## Materials and Methods

**Bacterial strains**. The *E. coli* strains used in this study are listed in Supplementary Table 1. The EcN (Mutaflor, DSM 6601) wild-type strain was kindly provided by Ardeypharm GmbH, Herdecke, Germany. All strains were routinely grown aerobically in LB broth (10 g/l tryptone, 5 g/l yeast extract, 10 g/l NaCl) or on LB agar plates (15 g/l agar) at 37°C. When appropriate, antibiotics were added to the media at the following concentrations: 0.03 mg/ml chloramphenicol (Cm), 0.1 mg/ml carbenicillin (Carb), 0.05 mg/ml kanamycin (Kan), and 0.01 mg/ml tetracycline (Tet).

**Construction of plasmids.** Standard molecular cloning techniques were performed as described previously (*22*) to generate the plasmids listed in Supplementary Table 1. The oligonucleotides used to amplify DNA fragments by PCR are listed in Supplementary Table 2. PCR products were routinely cloned into pCR2.1 using the TOPO TA cloning kit (Life Technologies, Carlsbad) and sequenced prior to subcloning.

pKD46 (*23*) was digested with AccI and ClaI, and the Klenow-treated, 3.2 kb DNA fragment was religated to create pSW172. To construct pSW224, pSW225, pSW237, and pSW248, respectively, DNA regions upstream and downstream of the EcN *napA*, *narG*, *narZ*, and *moaA* genes were amplified by PCR, digested with XbaI and ligated using T4 DNA ligase. DNA fragments comprising the linked flanking regions of each gene were PCR-amplified from the ligation products, cloned into pRDH10 using either the BamHI (pSW224), SphI (pSW225), EcoRI (pSW237), or SphI/SalI (pSW248) restriction enzyme sites. The *moaA* gene and the *narG* gene were PCR-amplified with their native promoter regions from the EcN chromosome and cloned into pWSK29 utilizing the restriction enzymes XhoI/HindIII (pMOA1) and XhoI (pNARG1) to generate pMOA1 and pNARG1, respectively. pSW268 was created by amplifying the EcN *napA*

gene including the upstream and downstream regions and cloning this DNA fragment into pRDH10 via BamHI restriction sites.

**Construction of mutants by allelic exchange**. Suicide plasmids were routinely propagated in DH5α λ*pir* and introduced into the *E. coli* strains HS, EcN(pSW172) or LF82(pSW172) by conjugation with S17-1 λ*pir* as donor strain. To ensure stable replication of pSW172, all conjugation experiments were performed at 30°C. Exconjugants in which the suicide plasmid had integrated into the chromosome by a single crossover event were recovered on LB plates containing carbenicilin and chloramphenicol. Subsequent sucrose selection was performed on sucrose plates (5 % sucrose, 8 g/l nutrient broth base, 15 g/l agar) to select for second crossover events leading to the unmarked deletion of the target gene. Deletion or incorporation of the gene of interest was confirmed by PCR. If appropriate, pSW172 was cured by cultivating the bacteria at 37°C.

The EcN Δ*narG* mutant SW895 was created by introducing pSW225 into EcN(pSW172) by conjugation and subsequent sucrose selection. The *napA* gene in SW895(pSW172) was deleted by introduction of pSW224 and sucrose selection, thus creating SW925. To generate the Δ*narG* Δ*napA* Δ*narZ* triple mutant (SW930), pSW237 was incorporated into the SW925(pSW172) chromosome by conjugation, sucrose selection was performed to delete the *narZ* gene. Similarly, the LF82 Δ*napA* Δ*narG* Δ*narZ* triple mutant (SW963) was generated by subsequent introduction of the suicide plasmids pSW224, pSW225, and pSW237 followed in each case by sucrose selection, generating SW934 (Δ*napA*) and SW595 (Δ*napA* Δ*narG*) in this process. To delete the *moaA* gene in the *E. coli* strains HS and EcN, pSW248 was introduced by conjugation and sucrose selection performed on exconjugants, thus creating SW1098 and SW1029, respectively. The *napA* deletion in the EcN Δ*narG* Δ*napA* Δ*narZ* triple mutant (SW930)

was restored with the *napA* wild-type allele by introducing pSW268 into SW930(pSW172) by a single crossover and subsequent sucrose selection. The resulting strain (EcN Δ*narG* Δ*narZ napA*[restored]) was termed SW1069.

**Anaerobic growth assays.** Mucin broth was prepared by dissolving hog mucin (0.5 % [w/v] final concentration; Sigma-Aldrich, St.Louis) in No-Carbon E medium (NCE) (*24*) supplemented with trace elements (*25*). Sodium nitrate, DMSO, and TMAO (Sigma Aldrich, St. Louis) were added at a final concentration of 40 mM as indicated. 3 ml of mucin broth was inoculated with each of the indicated *E. coli* strains at a final concentration of 1 x $10^4$ colony forming units (CFU)/ml of and incubated anaerobically (Bactron I Anerobic Chamber; Sheldon Manufacturing, Cornelius) for 24 h at 37°C. Bacterial numbers were determined by spreading serial ten-fold dilutions on selective LB agar plates. The competitive index was calculated by normalizing the ratio of recovered wild-type bacteria to mutant bacteria (output ratio) to the respective ratio in the inoculum (input ratio).

**Nitrate reductase activity assays.** Overnight cultures of *E. coli* strains were diluted 1:50 in fresh LB broth containing 40 mM sodium nitrate or in NCE minimal medium supplemented with 40 mM glucose and 40 mM sodium nitrate. Cultures were statically incubated for 3 h at 37°C and the relative nitrate reductase activity was measured as described previously (*26*).

**DSS colitis model.** All experiments involving mice were approved by the Institutional Animal Care and Use Committee at the University of California, Davis. Female, 9-12 week old C57BL/6J wild-type mice and *Nos2*-deficient mice (B6.129P2-Nos2tm1Lau/J) of were obtained from The Jackson Laboratory (Bar Harbor). To induce colitis, the following procedure was followed unless indicated otherwise (Fig S1D). The drinking water was replaced with either filter-sterilized water (mock-treatment) or with a

filter-sterilized solution of 4 % (w/v) dextran sulfate sodium (DSS; relative molecular mass 36,000 – 50,000; MP Biomedicals, Santa Ana) in water as indicated. For DSS-treated mice, the drinking water was switched for 24 h to regular, filter-sterilized water 3 days and 1 day prior to the end of the experiment. 4 days after the start of the DSS treatment, animals were orally inoculated with either 0.1 ml of LB broth or 1 x $10^9$ CFU of the indicated *E. coli* strains suspended in 0.1 ml LB broth. Animals were inoculated with 5 x $10^8$ CFU of each *E. coli* strain in the competitive colonization experiments. If appropriate, aminoguanidine chloride (AG) was added to the drinking water at a concentration of 1 mg/ml starting on the day of the inoculation and for the remainder of the experiment. Animals were euthanized 5 days after inoculation (Fig. S1D). For experiments shown in Fig. S7, mice were treated with 2 % (w/v) DSS for the entire duration of the experiment. To obtain the data displayed in Fig S3D and S3E, mice were orally inoculated with *E. coli* strains suspended in 0.1 ml LB broth, treated with 4 % DSS after 24 h for the rest of the experiment and animals sacrificed 9 days after inoculation. Colonic tissue for mRNA and protein expression analysis was removed, flash frozen and stored at -80°C. Fecal material, cecal content, and colonic content was harvested in sterile PBS and the bacterial load for the *E. coli* strains determined by plating serial 10-fold dilutions on selective agar plates. To facilitate recovery from biological samples, *E. coli* strains were differentially marked with the low-copy number plasmids pWSK29 and pWSK129. Both plasmids were stably replicated during the entire period and the use of two different antibiotic resistance markers did not alter the bacterial load of the relevant *E. coli* strains in the intestinal content (data not shown).

**Measurement of nitrate concentrations in cecal mucus extracts.** C57BL/6 wild-type mice were treated with 4% DSS and AG as described above. Animals were orally inoculated with 1 x $10^9$ CFU of the EcN Δ*narG* Δ*napA* Δ*narZ* triple mutant (SW930)

four days after the start of the DSS treatment. Five days after inoculation, animals were euthanized. The luminal content of the cecum was removed, and the cecal mucus layer was collected and weighed. Cecal mucus was extracted with 0.2 ml ultra-pure water (Gibco), larger particles removed by centrifugation at 20,000 g for 2 min at 4°C and the filter-sterilized supernatant stored at -80°C. The nitrate concentration was determined as described previously (*27*). Briefly, thawed samples were derivatized with modified Griess reagent (0.25 % (w/v) vanadium(III)chlorie, 0.1 % (w/v) sulfanilamide, 0.005 % (w/v) *N*-(1-Naphthyl)ethylenediamine dihydrochloride in 0.5 M HCl) for 8 h. The absorbance at a wavelength of 540 nm was measured. Nitrate concentrations were determined based on a standard curve derived from similarly treated dilutions of sodium nitrate.

**Genetic mouse colitis model.** The mouse strain *Il10*$^{flox/flox}$ *Cd4-cre*$^{+/+}$ mice (*28*) was a generous gift from Dr. Werner Müller, University of Manchester. The mice were further bred at UC Davis animal facilities. The genotype of all mice used in experiments was confirmed by PCR using the primers listed in Supplementary Table 2. Upon development of colitis, animals were orally inoculated with an equal mixture of 5 x $10^8$ CFU of each of the indicated *E. coli* strains. Five days later, animals were euthanized and samples collected as described above.

**Bovine ligated ileal loop model.** All experiments involving cattle were approved by the Institutional Animal Care and Use Committee at Texas A&M University. Bovine ligated ileal loop surgery was performed as described previously (*29*). Briefly, small intestinal loops were inoculated by intralumenal injection of 3 ml of LB broth containing an equal mixture of 5 x $10^8$ CFU of each strain as indicated. Thapsigargin (Sigma Aldrich) was initially resuspended in dimethyl sulfoxide and either added to the injected suspension at a final concentration of 0.1 mM or dimethyl sulfoxide was added instead at a final concentration of 1 % (v/v; vehicle control). Mucus scrapings and luminal fluid were

collected 8 h after inoculation. Serial dilutions of mucus samples and the luminal fluids were spread on selective media. Competitive indices were calculated by normalizing the ratio of recovered wild-type bacteria to mutant bacteria to the respective ratio in the inoculum.

**Quantification of mRNA levels in intestinal tissue.** The relative transcription levels of iNOS, KC, and TNF-α, encoded by the *Nos2*, *Kc*, and *Tnf* genes, respectively, was determined as described previously (*30*). Briefly, colonic tissue was homogenized in a Minibeadbeater (Biospec Products, Bartlesville) and RNA extracted by the TRI reagent method (Molecular Research Center, Cincinnati). RNA from DSS treated mice was further purified using the dynabeads mRNA direct kit (Life Technologies) according to the recommendations of the manufacturer. TaqMan reverse transcription reagents (Life Technologies) were employed to generate cDNA from all RNA samples. SYBR Green (Life Technologies)-based real-time PCR was performed in an 11 μl volume with 2 μl of cDNA as a template. The primers listed in supplementary table 2 were added at a final concentration of 250 nM. Data was acquired by a ViiA 7 real-time PCR system (Life Technologies) and analyzed using the comparative Ct method. Target gene transcription of each sample was normalized to the respective levels of *Gapdh* mRNA.

**Analysis of protein expression.** Colonic tissue was homogenized in a Minibeadbeater (Biospec Products) and colonic proteins were extracted using the TRI reagent method (Molecular Research Center). Isolated proteins were resuspended in 1 % (w/v) sodium dodecyl sulfate (SDS) and 10 mM 2-mercaptoethanol. Protein concentrations were measured using the Micro BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham) according to the recommendation of the manufacturer. 10 μg of protein from each samples was subjected to SDS-polyacrylamide gel electrophoresis and transferred (Semi-dry transfer, Bio-Rad laboratories, Hercules) to a polyvinylidene

fluoride membrane (Millipore, Billerica) by Western Blot. Immundetection of protein expression was performed using primary antibodies specific for murine iNOS and tubulin (Cell Signaling Technology, Beverly) as well as a horseradish peroxidase-conjugated goat-anti-rabbit secondary antibody (Jackson ImmunoResearch Laboratories, West Grove). Chemiluminescence (SuperSignal West Pico Chemiluminescent Substrate, ThermoFisher Scientific) was detected by a BioSpectrum Imaging System (UVP) and images were processed in Photoshop CS2 (Adobe Systems, Mountain View) to uniformly (linear) adjust brightness levels.

**Histopathology.** Murine colonic and bovine ileal tissue was fixed in phosphate-buffered formalin and 5 μm sections of the tissue were stained with hematoxylin and eosin. An experienced veterinary pathologist evaluated blinded sections according to the criteria listed in Figure S11. Images were taken with an Olympus BX41 microscope at a magnification of 20x. The contrast for the images was uniformly (linear) adjusted using Photoshop CS2.

**Abundance of Enterobacteriaceae.** The relative abundance of Enterobacteriaceae as part of the bacterial microbiota was analyzed as described previously (*11, 31*). Briefly, the cecum was homogenized in 0.5 ml Dulbecco's phosphate buffered saline (Life Technologies). The QIAamp DNA stool kit (Qiagen, Valencia) was used to extract genomic bacterial DNA from 0.25 ml of the homogenate. A 2 μl sample of the 1:10 diluted bacterial DNA served as the template for SYBR-green based real-time PCR reactions as described above. The gene copy number in the sample was determined based on a standard curve generated by using a DNA fragment of the respective bacterial 16S rRNA gene (Eubacteria: *R. productus* [ATCC 27340D], Enterobacteriaceae: *E. coli* K-12 [TOP10]) cloned into pCR2.1 (TOPO TA cloning kit, Life Technologies) as a template. The fraction of Enterobacteriaceae as part of the entire

bacterial population for each sample was calculated by dividing the gene copy number of the Enterobacteriacaea by the gene copy number determined using the eubacterial primers.

**Statistical analysis.** The statistical significance of differences between groups was determined by a Student's *t*-test. Nitrate concentrations, fold changes in mRNA levels, competitive indices, relative abundance of Enterobacteriaceae, and bacterial numbers underwent logarithmic transformation prior to analysis.

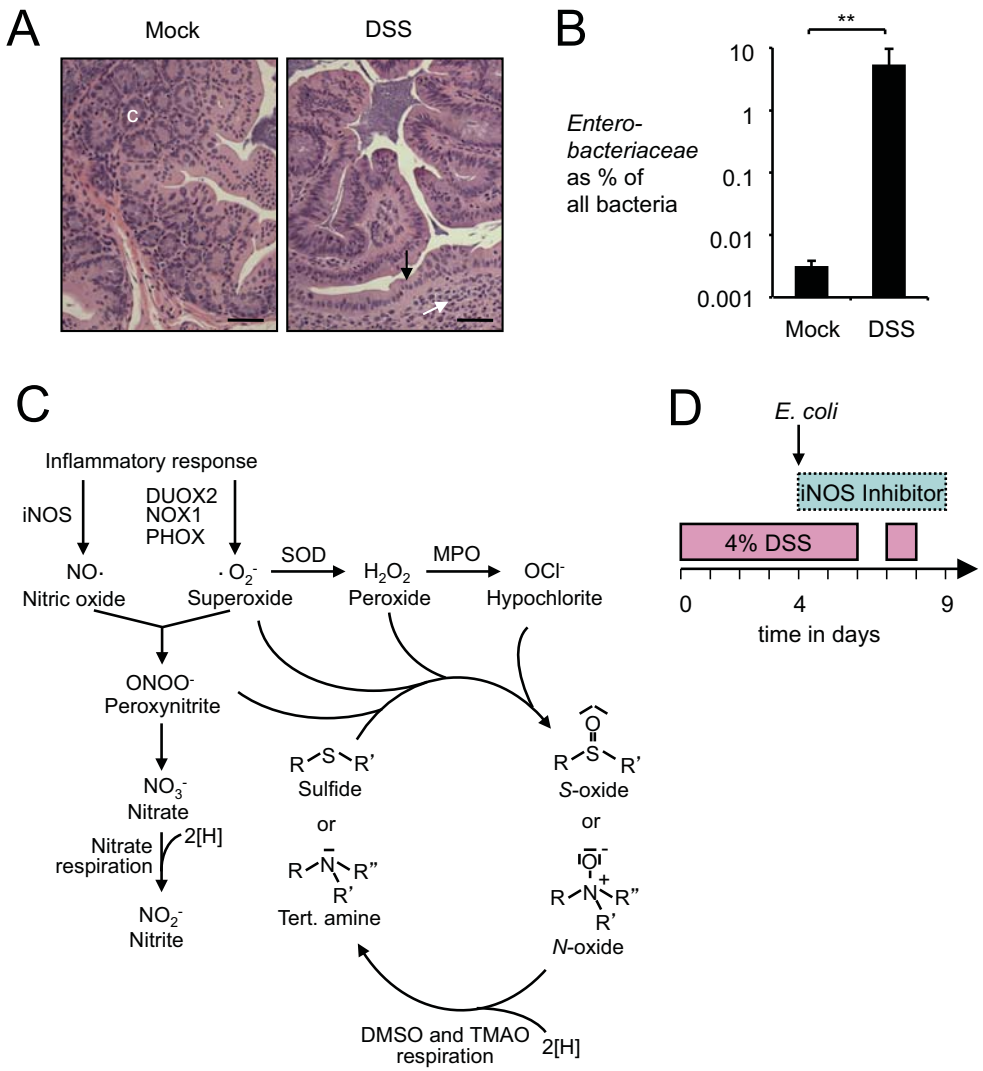


**Figure S1: Proposed mechanism for the dysbiosis observed during DSS-induced colitis.** (A and B) Samples from mock-treated mice (Mock, *N* = 4) or DSS-treated mice (DSS, *N* = 3) five days after inoculation with *E. coli* K-12. (A) Representative H&E stained colonic sections. Scale bar, 50 μm. (B) Fraction of *E. coli* as percentage of the cecal bacterial population using 16S rRNA gene qRT-PCR. **, $P < 0.01$ (Student's *t*-test). (C) Proposed model for the generation of respiratory electron acceptors during gut inflammation. (D) Schematic of DSS treatment, AG treatment and *E. coli* inoculation regiments used for experiments shown in figures 1, 2 and 3.

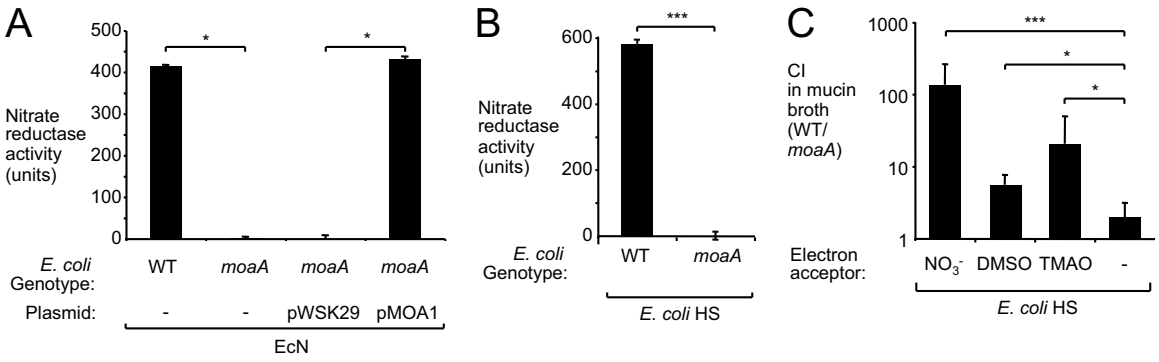


**Figure S2: Inactivation of *moaA* in *E. coli* strains Nissle 1917 (EcN) and HS.** (A) Detection of nitrate reductase activity in EcN wild type (WT), an isogenic *moaA* mutant (*moaA*) or *moaA* mutants carrying a vector control (pWSK29) or the cloned *moaA* gene (pMOA1) (*N* = 3). (B) Detection of nitrate reductase activity in the *E. coli* HS wild type (WT) and an isogenic *moaA* mutant (*moaA*) (*N* = 3). (C) Competitive index (CI) of the *E. coli* HS wild type (WT) and an isogenic anaerobic respiration-deficient mutant (*moaA*) after anaerobic growth in mucin broth supplemented with 40 mM of the indicated electron acceptors (*N* = 3). (A-C) Bars represent the geometric mean from three independent experiments ± standard error. *, $P < 0.05$; ***, $P < 0.001$ (Student's *t*-test).

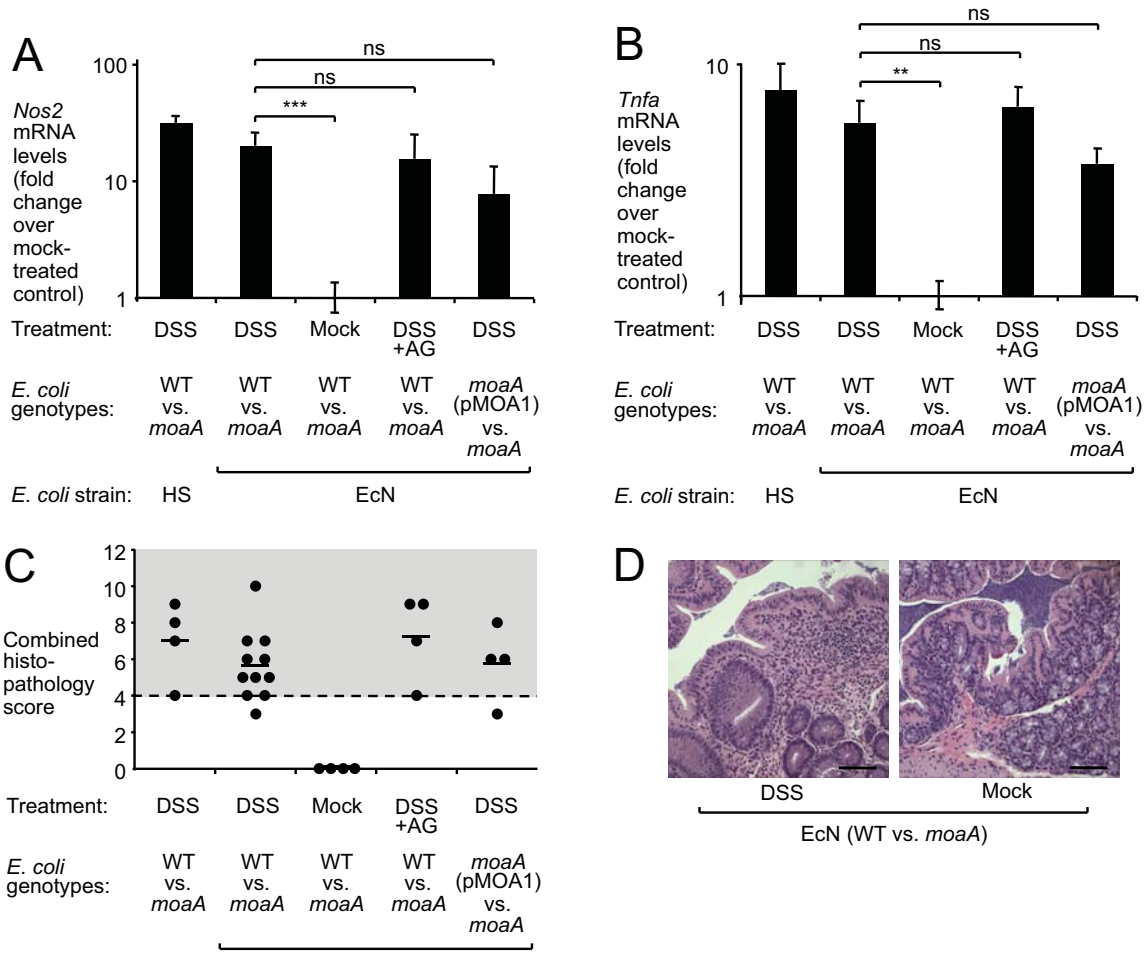


**Figure S3: Induction of inflammation in the colonic mucosa of DSS-treated mice.** (A - B) Levels of *Nos2* mRNA (A) and *Tnfa* mRNA (B) in colonic mucosa of mice used for experiments shown in figure 1 were determined by qRT-PCR (fold-increases over mock-treatment). (C) Combined histopathology score in the colon. Each dot represents one animal. (A-C) Mock-treated mice (Mock), DSS-treated mice (DSS) or mice treated with DSS and AG (DSS+AG) were inoculated with the indicated mixtures of *E. coli* strains and samples collected 5 days after inoculation. A plasmid (pMOA1) carrying the cloned *moaA* gene was used to complement the *moaA* mutant (*moaA*). (D) Representative H&E stained colonic sections for experiments shown in figure 1. Scale bar, 100 µm. (A and B) Bars represent geometric means ± standard error. **, $P < 0.01$; ***, $P < 0.001$; ns, not statistically significant (Student's *t*-test). *N* is given in panel C.

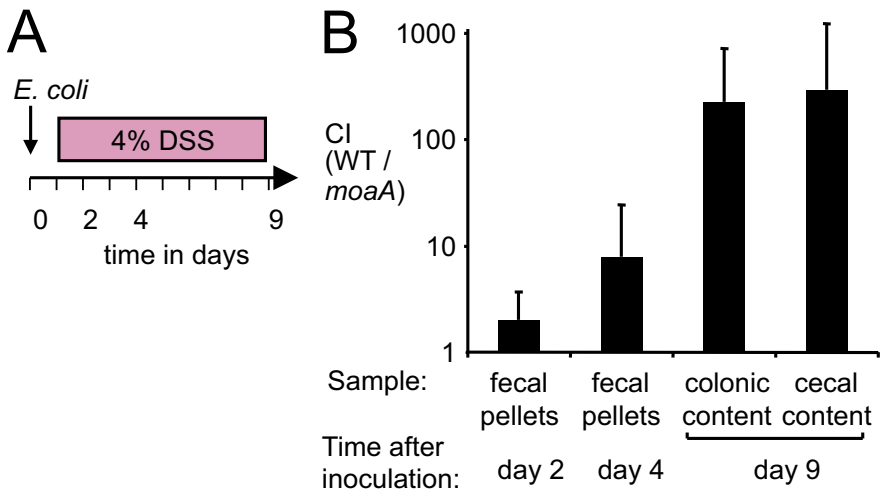


**Figure S4: Colonization of mice prior to induction of inflammation.** Animals (*N* = 4) were inoculated with an equal mixture of the EcN wild-type strain (WT) and the isogenic *moaA* mutant. DSS was administered beginning one day after inoculation of *E. coli* strains (A) and the competitive index (CI) in the feces, cecal contents, and colon contents were determined at the indicated time points (B). Bars represent geometric means ± standard error.

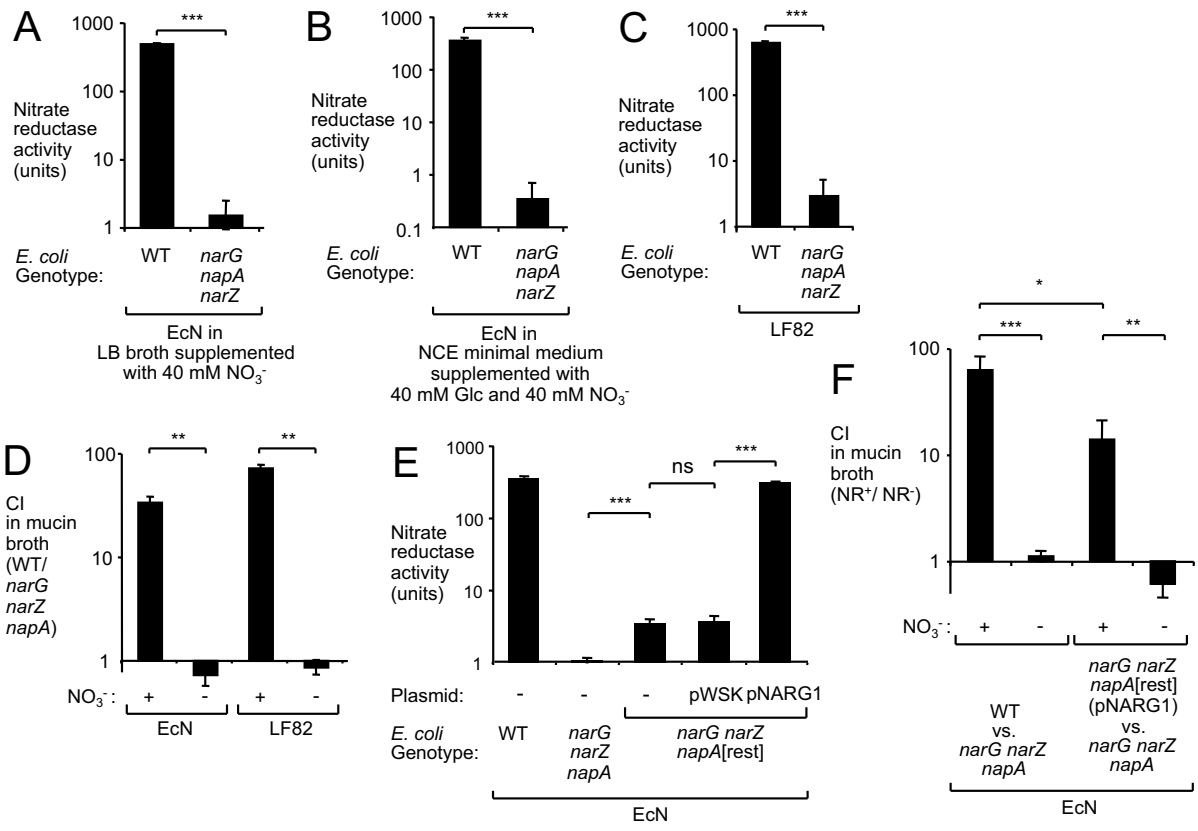


**Figure S5: Inactivation of nitrate reductases in *E. coli* strains EcN and LF82.** Wild-type isolates (WT) of EcN and *E. coli* LF82 were used to generate a triple mutant lacking all nitrate reductases (*napA nar narG*). The *narG narZ napA* mutant was complemented by introducing a functional chromosomal *napA* allele and a plasmid (pNARG1) carrying the cloned *narG* gene (*narG narZ napA*[rest] [pNARG1]). As a control, the *narG narZ napA* mutant in which the *napA* allele had been restored (*narG narZ napA*[rest]) was transformed with a plasmid vector control (pWSK; pWSK29). (A, B, C, and E) Detection of nitrate reductase activity in the indicated *E. coli* strains cultured in LB broth containing 40 mM nitrate (A, C, and E) or NCE minimal media containing 40 mM Glucose (Glc) and 40 mM nitrate (B) ($N$ = 3). (D and F) Competitive index (CI) of the indicated mixtures of *E. coli* strains during anaerobic growth in mucin broth in the presence (+) or absence (-) of nitrate ($NO_3^-$) ($N$ = 3). (A-F) Bars represent geometric means of three independent experiments ± standard error. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not statistically significant (Student's *t*-test).

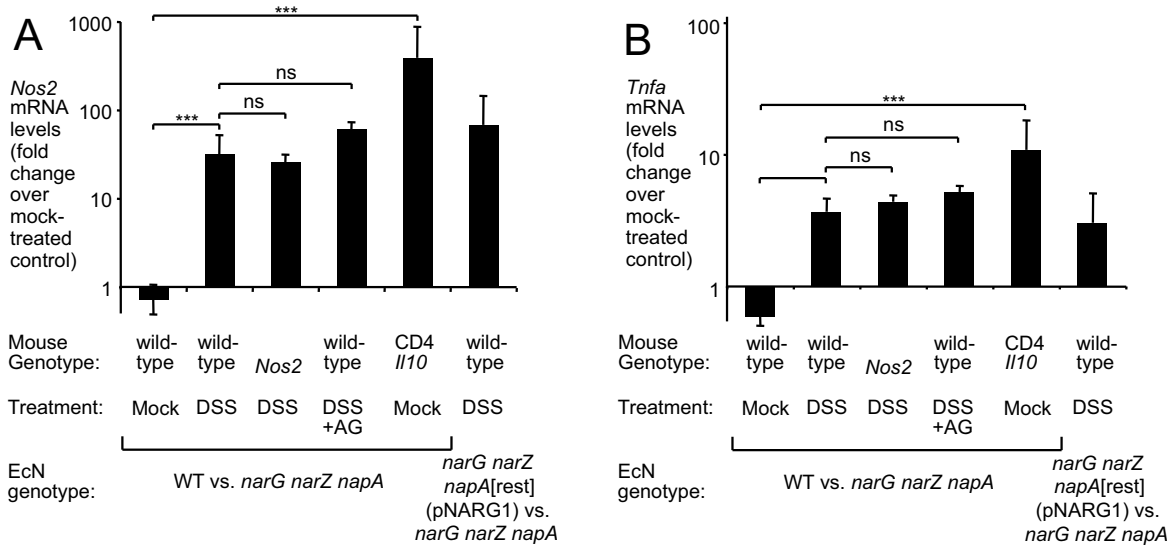


**Figure S6: Levels of pro-inflammatory markers in the colonic mucosa of DSS-treated mice.** (A and B) mRNA levels of *Nos2* (A) and *Tnfa* (B) in the colonic mucosa of mice used for experiments shown in Fig. 2A, 2B, and 2C were determined by qRT-PCR (fold-increases over mock-treatment). Mock-treated (Mock), DSS-treated (DSS) or DSS+AG-treated (DSS+AG) wild-type mice, *Nos2*-deficient mice (*Nos2*) or mice harboring T cells deficient for the production of IL-10 (*Cd4 Il10* mice) were inoculated with the indicated mixtures of *E. coli* strains. WT, *E. coli* wild type; *narG narZ napA*, *E. coli* nitrate respiration-deficient mutant. The *narG narZ napA* mutant was complemented by introducing a functional chromosomal *napA* allele and a plasmid (pNARG1) carrying the cloned *narG* gene (*narG narZ napA*[rest] [pNARG1]). (A-B) Bars represent geometric means ± standard error. ***, $P < 0.001$; ns, not statistically significant. *N* is given in Fig. 2A.

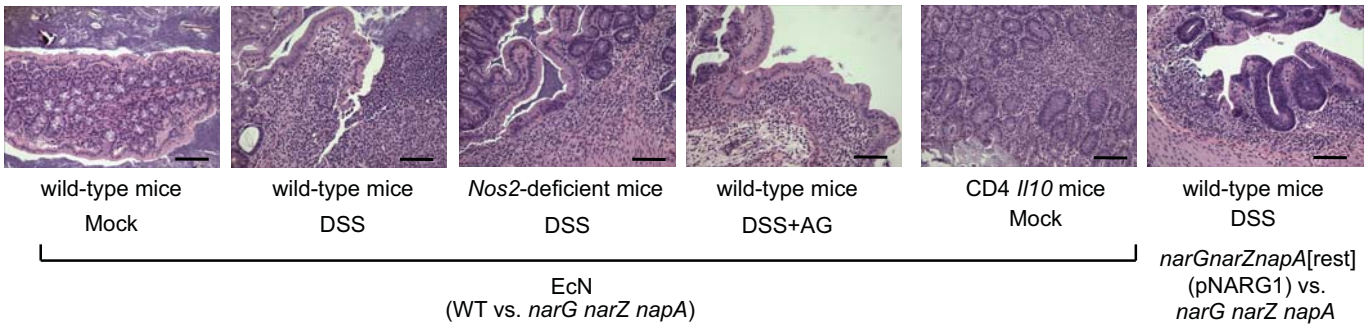


**Figure S7: Representative images of histopathological changes for experiments shown in Figure 2.** Mock-treated (Mock), DSS-treated (DSS) or DSS+AG-treated (DSS+AG) wild-type mice, *Nos2*-deficient mice (*Nos2*) or mice harboring T cells deficient for the production of IL-10 (*Cd4 Il10* mice) were inoculated with the indicated mixtures of *E. coli* strains (*N* is given in Fig. 2A). WT, *E. coli* wild type; *narG narZ napA*, *E. coli* nitrate respiration-deficient mutant. The *narG narZ napA* mutant was complemented by introducing a functional chromosomal *napA* allele and a plasmid (pNARG1) carrying the cloned *narG* gene (*narG narZ napA*[rest] [pNARG1]). H&E stained colonic sections. Scale bar, 100 mm.

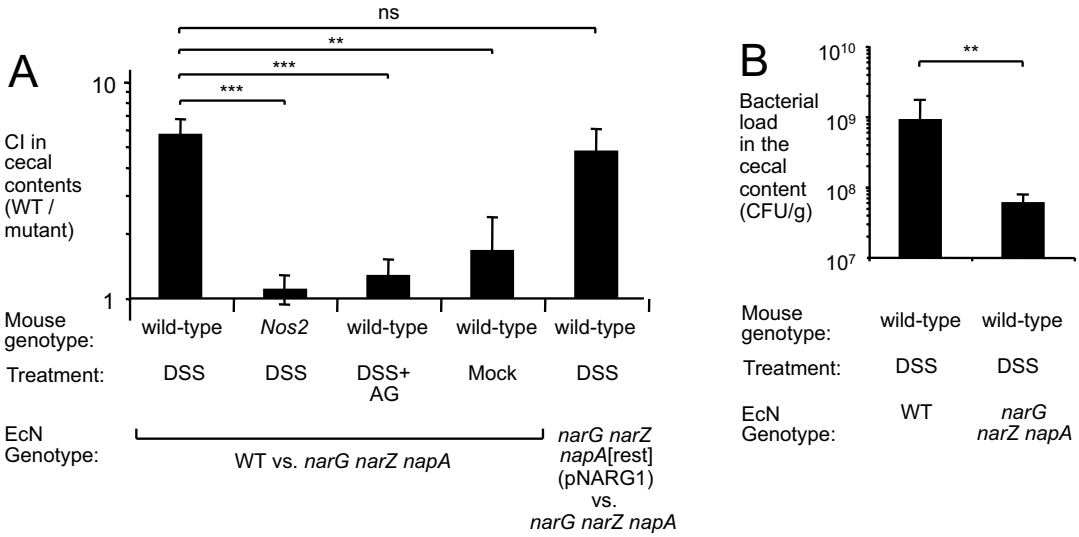


**Figure S8: Anaerobic respiration enhances luminal growth of *E. coli* in the cecum of DSS-treated mice.** (A) Mock-treated (Mock), DSS-treated (DSS) or DSS+aminoguanidine chloride-treated (DSS+AG) wild-type mice or *Nos2*-deficient mice (*Nos2*) were inoculated with the indicated mixtures of *E. coli* strains (*N* is given in Fig. 2A). The *narG narZ napA* mutant was complemented by introducing a functional chromosomal *napA* allele and a plasmid (pNARG1) carrying the cloned *narG* gene (*narG narZ napA*[rest] [pNARG1]). Five days after inoculation competitive indices (CI) were determined in the cecal content. (B) DSS-treated mice were inoculated either with EcN (WT) or with a nitrate respiration-deficient mutant (*narG napA narZ*) (*N* is given in Fig. 3F). Bacterial numbers recovered from cecal contents were determined 5 days after inoculation. (A-B) Bars represent geometric means ± standard error. **, $P < 0.01$; ***, $P < 0.001$; ns, not statistically significant (Student's *t*-test).

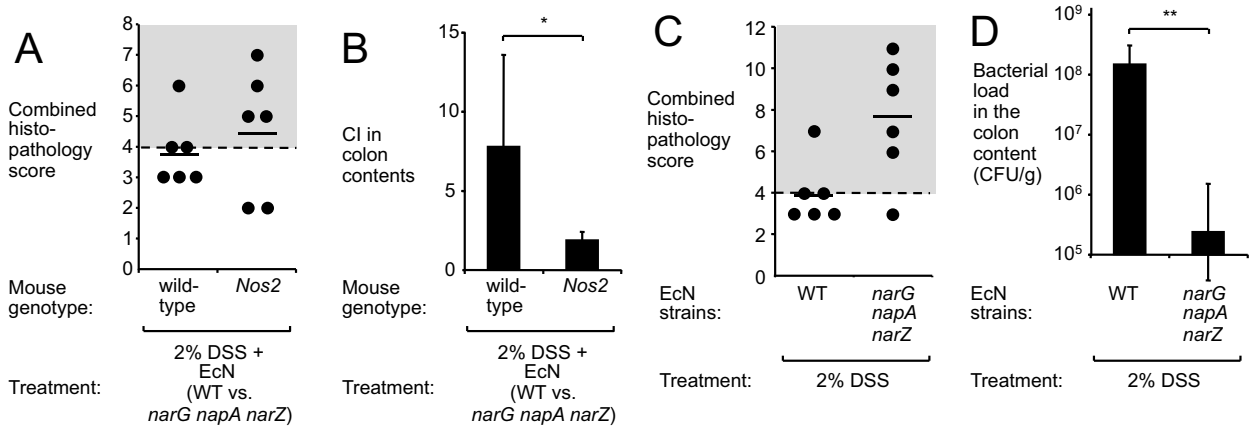


**Figure S9: Colitis induced by treatment with 2% DSS supports growth of EcN by nitrate respiration.** (A and B) Wild-type mice or *Nos2*-deficent mice (*N* is given in panel A) were treated with 2% DSS and inoculated with an equal mixture of the EcN wild type (WT) and a nitrate respiration-deficient mutant (*narG narZ napA*). Pathological changes in the colon (A) and the competitive index (CI) recovered from colon contents (B) were determined 5 days after inoculation. (C and D) Mice (*N* is given in panel C) were treated with 2% DSS and inoculated either with EcN (WT) or with a nitrate respiration-deficient mutant (*narG napA narZ*). Inflammation in the colon (C) and bacterial numbers recovered from colon contents (D) were determined 5 days after inoculation. (A and C) Each dot represents data from an individual animal. (B and D) Bars represent geometric means ± standard error. *, $P < 0.05$; **, $P < 0.01$ (Student's *t*-test).

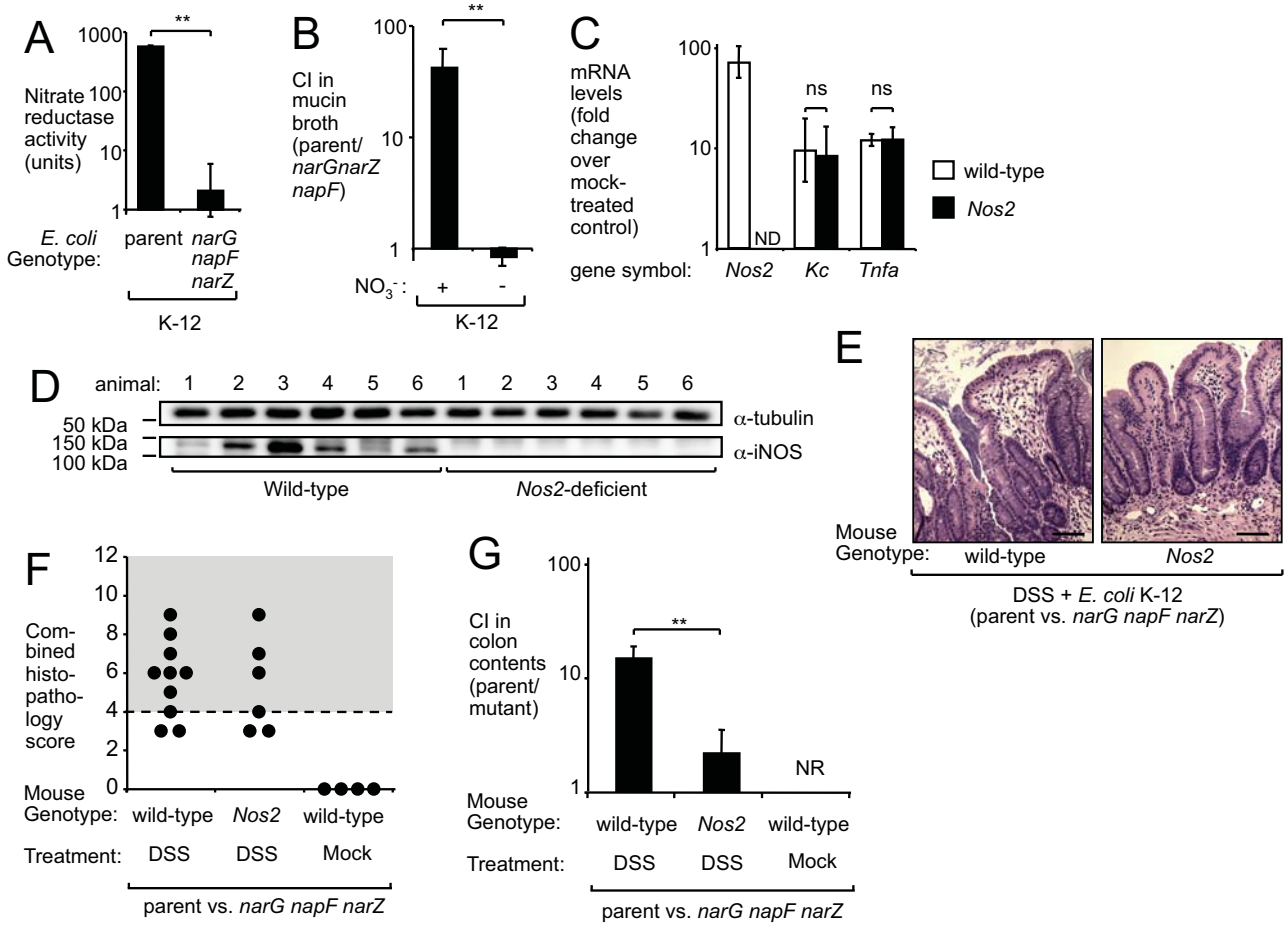


**Figure S10: DSS-induced colitis promotes luminal growth of *E. coli* K-12 by nitrate respiration.** (A) Detection of nitrate reductase activity in *E. coli* K-12 and mutants lacking all three nitrate reductases (*N* = 3). (B) Competitive index (CI) for anaerobic growth in mucin broth with (+) or without (-) nitrate (*N* = 3). (C-G) Samples from DSS-treated C57BL/6 mice (wild-type) or iNOS-deficient mice (*Nos2*) five days after inoculation with a mixture of *E. coli* K-12 (parent) and a nitrate respiration-deficient mutant (*narG napF narZ*) (*N* is given in panel F). (C) Expression of *Nos2*, *Kc* and *Tnf* in colonic RNA samples using qRT-PCR (fold-increases over mock-treatment). (D) Detection of iNOS (α-iNOS) or tubulin (α-tubulin) in colonic extracts (n=6). (E) Representative H&E stained colonic sections. Scale bar, 100 μm. (F) Combined histopathology score in the colon. Each dot represents data from an individual animal. (G) Competitive index (CI) in colon contents. (A, B, C, and G) Bars represent geometric means ± standard error. **, $P < 0.01$; ns, not statistically significant (Student's *t*-test); ND, not determined; NR, no bacteria were recovered.

A Histopathology scoring for murine colon

| Score | Infiltration inflammatory cells* | Submucosal edema | Epithelial damage | Exudate |
|---|---|---|---|---|
| 3 | severe multifocal todiffuse infiltration | severe (>40%) | multifocal to diffuse ulceration and/or severe multifocal to diffuse enterocyte hyperplasia with metaplasia | severe accumulation |
| 2 | moderate multifocal to diffuse infiltration | moderate (20-40%) | moderate multifocal to diffuse loss of goblet cells associated with enterocyte hyperplasia | moderate accumulation |
| 1 | mild multifocal to diffuse infiltration | mild (1-20%) | erosion/mild loss of goblet cells and enterocyte hyperplasia | mild accumulation |
| 0 | no infiltration | not present | not present | not present |

| Combined Score | Description |
|---|---|
| > 7 | severe inflammation |
| 4 to 6 | moderate inflammation |
| 2 to 3 | mild inflammation |
| 0 to 1 | normal |

B Histopathology scoring for bovine ileum

| Score | Infiltration inflammatory cells* | Submucosal edema | Epithelial damage | Exudate |
|---|---|---|---|---|
| 3 | severe multifocal todiffuse infiltration | severe (>40%) | severe multifocal to diffuse villus blunting associated with multifocal to diffuse ulceration and/or hemorrhage | severe accumulation |
| 2 | moderate multifocal to diffuse infiltration | moderate (20-40%) | moderate multifocal villus blunting associated with mild to moderate epithelial erosion | moderate accumulation |
| 1 | mild multifocal to diffuse infiltration | mild (1-20%) | mild multifocal villus blunting | mild accumulation |
| 0 | no infiltration | not present | not present | not present |

*Accumulation of mononuclear cells and neutrophils in the lamina propria

| Combined Score | Description |
|---|---|
| > 7 | severe inflammation |
| 4 to 6 | moderate inflammation |
| 2 to 3 | mild inflammation |
| 0 to 1 | normal |

**Figure S11: Evaluation of intestinal inflammation.** Chart indicating scoring criteria for blinded examination of H&E stained sections from the colon of mice (A) and for the ileum of calves (B) used in this study.

**Supplementary Table 1: Bacterial strains and plasmids used in this study**

| *E. coli* | | |
|---|---|---|
| Designation | Genotype* | Reference |
| VJS691 | K-12 parent strains; $\lambda^-$ $F^-$ Δ(*argF-lac*)*U169* Δ(*trpEA*)*2* | (*32*) |
| VJS5437 | VJS691 Δ*napF1*::$Kan^r$ Δ*narUZ*::$Spec^r$ *narG205*::Tn*10* | (*32*) |
| Nissle 1917 (EcN) | Wild-type strain (O6:K5:H1) | (*33*) |
| SW895 | EcN Δ*narG* | This study |
| SW925 | EcN Δ*narG* Δ*napA* | This study |
| SW930 | EcN Δ*narG* Δ*napA* Δ*narZ* | This study |
| SW1029 | EcN Δ*moaA* | This study |
| SW1069 | EcN Δ*narG* Δ*narZ napA*(restored) | This study |
| LF82 | Adherent invasive *E. coli* isolate (O83:H1) | (*34*) |
| SW934 | LF82 Δ*napA* | This study |
| SW959 | LF82 Δ*napA* Δ*narG* | This study |
| SW963 | LF82 Δ*napA* Δ*narG* Δ*narZ* | This study |
| HS | Human commensal *E. coli* isolate (O9:H4); $Carb^r$ | (*35*) |
| SW1098 | HS Δ*moaA* | This study |
| TOP10 | $F^-$ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) Φ*80lacZΔM15 lacX74 recA1 araD139* Δ(*ara - leu*)*7697 galU galK rpsL endA1 nupG* | Life Technologies |
| DH5α λ*pir* | $F^-$ *endA1 hsdR17* ($r^-m^+$) *supE44 thi-1 recA1 gyrA relA1* Δ(*lacZYA-argF*)*U189* Φ*80lacZΔM15* λ*pir* | (*36*) |
| S17-1 λ*pir* | *zxx*::RP4 2-($Tet^r$::Mu) ($Kan^r$::Tn7) λ*pir* | (*37*) |

| | | |
|---|---|---|
| | *recA1 thi pro hsdR* ($r^-m^+$) | |
| Plasmids | | |
| Designation | Relevant characteristics | Reference / source |
| pCR2.1 | Cloning vector | Life Technologies |
| pWSK29 | *ori*(pSC101) $Carb^r$ | (*38*) |
| pWSK129 | *ori*(pSC101) $Kan^r$ | (*38*) |
| pRDH10 | *ori*(R6K) *mobRP4 sacRB* $Tet^r$ $Cm^r$ | (*39*) |
| pMOA1 | *moaA* gene under control of its native promoter in pWSK29 | This study |
| pNARG1 | *narG* gene under control of its native promoter in pWSK29 | This study |
| pSW172 | *ori*(R101) *repA101ts* $Carb^r$ | This study |
| pSW225 | Upstream and downstream regions of the *narG* gene in pRDH10 | This study |
| pSW224 | Upstream and downstream regions of the *napA* gene in pRDH10 | This study |
| pSW237 | Upstream and downstream regions of the *narZ* gene in pRDH10 | This study |
| pSW248 | Upstream and downstream regions of the *moaA* gene in pRDH10 | This study |
| pSW268 | *napA* gene with upstream and downstream regions in pRDH10 | This study |

**Supplementary Table 2: Primers used in this study**

| Targeted mutagenesis and complementation constructs | | |
|---|---|---|
| Purpose | | Sequence* |
| Deletion of *moaA* | | 5'-GCATGCCACTTTCAGCAGATTACG-3'<br>5'-GTATCTAGACCAGATACGGGAGGCG-3'<br>5'-TGGTCTAGAGGCTAAAACGTCAAAAGG-3'<br>5'-GTCGACGCACGGTTTCCGCTTTGG-3' |
| Deletion of *narG* | | 5'-GCATGCCCGTAAACTGACAATGG-3'<br>5'-GAGTCTAGAGTGGGAGCCTGTCGG-3'<br>5'-GCGTCTAGAGTACAGGAGAGCGTAAAATG-3'<br>5'-GCATGCTCTCAGACTTACCGC-3' |
| Deletion and restoration of *napA* | | 5'-GGATCCGCGTTAATGATGGAAGG-3'<br>5'-GAGTCTAGATTCATGGTGTTTCCTCACC-3'<br>5'-GCGTCTAGAGGAGAAGGTGTAAGC-3'<br>5'-GGATCCGCCAACGGTGACTG-3' |
| Deletion of *narZ* | | 5'-GTCGACCTGTCTTACAACGCC-3'<br>5'-GAGTCTAGAGCTCCAGGAATGAATTGG-3'<br>5'-GCGTCTAGAAGGAGGCGAAAAAATG-3'<br>5'-GCATGCAGGAACACTTCGCAC-3' |
| Complementation of *moaA* | | 5'-CTCGAGATAGTTTCCTGGAGTCAG-3'<br>5'-AAGCTTCCTTTTGACGTTTTAGCC-3' |
| Complementation of *narG* | | 5'-CTCGAGCCGTAAACTGACAATGG-3'<br>5'-CTCGAGTGCCGACTTGTGAACG-3' |
| Quantitative real-time RT–PCR | | |
| Organism(s) | Target | Sequence |

| | | |
|---|---|---|
| | gene | |
| *Mus musculus* | *Gapdh* | 5'-TGTAGACCATGTAGTTGAGGTCA-3'<br>5'-AGGTCGGTGTGAACGGATTTG-3' |
| *Mus musculus* | *Kc* | 5'-TGCACCCAAACCGAAGTCAT-3'<br>5'-TTGTCAGAAGCCAGCGTTCAC-3' |
| *Mus musculus* | *Nos2* | 5'-CCAGCCTTGCATCCTCATTGG-3'<br>5'-CCAAACACCAAGCTCATGCGG-3' |
| *Mus musculus* | *Tnf* | 5'-TTGGGTCTTGTTCACTCCACGG-3'<br>5'-CCTCTTTCAGGTCACTTTGGTAGG-3' |
| *Eubacteria* | 16S<br>rRNA | 5-ACTCCTACGGGAGGCAGCAGT-3<br>5-ATTACCGCGGCTGCTGGC-3 |
| *Enterobacteriaceae* | 16S<br>rRNA | 5'-GTGCCAGCMGCCGCGGTAA-3'**<br>5'-GCCTCAAGGGCACAACCTCCAAG-3' |
| Genotyping of *Il10*$^{flox/flox}$ *Cd4-cre* mice | | |
| *Mus musculus* | *Il10* | 5'-CCAGCATAGAGAGCTTGCATTACA-3'<br>5'-GAGTCGGTTAGCAGTATGTTGTCCAG-3' |
| bacteriophage P1 | *Cre* | 5'-ACGACCAAGTGACAGCAA-3'<br>5'-CTCGACCAGTTTAGTTACCC-3' |

* Restriction enzyme sites are underlined

** M: A or C

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