and infections were carried out in the biosafety cabinet.

Faecal transplant into germ-free Swiss Webster mice. Previously frozen pooled faecal samples from cages of each C57BL/6 substrain were thawed on ice and delivered via oral gavage (200 µl) once. Colonization was allowed to proceed for a minimum of 5 d before sample collection and infection with *S.* Typhimurium. Bedding changes were performed before infection, carried out under strictly aseptic conditions, and samples were collected from individual mice using autoclaved beakers before infection.

S. Typhimurium infections in germ-free Swiss Webster mice. Concentrated overnight cultures were prepared as described above. Animals were colonized with faecal microbiota transplant for 5 d. Each ex-germ-free mouse received 0.1 ml of a suspension containing the indicated c.f.u. of *S.* Typhimurium via oral gavage. Fresh faecal pellets were collected aseptically using autoclaved beakers and plated on agar plates containing the appropriate antibiotics.

16S rDNA amplicon sequencing. Faecal samples were collected from individual mice before infection for analysis and frozen at −20 °C. DNA was extracted using the MoBio PowerSoil Kit according to the manufacturer's instructions, with the following two recommended modifications: (1) samples were heated at 70 °C for 10 min following addition of buffer 1 to enhance disruption of Gram-positive bacteria; (2) samples were homogenized using a mini-bead beater for 60 s to achieve greater mechanical lysis of bacterial cells. Paired-end library

construction was performed as previously described[13]. Primers 515 F and 806 R (Supplementary Table 2) were used to amplify the V4 domain of the 16S rRNA. Both forward and reverse primers contained a unique 8-nucleotide barcode (N), a primer pad (underlined), a linker sequence (italicized) and the Illumina adapter sequences (bold) (Supplementary Table 2). Each sample was barcoded with a unique forward and reverse barcode combination. PCR contained 1 U Kapa2G Robust Hot Start Polymerase (Kapa Biosystems), 1.5 mM $MgCl_2$, 10 µmol of each primer, 10 mM dNTPs and 1 µl DNA (20–30 ng). PCR conditions were: an initial incubation at 95 °C for 2 min, followed by 30 cycles of 95 °C for 20 s, 50 °C for 20 s, 72 °C for 20 s and a final extension at 72 °C for 3 min. The final product was quantified on a Qubit instrument using the Qubit High Sensitivity DNA kit and individual amplicon libraries were pooled, cleaned using Ampure XP beads (Beckman Coulter), and sequenced using a 250-bp paired-end method on an Illumina MiSeq instrument in the Genome Center DNA Technologies Core, University of California, Davis. Raw paired-end sequence data were de-multiplexed and trimmed, and then filtered for quality. Samples containing fewer than 1,000 quality reads were removed from dataset. Quantitative Insights into Microbial Ecology (QIIME) open-source software package (v.1.9) was initially used to perform sequence alignment and closed-reference operational taxonomic units picking against the Greengenes reference collection (v.13_8) at 97% identity. Data were later reanalysed when QIIME 2 became available (core 2018.2 distribution). Clustering, permutational multivariate analysis of variance (PERMANOVA), beta-diversity measures (principal coordinates analysis of unweighted UniFrac distances) and phylogenetic profiling were similar between QIIME 1.9 and QIIME 2. Additionally, taxonomy was also assigned with the Ribsosomal Database Project (RDP release 11) classifier using the DADA2 R package v.1.6 pipeline (https://benjjneb.github.io/dada2/tutorial.html) to resolve more sequences down to the species level.

Co-housing. Mice were received at five-to-seven weeks of age. After allowing one week to acclimate in our vivarium, mice from Charles River Laboratories and The Jackson Laboratories were placed in clean cages (with new food and water) at a ratio of 1:1 and co-housed for 14 d. Ear punching facilitated long-term identification. In one experiment, co-housed mice were subsequently separated after two weeks. Faecal samples were flash frozen at the start of co-housing and every week thereafter. For challenge experiments, animals remained co-housed after receiving 1×10^8 c.f.u. *Salmonella*. All mice were euthanized at 5 d after infection.

Enteric strain isolation and identification. Tenfold serial dilutions of previously frozen faecal transplants in glycerol or flash-frozen faeces were plated on MacConkey agar and incubated aerobically at 37 °C overnight. Single colonies with discernable unique morphologies were picked and streaked for isolation. Colonies were initially typed on the basis of colony appearance into lactose-fermenting red colonies (Lac^+) and lactose non-fermenting white colonies (Lac^-). Approximately 100 colonies (30–40 colonies from each of the three vendors harbouring Enterobacteriaceae) were isolated and identified biochemically using the EnteroPluri Test (BD Biosciences) per the manufacturer's instructions. Isolates used for animal experiments were subjected to full-length 16S rRNA gene Sanger sequencing.

Quantification of Lactobacilli by culturing. Faecal pellets from individual C57BL/6NCrl, C57BL/6NHsd, C57BL/6NTac, and C57BL/6J were plated on Difco *Lactobacilli* MRS Agar (10 g l^{-1} protease peptone no. 3, 10 g l^{-1} beef extract, 5 g l^{-1} yeast extract, 20 g l^{-1} dextrose, 1 g l^{-1} polysorbate 80, 2 g l^{-1} ammonium citrate, 5 g l^{-1} sodium acetate, 0.1 g l^{-1} magnesium sulfate, 0.05 g l^{-1} manganese sulfate, 2 g l^{-1} dipotassium phosphate, 15 g l^{-1} agar; pH, 5.5). Faeces were serially diluted in PBS and plates were pre-reduced for 2 d. Plates were incubated for 3 d at 37 °C in an anaerobic chamber (Shel Lab Bactron II; 5% hydrogen, 5% carbon dioxide, 90% nitrogen).

16S rDNA Sanger sequencing. Bacterial strains were grown in LB broth and pelleted. After resuspension in distilled water, tubes were boiled for 1 min and immediately cooled on ice. One microlitre of the lysed bacterial suspension containing genomic DNA was used in a PCR reaction to amplify the 16S rRNA gene using primers 63F-1387R. PCR conditions: 1 µl template, 1 µl each forward and reverse primers, 17 µl PCR Supermix high fidelity. Thermocycler (MJ Research PTC-200 Peltier Thermal Cycler) conditions: 30 cycles of denaturation at 95 °C for 1 min, annealing at 48 °C for 1 min, elongation at 72 °C for 2 min, followed an additional 15 min at 72 °C. DNA fragment sizes (1.5 kb) were estimated using agarose gel electrophoresis. Following the QIAquick PCR purification kit (Qiagen), 1 µl of DNA was ligated into pCR2.1-TOPO vector. Chemically competent TOP10 *E. coli* cells were transformed with ligation products by heat shock (30 s at 42 °C). Recombinant cells were selected on LB plates containing kanamycin and X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside). DNA preparations for sequencing were made with a QIAprep mini as specified by the manufacturer. Plasmids were eluted with 50 µl of water, and the products were stored at −20 °C. Sizes (5.4 kb) of plasmids containing inserts were checked by agarose gel electrophoresis and concentrations (200 ng μl^{-1}) were determined on a NanoDrop 1000 spectrophotometer (Thermo Scientific). Plasmid inserts were sequenced by the College of Biological Sciences UCDNA Sequencing Facility using M13 primers. Two contigs were aligned for each strain using Snapgene. Evolutionary analyses were conducted in MEGA7[29]. The evolutionary history was inferred by using the maximum-likelihood method, which is based on the Tamura–Nei model[30]. The analysis involved ten nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1,357 positions in the final dataset.

Colonization of C57BL/6J mice with Enterobacteriaceae. Isolates from frozen faecal transplants of C57BL/6NCrl, C57BL/6NHsd, C57BL/6NTac and C57BL/6J on MacConkey agar were maintained individually. Cultures were grown in LB broth overnight and 1×10^{10} c.f.u. per ml preparations were made. In some experiments, strains were mixed in equal volumes to prepare CR mix (*E. coli* Crl141, *E. coli* Crl142 and *P. mirabilis* Crl143), Har mix (*E. coli* Hsd145 and *E. cloacae* Hsd146) or Tac mix (*K. oxytoca* Tac148 and *P. vulgaris* Tac149). A single dose of 100 µl was delivered via gavage to C57BL/6J mice. Colonization levels were determined by plating faeces on MacConkey agar. *E. coli* Nissle 1917 and the *E. coli* Nissle 1917 *cydAB* mutant were transformed with plasmids (pCAL61 or pCAL62) before inoculation of mice to facilitate recovery from the faeces.

In vitro growth assays. For Supplementary Fig. 9a–c, an overnight liquid culture of *S.* Typhimurium was diluted in PBS to a concentration of 1×10^3 c.f.u. per ml and 100 µl aliquots were added to 900 µl of mouse faecal homogenate in glass tubes with loose-fitting caps. Faeces were processed as follows: approximately 100 to 300 mg of faeces were collected from one cage of mice pooled. Samples were diluted 1:100 in PBS and homogenized by vortexing for 10 min at room temperature. Large particles were pelleted by centrifugation at 500*g* for 1 min. Half of the sample was filtered through a 0.45 µm low-protein-binding membrane. Faecal homogenates were stored at 4 °C for up to 1 h before adding *S.* Typhimurium. Tubes were then incubated for 24 h at 37 °C while shaking under atmospheric oxygen. For Supplementary Fig. 9d,e, pooled faeces from C57BL/6J mice were diluted 1:100 in PBS, homogenized, briefly centrifuged, stored at 4 °C for up to 1 h before adding *S.* Typhimurium to achieve a final concentration of 1×10^3 c.f.u. per ml faecal homogenate. Aliquots (900 µl) of faeces from C57BL/6 J mice containing *S.* Typhimurium were distributed to glass tubes with loose-fitting caps. Overnight cultures of each commensal Enterobacteriaceae strain were diluted in PBS to a concentration of 1×10^4 c.f.u. per ml and 100 µl of this suspension was added to tubes containing 900 µl of *S.* Typhimurium in faecal homogenate. For anaerobic conditions, a duplicate set of tubes was prepared and immediately moved into the anaerobic chamber (Shel Lab Bactron II; 5% hydrogen, 5% carbon dioxide and 90% nitrogen) and incubated without shaking at 37 °C. The number of c.f.u. per ml was determined by selective plating of serial dilutions

Construction of mutants by allelic exchange. The *E. coli* Nissle *cydAB* mutant was constructed as described previously[19]. Complementation was achieved by introducing *cydAB* on a plasmid under expression of the native promoter as follows. Primer pair EcNcomp Fwd and EcNcomp Rev (Supplementary Table 2) was used to amplify the coding sequence of *E. coli* Nissle 1917 *cydAB* operon plus 500 bp upstream and downstream. The resulting PCR product was visualized by agarose gel electrophoresis and purified using Zymoclean Gel DNA Recovery kit (Zymo Research). The fragment was then cloned into the EcoRV restriction site of the low-copy-number plasmid pWSK129[31] to generate the plasmid pBMM17 via Gibson Assembly Master Mix (NEB).

Quantification and statistical analyses. The investigators were not blinded to allocation during experiments and outcome assessment. Sample size was estimated on the basis of previous studies. Mice that were euthanized early due to health concerns were excluded from analysis, except in experiments determining lethal morbidity after challenge. Data analysis was performed in Microsoft Excel and GraphPad Prism v.7.0. Precision measures are described in figure legends and details regarding the statistics of each experiment are reported in Supplementary Table 1. For survival analysis, the log-rank (Mantel–Cox) test was used. The limit of detection via plating was 10 c.f.u. per g. The natural logarithm of the number of c.f.u. per g faeces was used to normalize the data. When samples were collected over time and matched to individual mice, a two-way repeated-measures analysis of variance (ANOVA) was used, followed by Tukey's multiple-comparison test. When samples were not collected for all mice across time or were not matched to individual animals, then an ordinary one-way ANOVA was used if variances were equal between groups. Tukey's multiple-comparison tests were used when all pairwise comparisons were of interest; Dunnet's multiple-comparison tests were used when comparisons to a reference were of interest. Otherwise, a Kruskal–Wallis test was used when unequal variance was observed across groups (determined by Browne–Forsythe test and demonstrated by box and whisker plots) followed by Dunn's multiple-comparison test. All family-wise significance was set to 0.05 (95% confidence interval). For calculations of ID_{50}, a constrained nonlinear regression model was used. For analysis of community 16S rRNA amplicon sequences (microbiome), PERMANOVA was used to evaluate beta diversity. For association testing, linear regression was used. For biomarker

discovery, LEfSe was used with the following parameters: alpha = 0.05 for factorial Kurskal–Wallis test among classes, threshold = 2.0 on the logarithmic LDA score for discrimative features, and all-against-all multi-class analysis. For analysis of strain-specific full-length 16S rRNA Sanger sequences, MEGA was used to align sequences by MUSCLE with the following parameters: gap open penalty = −400, clustering method = UPGMC. A maximum-likelihood tree was constructed under the Tamura–Nei model, assuming uniform variation rates among sites, gap deletion, and a bootstrap method (1,000 replications) was used to test phylogeny. Differences with $P \leq 0.05$ were considered to be significant.

Software. The following open-source software was used: QIIME v.1.91 (http://qiime.org/) and v.2 (https://qiime2.org/), NIH nBLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi), MEGA v.7 (http://www.megasoftware.net/), R (http://cran.us.r-project.org/), DADA2 plugin for R v.1.6 (https://benjjneb.github.io/dada2/index.html), Greengenes v.13_5 (http://greengenes.secondgenome.com/), RDP Project (https://rdp.cme.msu.edu/) and LEfSE (http://huttenhower.sph.harvard.edu/galaxy). The following commercial software was used: Microsoft Excel 2011 for Mac and 2010 for Windows; www.microsoft.com/Buy/Excel), Graphpad Prism v.7 for Mac (https://www.graphpad.com/scientific-software/prism/) and Snapgene v.4.1 (http://www.snapgene.com/).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Illumina sequences obtained in the present study were deposited in the Sequence Read Archives (SRA) NCBI database under accession number SRP148888. Sanger sequences were deposited in GenBank under accession numbers MH759762 to MH759768.

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Author contributions

E.M.V., H.N., K.T.H., L.M.G., A.W.L.R., B.M.M. M.J.L. and C.H.S performed and analysed the experiments. E.M.V., H.N., K.T.H., Y.L., C.A.L., F.F., D.N.B., C.R.T., M.X.B. and A.J. Byndloss performed experiments involving germ-free mice. E.M.V. and M.R.R. analysed 16S profiling data. E.M.V. and A.J.Bäumler designed the experiments, interpreted the data and wrote the manuscript with contributions from all authors.

Competing interests

The authors declare no competing interests.

Additional information

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☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	No software was used.
Data analysis	For analysis of 16S rRNA amplicon reads, freely available online tutorials were used. For QIIME2, see: https://docs.qiime2.org/2018.2/tutorials/moving-pictures/. For DADA2, see: https://benjjneb.github.io/dada2/tutorial.html.

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Illumina sequences obtained in the present study were deposited in the Sequence Read Archives (SRA) NCBI database under accession number SRP148888. Sanger sequences were deposited in Genbank under accession numbers MH759762-MH759768.

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Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample-size calculation was not performed. It is impossible to predict the magnitude of the variation between animals for a particular parameter based on our current knowledge. The group sizes determined for each experimental design (at least 4 animals per treatment group) represent the minimal number of animals needed to detect a difference in means.
Data exclusions	Mice that were euthanized early due to health concerns were excluded from the analysis, except for experiments determining lethal morbidity after challenge.
Replication	Most experiments were independently repeated at least twice. All attempts to replicate the experiments were successful.
Randomization	Animals were randomly assigned to groups (cages) prior to any experimentation (see methods section: Specific-pathogen free mouse husbandry).
Blinding	Microbiota sample preparation and analysis performed in a blinded manner.

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study	
☒	☐	Unique materials
☒	☐	Antibodies
☒	☐	Eukaryotic cell lines
☐	☒	Research animals
☒	☐	Human research participants

Research animals

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Animals/animal-derived materials	Male and female Swiss Webster were used for experiments involving germ-free animals with ages ranging 6 to 12 weeks. Female mice from Charles River (C57BL/6NCrl), Envigo (C57BL/6NHsd), Taconic (C57BL/6NTac) and Jackson (C57BL/6J and C57BL/6NJ) were purchased and infections were carried out concurrently when animals reached 8 to 12 weeks of age. Full husbandry and experimental details provided in methods sections: Mouse lines, Specific-pathogen free mouse husbandry, S. Tm infection in C57BL/6 mice, Germ-free mouse husbandry, and S. Typhimurium infections in Germ-free Swiss Webster mice.

Method-specific reporting

n/a	Involved in the study	
☒	☐	ChIP-seq
☒	☐	Flow cytometry
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