Microcins mediate competition among Enterobacteriaceae in the inflamed gut

Martina Sassone-Corsi1,2, Sean–Paul Nuccio1,2, Henry Liu1, Dulcemaria Hernandez1, Christine T. Vu1, Amy Y. Takahashi1, Robert A. Edwards2,3 & Manuela Raffatellu1,2

The Enterobacteriaceae are a family of Gram-negative bacteria that include commensal organisms as well as primary and opportunistic pathogens that are among the leading causes of morbidity and mortality worldwide. Although Enterobacteriaceae often comprise less than 1% of a healthy intestine’s microbiota, some of these organisms can bloom in the inflamed gut2–5; expansion of enterobacteria is a hallmark of microbial imbalance known as dysbiosis6. Microcins are small secreted proteins that possess antimicrobial activity in vitro, but whose role in vivo has been unclear. Here we demonstrate that microcins enable the probiotic bacterium Escherichia coli Nissle 1917 (EcN) to limit the expansion of competing Enterobacteriaceae (including pathogens and pathobionts) during intestinal inflammation. Microcin-producing EcN limits the growth of competitors in the inflamed intestine, including commensal E. coli, adherent–invasive E. coli and the related pathogen Salmonella enterica. Moreover, only therapeutic administration of the wild-type, microcin-producing EcN to mice previously infected with S. enterica substantially reduced intestinal colonization by the pathogen. Our work provides the first evidence that microcins mediate inter- and intraspecies competition among the Enterobacteriaceae in the inflamed gut. Moreover, we show that microcins can act as narrow-spectrum therapeutics to inhibit enteric pathogens and reduce enterobacterial blooms.

Broad-spectrum antibiotics are largely unsuccessful in treating enterobacterial gut infections. Efforts to identify novel therapeutic treatments can be aided by identifying the mechanisms employed by beneficial microbes to mediate colonization resistance to pathogens.9,10. Reports of microcin-producing Enterobacteriaceae in human stool led to suggestions that microcins contribute to gut microbial ecology11. We therefore sought to identify an in vivo role for microcins by studying EcN12,13 and its interplay with related competitors in the gut. Microcins can exhibit potent antibacterial activity in vitro, similar to bacteriocins produced by Gram-positive bacteria14–16. In contrast to bacteriocins15, however, a compelling in vivo role for microcins has not previously been demonstrated17.

The EcN microcin gene cluster resides on genomic island I (Extended Data Fig. 1a and Supplementary Table 1) and encodes for microcin M (mcmM), microcin H47 (mchB) and their cognate immunity genes (mcmL and mchI, respectively). To understand whether the microcins of EcN promote colonization of the healthy intestine, we intragastrically inoculated specific-pathogen-free (SPF) mice with an equal mixture of a mouse-commensal E. coli (Ec) strain18 and either wild-type EcN or a mutant unable to secrete both microcins (EcN mchDEF; all strains are described in Supplementary Table 3)14. Although all strains poorly and transiently colonized the healthy intestine (enterobacteria typically colonize at low levels in this setting), Ec colonization was not significantly impacted by either wild-type EcN or EcN mchDEF, and Ec outcompeted both strains to a similar extent (Extended Data Fig. 1b–g). To determine whether low colonization masked a microcin phenotype, we performed similar studies in germ-free mice and in streptomycin-treated SPF mice. We administered Ec alone or in competition with wild-type EcN, EcN mchDEF, or EcN mcmA mchB, a mutant that lacks both microcin genes. All strains colonized the gut to high levels in these models, but Ec colonization was again unaffected by competition with EcN wild-type or microcin mutants (Fig. 1a, b). Moreover, there were no significant differences in colonization among EcN strains (Fig. 1c, d), and Ec outcompeted EcN wild-type and microcin mutants to a similar extent (Fig. 1e, f). Together, these results indicate that the microcins of EcN do not provide a competitive advantage under homeostatic conditions (Fig. 1g).

Prior work has shown that the microcins of EcN possess antibacterial activity in iron-deprived medium14. Moreover, a subset of microcins are post-translationally modified at the C terminus with a siderophore moiety19–21 and EcN microcins are thought to be modified by a similar mechanism14,21. In response to iron starvation, some commensals and pathogens synthesize and secrete siderophores to acquire this essential metal nutrient22. Bacterial uptake of iron-bound siderophores occurs via specific membrane receptors14. Microcins conjugated to siderophores are also synthesized during iron starvation and can target non-immune bacteria in vitro through cognate siderophore receptors14,16, a mechanism described as a ‘Trojan horse’ mode of action23. Incorporating these observations, we proposed that microcins contribute to competition among enterobacteria in the inflamed gut, where iron is limited23.

We confirmed that EcN microcin genes (mcmM, mchB) are expressed in iron-restricted medium, and not in iron-rich medium (Fig. 1h). To identify a role for microcins during intestinal inflammation, we used the dextran sulphate sodium (DSS)-treated mouse model of colitis (Fig. 2a). First, we inoculated DSS-treated SPF mice intragastrically with an equal mixture of Ec and either wild-type EcN, EcN mchDEF, or EcN mcmA mchB. Although Ec initially outcompeted (~10-fold) wild-type EcN, both strains colonized the intestine equally well by 5 days after inoculation. By contrast, Ec greatly outcompeted the microcin mutants (up to 10,000-fold; Fig. 2b and Extended Data Fig. 2a). Whereas intestinal colonization of Ec was similar when alone or in competition with wild-type EcN or a microcin mutant at day 1–3 following inoculation, only wild-type EcN significantly reduced Ec colonization at later time points (Fig. 2c and Extended Data Fig. 2b, h). Consistent with this, the in vitro killing of Ec was only observed in iron-restricted medium when competing with wild-type EcN, not the microcin mutants (Extended Data Fig. 3a–d). As the EcN microcin mutants still express cognate microcin immunity proteins, their competitive growth in vitro was not impaired (Extended Data Fig. 3c–g) until the immunity genes were deleted (Extended Data Fig. 3h, i). Nevertheless, gut colonization of microcin mutants was similarly reduced in competition with Ec (Fig. 2d and Extended Data Fig. 2c, i), but complementation of EcN mchDEF with mchDEF (EcN mchDEF pWSK29::mchDEF) restored colonization.

1Department of Microbiology and Molecular Genetics, University of California Irvine, Irvine, California 92697, USA. 2Institute for Immunology, University of California Irvine, Irvine, California 92697, USA. 3Department of Pathology and Laboratory Medicine, University of California, Irvine, California 92697, USA.
Microcins do not promote bacterial competition in the absence of intestinal inflammation. a–g, SPF streptomycin-treated (at 1 day prior to infection) mice (a, c, e, g) or germ-free mice (b, d, f) were intragastrically inoculated with the indicated E. coli strains. a–d, Colony-forming units (CFU) mg⁻¹ in the faeces of SPF (a, c) or germ-free (b, d) mice on days 1–5 after inoculation. e, f, Ratio (EcN:cEc) of panels a and c (in e) or panels b and d (in f); bars represent the geometric mean ± s.e.m. g, Caecal histopathology scores for mice in panels a and c. a, c, d–g, Each symbol represents an individual mouse, bars represent the geometric mean. h, Levels of mcmA (microcin M) and mchB (microcin H47) mRNA in iron-rich and iron-limited medium, normalized to the gapA housekeeping gene; bars represent the geometric mean from three independent experiments ± s.e.m. WT, wild type. *P < 0.05, **P < 0.01; NS, not significant. Unpaired Student’s t-test was used for statistical comparisons in panel h. Mann–Whitney–Wilcoxon for all other comparisons; P values are presented in Supplementary Table 7.

As cEc was isolated from the murine gut, cEc is plausibly better adapted to growth in this environment than is EcN, a human isolate. Microcins thus give EcN an edge by which to compete. Colonization of microcin mutants was not impaired in DSS-treated mice when strains were administered alone or in competition with wild-type EcN, in contrast to competition with cEc (Fig. 3a–c and Extended Data Fig. 4a–d). Microcin mutants exhibited a 5–10-fold advantage over wild-type EcN at day 3 or 5 after inoculation (Fig. 3c). This observation may result from the mutants not having to expend energy on microcin production, yet still being immune to microcin activity; relatedly, wild-type EcN rescued the colonization of microcin mutants when co-administered with cEc in DSS-treated mice (Extended Data Fig. 4e–h). Microcin mutants are thus not intrinsically attenuated. Moreover, our findings indicate that microcins enable EcN to limit the expansion of a related commensal in the inflamed gut (Extended Data Fig. 2b–d). As cEc was isolated from the murine gut, cEc is plausibly better adapted to growth in this environment than is EcN, a human isolate. Microcins thus give EcN an edge by which to compete.

Moreover, our findings indicate that microcins enable EcN to limit the expansion of a related commensal in the inflamed gut (Extended Data Fig. 2b–d). As cEc was isolated from the murine gut, cEc is plausibly better adapted to growth in this environment than is EcN, a human isolate. Microcins thus give EcN an edge by which to compete. Colonization of microcin mutants was not impaired in DSS-treated mice when strains were administered alone or in competition with wild-type EcN, in contrast to competition with cEc (Fig. 3a–c and Extended Data Fig. 4a–d). Microcin mutants exhibited a 5–10-fold advantage over wild-type EcN at day 3 or 5 after inoculation (Fig. 3c). This observation may result from the mutants not having to expend energy on microcin production, yet still being immune to microcin activity; relatedly, wild-type EcN rescued the colonization of microcin mutants when co-administered with cEc in DSS-treated mice (Extended Data Fig. 4e–h). Microcin mutants are thus not intrinsically attenuated. Moreover, our findings indicate that microcins enable EcN to limit the expansion of a related commensal in the inflamed gut (Extended Data Fig. 2b–d). As cEc was isolated from the murine gut, cEc is plausibly better adapted to growth in this environment than is EcN, a human isolate. Microcins thus give EcN an edge by which to compete.

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EcN (but not EcN \textit{mchDEF} or EcN \textit{mcmA mchB}) only killed STm in iron-limited medium (Extended Data Fig. 6a–e). When streptomycin-treated mice (a \textit{Salmonella} colitis model) were co-administered STm and either wild-type EcN or EcN \textit{mchDEF}, only wild-type EcN outcompeted STm beyond day 4 after infection (Fig. 3d, e). Although all strains initially colonized the gut to similar levels, reduction of STm colonization was significantly enhanced by microcin-producing EcN (Fig. 3f). By day 7, wild-type EcN and EcN \textit{mchDEF} had similarly reduced STm colonization, probably because EcN has other mechanisms to outcompete STm, including a greater ability to acquire iron\textsuperscript{22}. Nevertheless, in agreement with our cEc results (Fig. 2a–d), gut colonization of EcN \textit{mchDEF} was drastically reduced in competition with STm 7 days after co-administration (Fig. 3g). Moreover, wild-type EcN’s competitive advantage over STm was again only observed during intestinal inflammation (Extended Data Fig. 7a, b). Relatedly, wild-type EcN and EcN \textit{mchDEF} exhibited similar levels of intestinal colonization and no competitive advantages when competed with STm \textit{invA spiB}, a mutant in both type-three secretion systems that is unable to trigger gut inflammation as it cannot invade the intestinal epithelium or replicate intracellularly (Extended Data Fig. 7c–e).

Consistent with our results from STm and cEc experiments, microcins impaired growth of adherent invasive \textit{E. coli} (AIEC), a pathobiont frequently isolated from patients with Crohn’s disease. Wild-type EcN (but not EcN \textit{mchDEF}) was able to reduce AIEC growth in vitro (Extended Data Fig. 6f–i) and in the DSS–colitis model (Extended Data Fig. 8). Although AIEC and wild-type EcN colonized the inflamed gut to similar levels, AIEC significantly outcompeted EcN \textit{mchDEF} in this environment (Extended Data Fig. 8). Collectively, our data indicate that EcN produces microcins to limit growth of competing enterics in the inflamed gut.

We next investigated whether microcins could therapeutically treat an active enterobacterial infection by administering wild-type EcN or EcN \textit{mchDEF} to mice previously infected with STm (Fig. 4a). Mice receiving wild-type EcN at days 2 and 5 after infection with STm showed substantially reduced levels of STm gut colonization (average 1,300-fold; up to 100,000-fold), inflammation and weight loss (Fig. 4b–d and Extended Data Fig. 9). This phenotype largely depended on microcins, as EcN \textit{mchDEF} only minimally reduced STm intestinal burden (approximately 17-fold) and did not reduce inflammation or weight loss (Fig. 4b–d and Extended Data Fig. 9).

To understand the therapeutic phenotype better, we assayed the antibacterial activity of each microcin. Although both M and \textit{H47} microcins showed antibacterial activity against AIEC, only M was effective against STm and cEc (Extended Data Fig. 10a–c); the \textit{H47}–resistance mechanism is unknown as neither strain carries the cognate immunity gene \textit{mchl}. Accordingly, STm engineered to express \textit{mchl} (STm pMchl) was not rescued in competition with wild-type EcN, whereas STm engineered to express \textit{mcmI} (STm pMcml) was rescued (Extended Data Fig. 10d, e). Notably, the therapeutic administration of EcN only
minimally impaired colonization of the microcin-resistant STn pMcmI (Fig. 4b and Extended Data Fig. 9). Furthermore, inflammation, weight loss and STn colonization were comparable when either EcN mchDEF or STn pMcmI were used in the therapeutic experiment (Fig. 4b–d and Extended Data Fig. 9).

Together, our data provide in vivo evidence that microcin mediate competition among Enterobacteriaceae in the inflamed gut. We show that the probiotic bacterium EcN employs microcin to compete with related species, colonize the inflamed gut, and therapeutically displace an enteric pathogen from its niche. The microcins produced by EcN target microbes that express particular siderophore receptors and do not severely affect the microbiota. Although siderophore-receptor mutants are resistant to microcins14 (Extended Data Fig. 10f–h), they are themselves attenuated in the inflamed intestine24 and thus unlikely to be selected for. As microcins were particularly effective upon therapeutic EcN administration to pathogen-infected, inflamed mice, these or similar compounds (for example, siderophore-conjugated antibiotics25) could conceivably be used as a targeted strategy to treat enterobacterial colitis.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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METHODS

Bacterial strains, culture conditions and chemicals. Strains of the probiotic E. coli Nissle 1917 (EcN; Mutaflor, DSM 66001), the mouse commensal E. coli (Ec), the adherent invasive E. coli (AIEC), and the pathogen S. enterica serovar Typhimurium (STm) used in this study are listed in Supplementary Table 3. All plasmids used in this study are listed in Supplementary Table 4. EcN was provided by Ardeypharm GmbH. The commensal E. coli strain was isolated from mice in our vivarium and does not appear to encode for known antibacterial exoproducts.

The AIEC strain is a human isolate from a patient with Crohn's disease (isolate NRG857c O83:H1) and was provided by A. Torres. The STm strain background we employed (IR/715) is a fully virulent, nalidixic acid-resistant derivative of STm wild-type isolate ATCC 14028 and does not encode for known antibacterial exoproducts. All strains were routinely grown aerobically in Luria–Bertani (LB) broth (10 g l⁻¹ tryptone, 5 g l⁻¹ yeast extract, 10 g l⁻¹ NaCl) or on LB agar plates at 37 °C. When indicated, antibiotics were added to the medium at the following concentrations: 0.03 mg ml⁻¹ chloramphenicol (Cm); 0.1 mg ml⁻¹ carbenicillin (Car); 0.05 mg ml⁻¹ kanamycin (Kan); 0.01 mg ml⁻¹ tetracycline (Tet). For animal infections or bacterial administration, all strains were grown in LB medium aerobically at 37 °C overnight. For in vitro growth assays, strains were grown in iron-limiting conditions (nutrient broth supplemented with 0.2 mM 2,2'-dipyridyl dissolved in ethanol; Sigma) aerobically at 37 °C overnight. Restriction enzymes and Phusion High Fidelity DNA Polymerase were purchased from New England Biolabs. Oligonucleotides were synthesized by Fisher Scientific and are listed in Supplementary Tables 5 and 6.

Generation of bacterial mutants. Mutants in EcN and STm were constructed using the lambda red recombinase system. In brief, primers (Supplementary Table 5) homologous to sequences flanking the 5′ and 3′ ends of the target regions were designed (H1 and H2 primers, respectively; Supplementary Table 5) and were used to replace the selected genes with a chloramphenicol- (derived from pKD3), kanamycin- (derived from pKD4) or tetracycline-resistance cassette (Supplementary Table 4). Strain names for the mutants are listed in Supplementary Table 3. To confirm integration of the resistance cassette and deletion of the target, mutant strains and wild-type controls were each assayed using three PCR amplifications (5′ end, 3′ end, deleted target) to validate mutations. Primers (Supplementary Table 5) that flank the target sequence were used in conjunction with a common test primer (C1, C2, K1, K2 or primers for the tetR cassette) to test for both new-junction fragments.

Complementation studies. The mchDEF region was amplified from EcN genomic DNA using primers listed in Supplementary Table 5. A region of 4,148 bp was amplified to ensure all regulatory elements were included. The PCR fragment was cloned into plasmid pCR-XL-TOPO using Zero Blunt TOPO PCR Cloning Kit (Invitrogen) according to the manufacturer's protocol, subcloned into the multiple cloning site of low-copy plasmid pWSK29, and confirmed by Sanger sequencing (Eton Bioscience). The mchDEF mcm1 and mchDEF mchl constructs were amplified from EcN genomic DNA using primers listed in Supplementary Table 5. PCR fragments of 4,321 bp (mchDEFmcm1), 4,000 bp (mchDEF) and 400 bp (mchl) were directly assembled with plasmid pVW330 using the Gibson assembly method.

In vitro growth assays. The role of EcN microorganisms against C. jejuni, AIEC and STm was tested by an in vitro competitive growth assay in iron-rich and iron-limiting conditions. Strains were grown in nutrient broth supplemented with 0.2 mM 2,2'-dipyridyl (Sigma) aerobically at 37 °C overnight. Approximately 5 × 10⁸ CFU ml⁻¹ from an overnight culture were inoculated into 0.1 ml of tissue-culture medium (DMEM/F12 with 10% fetal bovine serum (FBS); Invitrogen), as previously described. When indicated, medium was prepared with 1 mM ferric iron citrate (Sigma). Wild-type EcN or EcN mutants were inoculated in competition with wild-type STm, STm mutants, EcN, or AIEC. CFUs of each strain were enumerated by plating serial dilutions at 0, 5, 8, 11 and (for some assays) 16 h after incubation at 37 °C.

Mice. The Institutional Animal Care and Use Committee at the University of California, Irvine approved all mouse experiments. Eight-to-ten-week-old male and female C57BL/6 Sle1(+ or −) mice were bred and housed in a vivarium. For some experiments, mice were purchased from Jackson laboratory and, upon arrival, allowed to acclimate to the new environment for at least one week before the start of an experiment. All mice were housed under specific pathogen-free conditions. Mice were fed an irradiated 2920X Teklad diet (Envigo). For one experimental procedure, mice were fed an irradiated iron-rich diet (1% diet FeSO₄ 2020, Teklad TD 160234; Envigo). Culturing faeces on MacConkey agar indicated that culturable enterobacteria were absent from our mice. Five to ten mice were used per experimental group, as indicated by the number of symbols in each figure panel. Group sizes were based on previous studies that detected a tenfold difference with statistical significance. For one experiment, ten-week-old Swiss Webster germ-free mice were used. Swiss Webster germ-free mice were maintained in sterile isolators and were fed a double-irradiated diet (Purina 5066, Charles River Rodent 18% Vac Pac). For this experimental procedure, three mice per experimental group were used (see figure panels). Mice were randomly grouped in cages of a maximum five animals per cage. Similar numbers of male and female mice were used in each experimental group. No blinding was performed, with the exception of histopathology.

Dextran sulphate sodium-induced colitis mouse model. Drinking water was replaced with either filter-sterilized water (mock treatment) or with a filter-sterilized solution of 4% (w/v) dextran sulphate sodium (DSS; relative molecular mass 36,000–50,000; MP Biomedicals) in water as indicated. For DSS-treated mice, 3 days before the end of the experiment, drinking water was switched for 24 h to filter-sterilized water. Drinking water was then replaced with either filter-sterilized water (mock treatment) or with a filter-sterilized solution of 2% (w/v) DSS. At 4 days after the start of DSS treatment, animals were orally inoculated with a 1:1 ratio of 5 × 10⁹ CFU of wild-type EcN and mutants in competition with EcN and AIEC strains (resuspended in 0.1 ml LB broth). Faecal material was collected each day after bacterial administration, resuspended in sterile PBS and the bacterial load was determined by plating serial tenfold dilutions on selective agar plates. Animals were euthanized 5 days after inoculation. Caecal tissue was collected and then flash-frozen in liquid nitrogen and stored at −80 °C for later isolation of mRNA and protein. The caecal tip was fixed in 10% formalin for histopathology. Faecal material and caecal content were collected in sterile PBS and the bacterial load for the E. coli strains was determined by plating serial tenfold dilutions on selective agar plates. At some time points, a mouse did not produce faecal material, hence an n value is displayed for a given time point that is less than the experiment's indicated n value for that group. To differentiate EcN from other E. coli strains in biological samples, the strains were differentially marked with the low-copy number pACY-omega (Cm) or pPH54omega (Carb) (Supplementary Table 4), and markers were swapped in some experiments. Plasmids were stably maintained throughout the experiment and similar colonies were counted in other differential media such as on MacConkey agar (not shown). When noted, the ratio of two strains in the faeces and caecal content was calculated by dividing the output ratio (EcN CFU/EcN or AIEC CFU) by the input ratio (EcN CFU/EcN or EcN CFU). The competitive index was calculated for isogenic strains by dividing the output ratio (EcN wild-type CFU/mutant CFU) by the input ratio (EcN wild-type CFU/mutant CFU).

5. Typhimurium-induced colitis. C57BL/6 Nnap1-/- mice were treated with streptomycin (100 µl of a 200 mg ml⁻¹ solution in sterile water) one day before infection. The following day, mice were orally inoculated with 1 × 10⁹ CFU of STm (resuspended in 0.1 ml LB broth) or with a 1:1 ratio of 5 × 10⁸ CFU each of STm and wild-type EcN, or EcN mchDEF strain for competitive colonization experiments. For therapeutic administration of EcN after STm infection, C57BL/6 Nnap1-/- mice were treated with streptomycin (100 µl of a 200 mg ml⁻¹ solution in sterile water) one day before STm infection. The following day, mice were orally inoculated with 1 × 10⁶ CFU of STm. At days 2 and 5 after STm infection, mice were either mock-treated or treated with 1 × 10⁸ CFU of wild-type EcN or EcN mchDEF. Faecal material was collected each day after bacterial administration and resuspended in sterile PBS. Bacterial load for STm and EcN strains was then determined by plating serial tenfold dilutions on selective agar plates. At some time points, a mouse did not produce faecal material, hence an n value is displayed for a given time point that is less than the experiment's indicated n value for that group. At day 7 following infection, mice were euthanized and a portion of the caecum was flash-frozen in liquid nitrogen then stored at −80 °C for later isolation of mRNA and protein. The caecal tip was fixed in 10% formalin for histopathology. Bacteria in the faecal content were counted by plating serial tenfold dilutions on LB agar plates containing the appropriate antibiotics. To selectively identify STm from EcN strain in the faecal content, strains were differentially marked with the low-copy number pACYomega (Cm) or pPH54omega (Carb) plasmids. Plasmids were stably maintained throughout the experiment and similar colonies were counted in other differential media such as MacConkey agar (not shown). When noted, the ratio of two strains was calculated by dividing the output ratio (EcN CFU/STm CFU) by the input ratio (EcN CFU/STm CFU).

Extraction of bacterial DNA from faecal samples. Faecal samples were collected before DSS treatment (day −4), at day 4 after DSS treatment (day 0; before inoculation of bacteria) and at day 5 after administration of commensal E. coli in competition with wild-type EcN or EcN mchDEF. Faeces were snap-frozen in liquid nitrogen, then DNA was later extracted using the QIAamp DNA Stool Kit (Qiagen) according to the manufacturer's instructions with modifications as previously described. Briefly, DSS interferes with PCR, we used the following protocol (developed by W. Zhu and S. Winter) to eliminate DSS from samples. Samples were eluted in 200 µl of ultra-pure water and combined with 80 mg of KCl followed by vortexing for 2 min. The mixture was then incubated on ice for 30 min and centrifuged at 16,800 × g at 4 °C for 30 min. The supernatant was then transferred
to a new tube and 1/10 volume of 3 M NaAc (pH 5.2) was added. We then added 2.5 mL of pure ethanol to the mixture, vortexed and centrifuged at 16,800 × g for 30 min. The supernatant was removed and 1 mL of 70% ethanol was added to the pellet, vortexed and centrifuged at 16,800 × g for 5 min at room temperature. This wash step was repeated 3–4 times. The pellet was then air-dried at 55 °C and resuspended in 30 μL of ultra-pure water.

Analysis of microbiota. DNA extracted from faecal samples was amplified by a two-step PCR enrichment of 16S rDNA (V4 region) with primers 515F and 806R modified by addition of barcodes for multiplexing, then sequenced on an Illumina MiSeq system (UC Davis HMSC Facility). Sequences were processed and analysed by employing the QIIME pipeline v1.9.1 (ref. 28) with default settings, except as noted. In brief, paired-end sequences were joined, quality-filtered, reverse-complemented and chimaera-filtered (usearch61 option; RDP gold database); operational taxonomic units (OTUs) were picked (usearch61 option; enable_rev_strand_match True) at 97% similarity; taxonomy was assigned (confidence 0.8) with the RDP classifier. Greengenes database v13.8 was used in the open-reference OTU picking workflow.

Bacterial RNA extraction. A bacterial RNA mini-kit (Bio-Rad Aurum Total) was used to extract RNA from bacterial cultures. EcN strains were grown in nutrient broth supplemented with 0.2 mM 2,2′-dipyridyl aerobically at 37 °C overnight. Approximately 10^6 CFU mL ^{-1} from an overnight culture were inoculated into 5 mL of nutrient broth medium (DMEM/F12 plus 10% FBS, Invitrogen), as previously described. When indicated, iron citrate was added to the medium at a final concentration of 1 μM. At 7 h post-inoculation, 2 × 10^9 CFU were used to extract RNA. An additional DNase treatment (Ambion) was done before the generation of cDNA with reverse transcription reagents (Roche).

Quantitative real-time PCR. For analysis of gene expression by quantitative real-time PCR, total RNA was extracted from caecal tissue with TRI Reagent (Molecular Research Center). RNA from DSS-treated mice was further purified using the Dynabeads mRNA DIRECT Purification Kit (Life Technologies) according to manufacturer recommendations. Reverse-transcription reagents (Roche) were employed to generate cDNA from all RNA samples. Real-time PCR was performed using SYBR Green (Roche) and the Roche Lightcycler 480 Instrument II system (Roche). Data were analysed using the comparative −2ΔΔC_{t} method. Target gene transcription of each tissue sample was normalized to the respective levels of Actb mRNA (β-actin). For qPCR analysis of bacterial transcripts, transcription of mcmA and mchB was normalized to bacterial gapA mRNA levels. Data represent at least three independent experiments. DNA contamination was less than 1% for all bacterial amplicons, as determined by separate mock reactions lacking reverse transcriptase. All primers used are listed in Supplementary Table 6.

Histopathology analysis. Tissue samples were fixed in 10% formalin, processed according to standard procedures for paraffin embedding, sectioned at 5 μm, and stained with haematoxylin and eosin. The pathology score of caecal samples was determined by blinded examination of caecal sections from a board-certified pathologist using previously published methods. Each section was evaluated for the presence of neutrophils, mononuclear infiltrate, submucosal oedema, surface erosions, inflammatory exudates, and cryptitis. Inflammatory changes were scored from 0 to 4 according to the following scale: 0, none; 1, low; 2, moderate; 3, high; 4, extreme. The inflammation score was calculated by adding up all scores obtained for each parameter and interpreted as follows: 0–2, within normal limit; 3–5, mild; 6–8, moderate; ≥9, severe.

Statistical analysis of data. The experiments were not randomized. No statistical methods were used to predetermine sample size. Prism 6 and 7 software (GraphPad) was used for statistical analysis. Bacterial growth curves were analysed by unpaired Student’s t-test. To compare bacterial CFUs in faeces and caecal content, mRNA expression, and eubacterial taxa present at greater than 0.5% relative abundance in at least one sample, we applied a non-parametric Mann–Whitney–Wilcoxon test. We chose this test because it can be applied to data with normal or unknown distribution. P-values for all statistical comparisons presented in the main text, figures and Extended Data are available in Supplementary Table 7.

Data availability. The data that support the findings of this study are available from the corresponding author upon request. Faecal microbiota 16S rRNA gene sequencing data were deposited in the European Nucleotide Archive under accession PRJEB15700.
Extended Data Figure 1 | Wild-type EcN and microcin mutant (EcN mchDEF) gut colonization in competition with commensal E. coli in the absence of intestinal inflammation. a, Illustration of the microcin gene cluster in EcN. b–g, Intragastric inoculation of SPF mice with cEc alone or in competition with wild-type EcN or EcN mchDEF at a 1:1 ratio. b, Ratio (EcN over cEc) in faecal content at days 1–5 after inoculation (n = 5 per group). c, d, CFU mg⁻¹ of (c) cEc or (d) wild-type EcN or EcN mchDEF in faecal content at days 1–5 after inoculation, when cEc was administered alone or in competition as indicated. e, f, Caecal histopathology scores (e) or gene expression (f) (data are expressed as fold change over mock-treated mice) at day 5 after inoculation for panels b–d (n = 5 per group). g, Haematoxylin and eosin-stained sections from representative animals at day 5 after inoculation. Scale bar, 100 μm. Each individual symbol represents one mouse (c–e). Bars represent the geometric mean ± s.e.m. (b, f), geometric mean (c, d), or mean (e). n.s., not significant.
Extended Data Figure 2 | Colonization and histopathology of wild-type EcN and microcin mutants (mchDEF and mcmA mchB) in competition with commensal E. coli, in mice with DSS-mediated colitis.

a–i. Experimental design as in Fig. 2a with SPF mice. a, Ratio (EcN over cEc) in caecal content at day 5 after intragastric inoculation (n = 6 per group). b, c, CFU mg⁻¹ of (b) cEc or (c) wild-type EcN or EcN mchDEF in faecal content at day 5 after inoculation when in competition as indicated (n = 6 per group). d, e, Caecal (d) gene expression (n = 5 per group; data are presented as fold change over mock-treated mice) or (e) histopathology scores at day 5 after inoculation from mice shown in panels b, c and Fig. 2b–d (DSS only, n = 3; all others, n = 5).

Scale bar, 100 μm. f, Detailed histopathology scoring of mice in e. g, Haematoxylin and eosin-stained sections from representative animals at day 5 following inoculation. h, i, CFU mg⁻¹ of (h) cEc or (i) wild-type EcN or EcN mchDEF in sample content at days 1–5 following intragastric inoculation when cEc was administered alone or in competition as indicated (n = 5 per group). Each individual symbol represents one mouse (b, c, e, h, i). Bars represent the geometric mean ± s.e.m. (a, d), geometric mean (b, c, e, h, i) or mean (e). *P < 0.05, **P < 0.01; n.s., not significant.
Extended Data Figure 3 | *In vitro* growth curves of EcN and microcin mutants when grown alone or in competition with EcN mutants or *commensal* *E. coli*. a–i, Strains were grown overnight in nutrient broth supplemented with 0.2 mM 2,2′-dipyridyl. Growth assays were performed in iron-limiting conditions (DMEM/F12 supplemented with 10% FBS) or in medium supplemented with 1 μM iron citrate. Time points at 0, 5, 8 and 11 h after inoculation were collected. a, b, cEc CFU ml⁻¹ when grown alone or in competition with wild-type or mutant EcN in iron-limiting conditions (a) or in medium supplemented with 1 μM iron citrate (b). c, d, CFU ml⁻¹ of wild-type or mutant EcN when grown alone or in competition with cEc in iron-limiting conditions (c) or in medium supplemented with 1 μM iron citrate (d). e–g, Under iron-limiting conditions, CFU ml⁻¹ of wild-type or mutant EcN when grown alone (e), of wild-type EcN (f) or EcN mchDEF or EcN mcmA mchB (g) when grown competitively as indicated. h, i, CFU ml⁻¹ of the indicated EcN mutants (immunity gene(s) and/or mchDEF) grown in competition with wild-type EcN in (h) iron-limiting conditions or in (i) medium supplemented with 1 μM iron citrate. Symbols represent the geometric mean of three independent experiments ± s.e.m. **P < 0.01, ***P < 0.001, ****P < 0.0001.
Extended Data Figure 4 | Gut colonization of wild-type EcN and microcin mutants in the DSS–colitis model. a, Experimental design for b–d with SPF mice. b, CFU mg−1 of wild-type EcN or EcN mchDEF in faecal content at days 1–5 following intragastric inoculation (n = 5 per group). c, Caecal gene expression (c; n = 5 per group; data are expressed as fold change over mock-treated mice) or haematoxylin and eosin-stained sections (d; representative) from panel b mice at day 5 following inoculation. Scale bar, 100 μm. e, Triple co-administration design for f–h with SPF mice. f, g, CFU mg−1 of cEc (f) or indicated EcN strain (g) in faecal content at days 1–5 following intragastric inoculation with cEc, wild-type EcN and the indicated EcN mutant (n = 5 per group). h, Competitive index (CI; EcN wild-type over mutant) for EcN data presented in panel g (n = 5 per group). b, f, g, Each individual symbol represents one mouse. Bars represent the geometric mean or the geometric mean ± s.e.m (c, h). *P < 0.05, **P < 0.01; n.s., not significant.
Extended Data Figure 5 | Impact of microcins on the microbiota and impact of a high iron diet on microcin-mediated competition.

a, High-iron diet design for b–e with SPF mice (n = 5 per group). b, Ratio (EcN over cEc) in faecal content at days 1–5 after intragastric inoculation. c, Caecal gene expression at day 5 following inoculation (n = 3–4 per group). d, e, CFU mg⁻¹ of cEc (d) or wild-type EcN or EcN mchDEF (e) in indicated samples at days 1–5 after inoculation when cEc was administered alone or in competition as indicated (n = 5 per group). f, g, See Fig. 2a for experimental design with SPF mice. 16S ribosomal rRNA gene sequence analysis (V4 region) of faecal DNA obtained from mice prior to DSS administration (day −4), after DSS administration (Day 0), and day 5 after intragastric inoculation of cEc with either wild-type EcN or EcN mchDEF at a 1:1 ratio. f, Eubacterial alpha diversity (inverse Simpson index); Shannon index yielded similar results. g, Principal coordinates (PCo) analysis plot (PCo1 versus PCo2) of eubacterial beta diversity (weighted UniFrac); symbols as in panel f. Each individual symbol represents one mouse (d, e, f, g). Bars represent the geometric mean ± s.e.m. (b, c), geometric mean (d, e) or mean ± s.d. (f). n.s., not significant. Black symbols represent comparisons within same day; red symbols represent comparisons between days.
Extended Data Figure 6 | In vitro activity of EcN microcins against S. Typhimurium and AIEC. a–i, Strains were grown overnight in nutrient broth supplemented with 0.1 mM 2,2'-dipyridyl. Growth assays were performed in iron-limiting conditions (DMEM/F12 supplemented with 10% FBS) or in medium supplemented with 1 μM iron citrate. Time points at 0, 5, 8 and 11 h after inoculation were collected. a, b, STm CFU ml⁻¹ when grown alone or in competition with wild-type or mutant EcN in iron-limiting conditions (a) or in medium supplemented with 1 μM iron citrate (b). c, STm CFU ml⁻¹ in iron-limiting conditions when in competition with wild-type EcN or an EcN mchDEF strain harbouring either pWSK29::mchDEF or the empty-vector control. d, e, CFU ml⁻¹ of wild-type or mutant EcN when grown alone or in competition with STm in (d) iron-limiting conditions or in (e) medium supplemented with 1 μM iron citrate. f, g, AIEC CFU ml⁻¹ when grown alone or in competition with wild-type or mutant EcN in iron-limiting conditions (f) or in medium supplemented with 1 μM iron citrate (g). h, i, CFU ml⁻¹ of wild-type or mutant EcN when grown alone or in competition with AIEC in iron-limiting conditions (h) or in medium supplemented with 1 μM iron citrate (i). Symbols represent the geometric mean (three independent experiments) ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
Extended Data Figure 7 | Co-administration of S. Typhimurium with wild-type EcN or EcN mchDEF. a–e, See Fig. 3d for co-administration design with SPF mice. a, b, Intragastric inoculation with wild-type STm. Cecal (a) gene expression (STm only, *n* = 7; all others, *n* = 9); data are expressed as fold change over mock-treated mice or (b) histopathology (STm only and STm + wild-type EcN, *n* = 5; STm + EcN mchDEF, *n* = 4) from mice shown in Fig. 3f, g at Day 7 following infection. c–e, Intragastric inoculation with STm invA spiB. c, d, CFU mg⁻¹ of cecal mRNA (fold change) day 7 p.i. n.s., not significant. e, Ratio of wild-type EcN or EcN mchDEF over STm invA spiB in faecal content at designated time points after infection (n = 5 per group). Each individual symbol represents one mouse (b–d). Bars represent the geometric mean ± s.e.m. (a, e), mean (b), or geometric mean (c, d).
Extended Data Figure 8 | Gut colonization of AIEC in DSS-treated mice when competing with wild-type EcN or EcN mchDEF. a, Experimental design for b–f with SPF mice. b, Ratio of wild-type EcN or EcN mchDEF over AIEC in faecal content at days 1–5 following intragastric inoculation (n = 9 per group). c, d, CFU mg⁻¹ of (c) AIEC or (d) wild-type EcN or EcN mchDEF in faecal content at days 1–5 following inoculation when AIEC was administered alone or in competition as indicated (n = 9 per group). e, Caecal histopathology scores at day 5 after inoculation for panels c, d (n = 5 per group). f, Detailed histopathology scoring of panel e mice. Each individual symbol represents one mouse (c–e). Bars represent the geometric mean ± s.e.m. (b), geometric mean (c, d) or mean (e). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; n.s., not significant.
Extended Data Figure 9 | Therapeutic administration of wild-type EcN, EcN mchDEF or mock during S. Typhimurium infection. a–j. See Fig. 4a for therapeutic design with SPF mice. 
a–f, Intragastric inoculation with wild-type STm in faecal content on days 4–7 after infection with STm (n = 8 per group). 
b, c, e, STm CFU mg⁻¹ at designated time points after infection in faecal content of mice therapeutically treated with mock (b; n = 7), wild-type EcN (c; n = 8) or EcN mchDEF (e; n = 8). d, f, CFU mg⁻¹ of wild-type EcN (d) or EcN mchDEF (f) in faecal content at designated time points after STm infection. 
g, h, Intragastric inoculation with STm pMcmI (n = 10 per group). 
cFu mg⁻¹ of STm pMcmI (g) or wild-type EcN (h) in faecal content at designated time points following STm pMcmI infection. 
grey box represents average STm CFU mg⁻¹ in mock-treated mice (panel b). 
i, Caecal histopathology scores for panels b–f (STm alone, n = 6; all others, n = 7). j, Detailed histopathology scoring of mice in i. Each individual symbol represents one mouse (b–h). Bars represent the geometric mean ± s.e.m. (a) or mean (i). *P < 0.05, **P < 0.01, ***P < 0.001; n.s., not significant.
Extended Data Figure 10  |  *In vitro* growth curves of microcin M (mcmA) and microcin H47 (mchB) EcN mutants in competition with commensal *E. coli*, *S. Typhimurium*, or AIEC. a–h. Strains were grown overnight in Nutrient Broth supplemented with 0.2 mM 2,2′-dipyridyl. Growth assays were performed in iron-limiting conditions (DMEM/F12 supplemented with 10% FBS) and time points at 0, 5, 8 and 11 h following inoculation were collected. a–c, CFU ml⁻¹ of commensal *E. coli* (a), STm (b) or AIEC (c) when grown alone or in competition with the indicated EcN strain. d, CFU ml⁻¹ of complemented and uncomplemented EcN microcin immunity gene mutants when in competition with EcN wild-type. e, CFU ml⁻¹ of wild-type STm or STm harbouring pMchI or pMcmI when in competition with EcN wild-type. f–h, CFU ml⁻¹ of STm *fepA* (f), STm *iroN* (g) and STm *fepA iroN* (h) in competition with either wild-type EcN or EcN *mcmA mchB*. Symbols represent the geometric mean for three independent experiments ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.