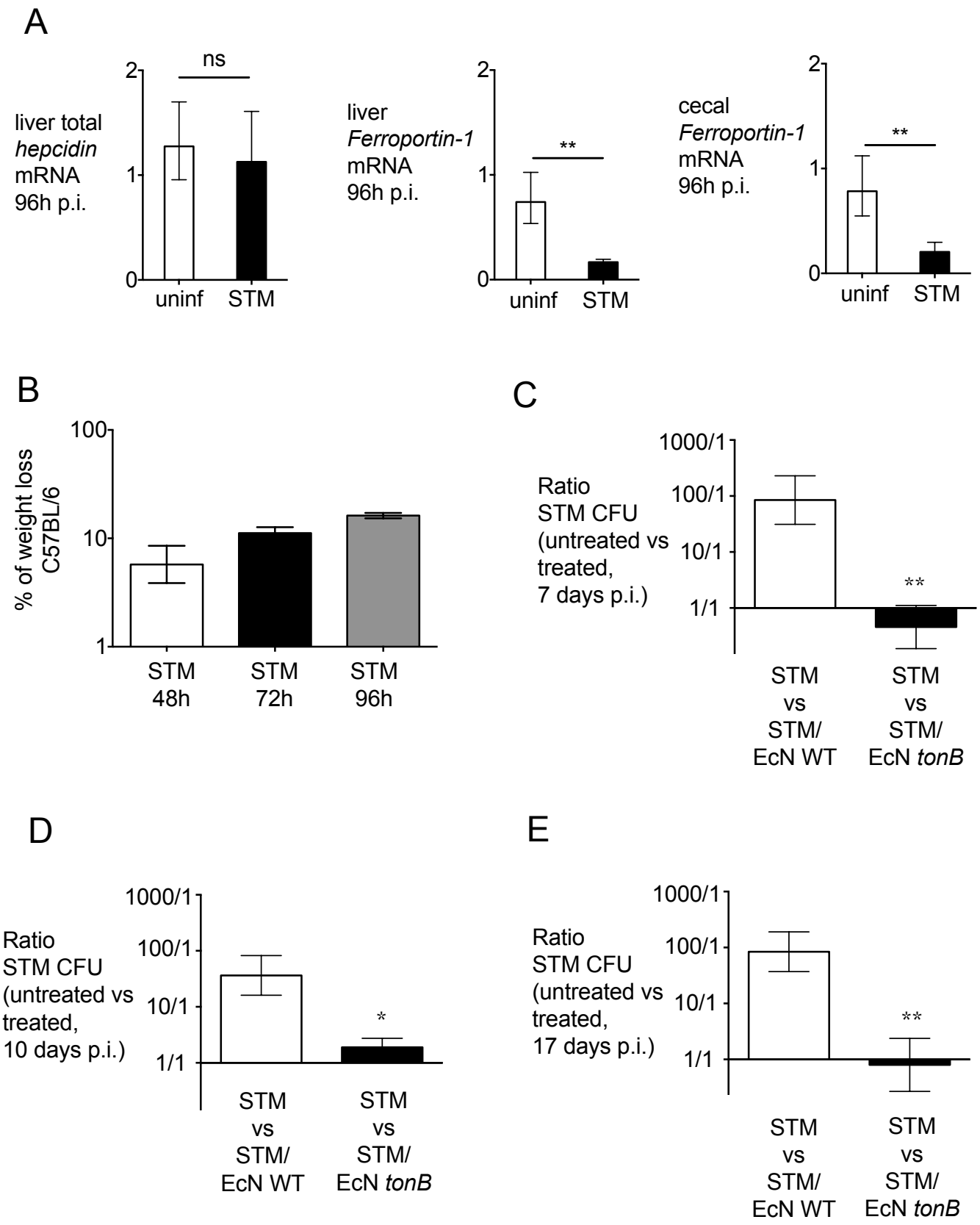
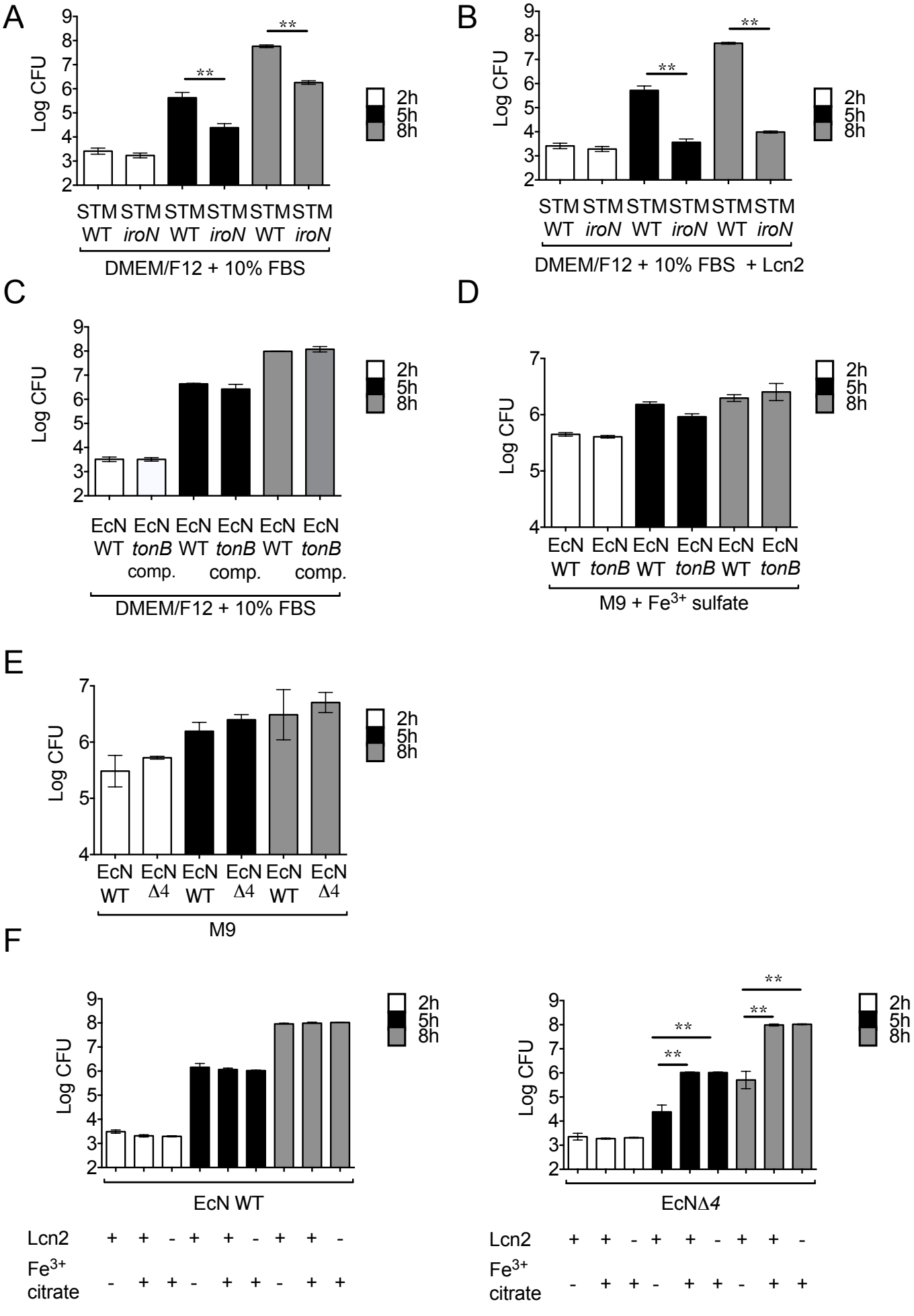
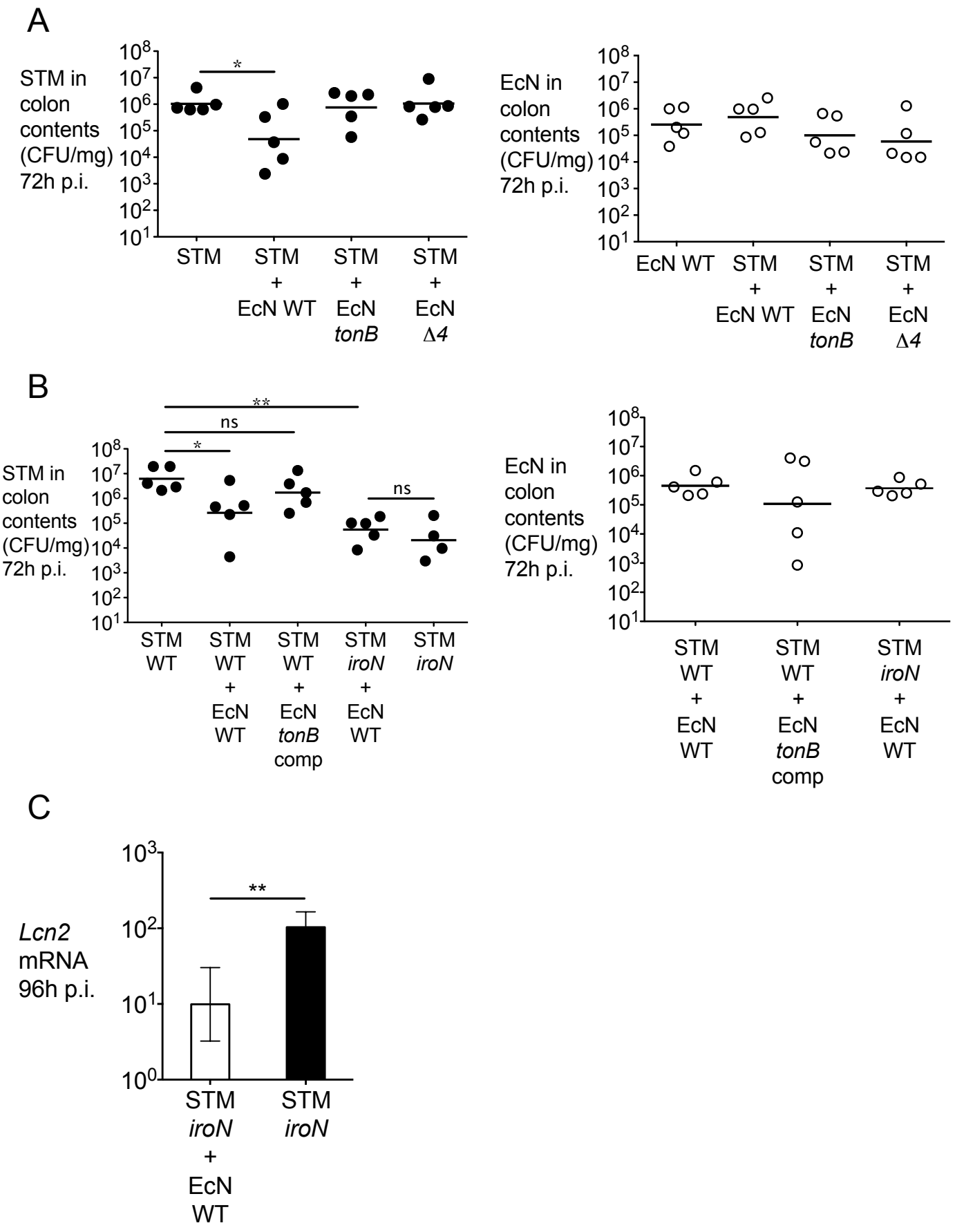


Supplementary Figure 1 - Related to Fig 1

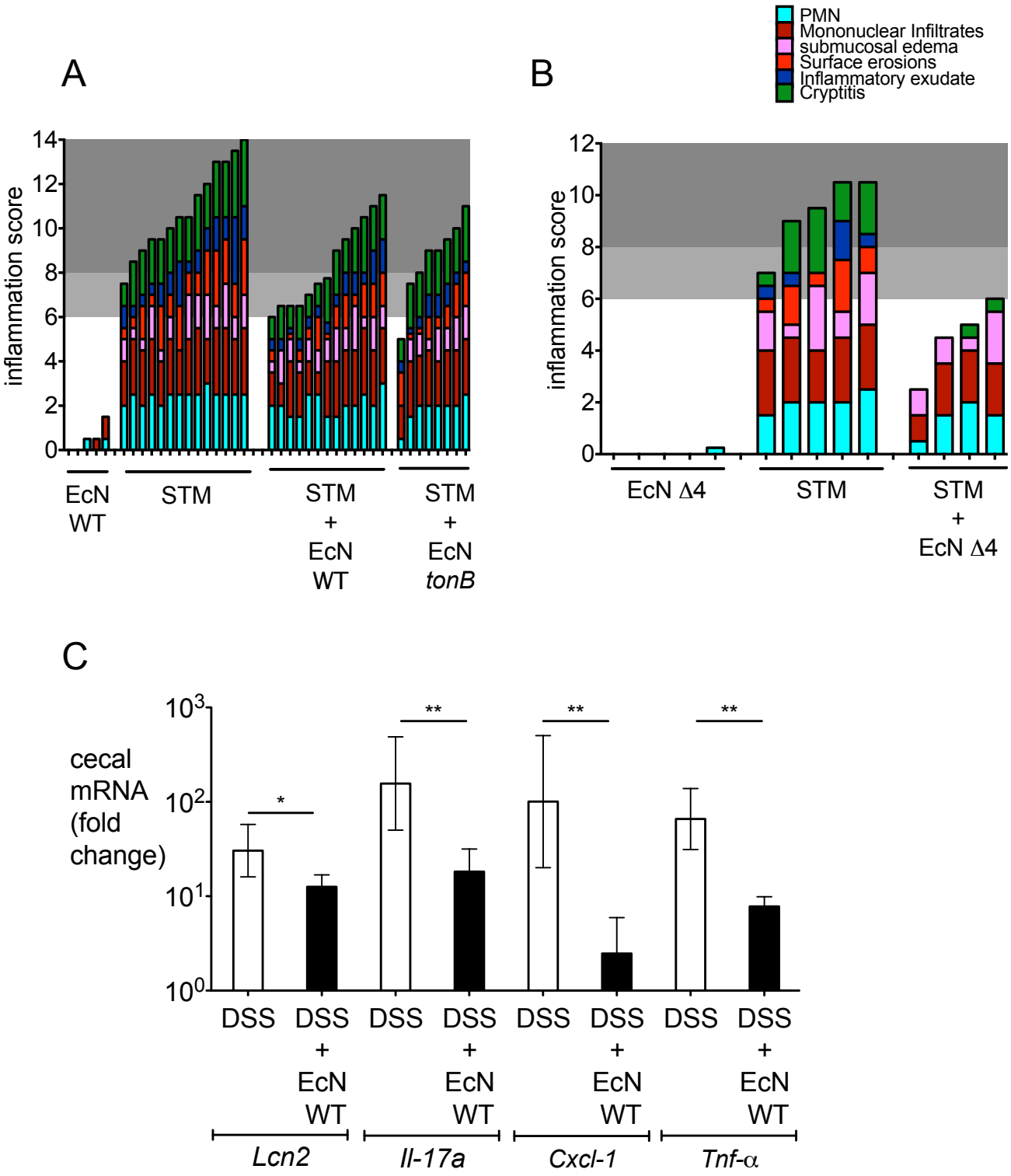
Supplemental Figure 2



Supplementary Figure 2 – Related to Fig 3

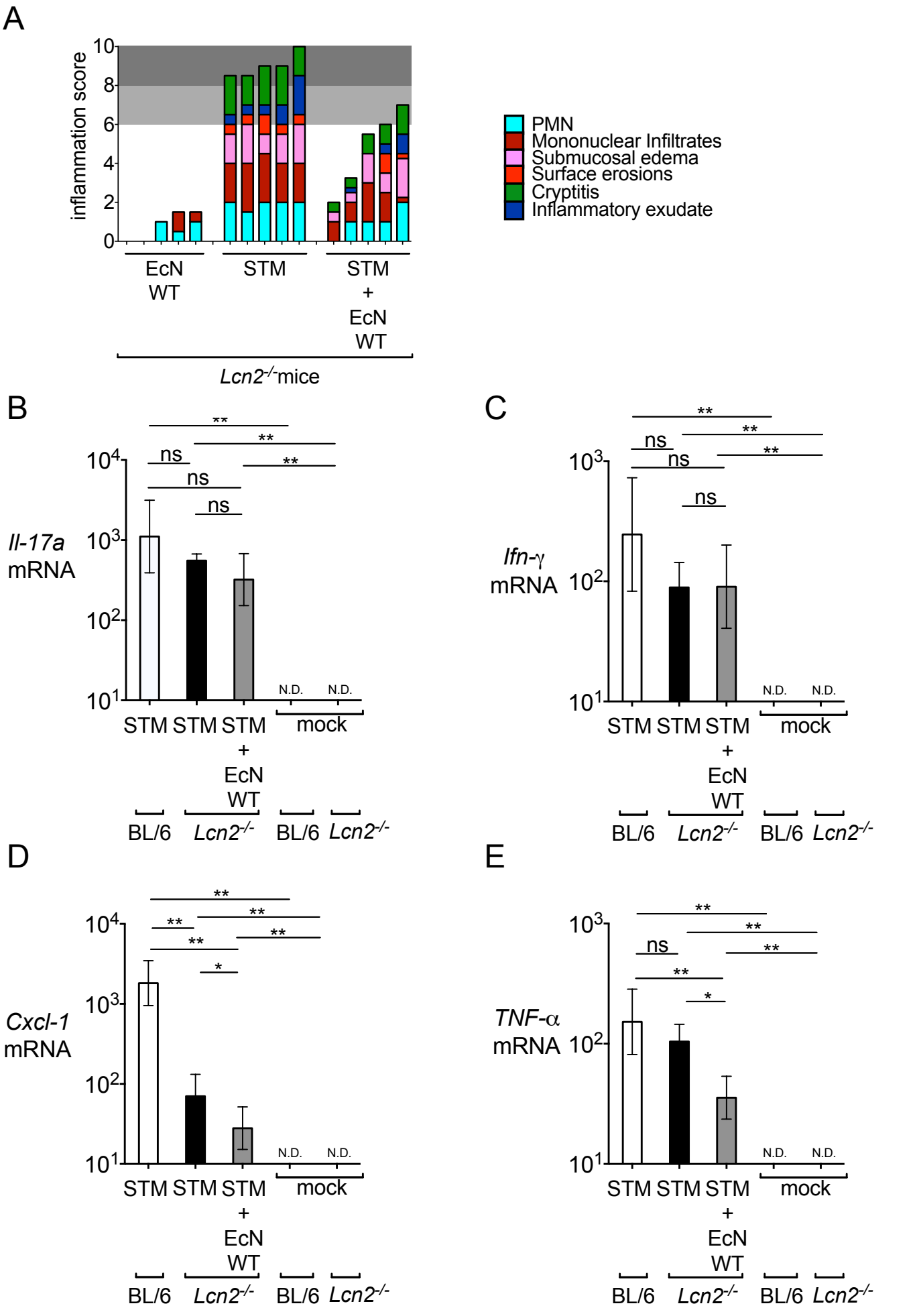


Supplementary Figure 3 – Related to Fig 4



Supplementary Figure 4 – Related to fig 5

Supplemental Figure 5



Supplementary Figure 5 – Related to fig 6

Probiotic Bacteria Reduce *Salmonella* Typhimurium Intestinal Colonization by Competing for Iron

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Supplementary information

Supplementary Figure 1 (Related to Figure 1)

Probiotic *E. coli* Nissle 1917 reduces *S*. Typhimurium fecal shedding.

(**A**) Transcript levels of total *hepcidin and ferroportin-1* were determined in the ceca and livers of mice uninfected (white bars) or infected with *S*. Typhimurium (black bars) 96h post infection. Data are expressed as fold-increase over uninfected mice. Bars represent geometric means ± standard deviation. (**B**) Effect of *S.* Typhimurium infection on weight loss in C57BL/6 mice. Percentage weight loss in streptomycin-pretreated mice infected with 10^9 CFU *S.* Typhimurium at 48h (white bar), 72h (black bar) and 96h (gray bar) post infection. Bars represent geometric means ± standard deviation. (**C, D, E**) Ratio of the colony forming units (CFU) recovered from the fecal samples of 129X1SvJ mice infected with *S*. Typhimurium that were untreated in comparison with mice that were treated with one dose of *E. coli* Nissle wild-type or *tonB* mutant three days after infection. The ratios at day 7 (**C**), 10 (**D**) and 17 (**E**) after infection are shown. Bars represent geometric means ± standard deviation. A significant difference is indicated by * (*P* value $\leq$ 0.05) or ** (*P* value $\leq$ 0.01). uninf=uninfected; STM=*S*. Typhimurium; EcN= *E. coli* Nissle.

Supplementary Figure 2 (Related to Figure 3)

Growth of *S*. Typhimurium and *E. coli* Nissle strains in iron-limited media. Growth of *S*. Typhimurium wild-type and *iroN* mutant in DMEM/F12 supplemented with 10% fetal bovine serum in the absence (**A**) or presence (**B**) of 1μg/ml lipocalin-2 (Lcn2). (**C**) Growth of *E. coli* Nissle wild-type or *tonB* mutant complemented with *tonB in trans* in DMEM/F12 supplemented with 10% fetal bovine serum. (**D**) Growth of a 1:1 mixture of *E. coli* Nissle wild-type and *tonB* mutant in M9 minimal media supplemented with 200μM iron (III) sulfate (Fe^{3+} sulfate). (**E**) Growth of a 1:1 mixture of *E. coli* Nissle wild-type and Δ4 mutant in M9 minimal media. (**F**) Growth of *E. coli* Nissle wild-type (left panel) and *E. coli* Nissle Δ4 (right panel) in DMEM/F12 supplemented with 10% fetal bovine serum in presence of 1μg/ml lipocalin-2 (Lcn2) and/or 1mM iron (III) citrate (Fe^{3+} citrate). Bacteria were enumerated by plating serial dilutions at 2h, 5h, and 8h after inoculation. Data represents the geometric mean ± standard error. A significant difference is indicated by * (*P* value ≤ 0.05) or ** (*P* value ≤ 0.01). STM=*S*. Typhimurium; EcN=*E. coli* Nissle

Supplementary Figure 3 (Related to Figure 4)

***E. coli* Nissle 1917 requires iron uptake systems to reduce *S*. Typhimurium intestinal colonization.**

(**A**) C57BL/6 mice were infected with *S*. Typhimurium alone or co-administered with either wild-type *E. coli* Nissle wild-type, the *tonB* mutant or the *iroN fuyA iutA chuA* mutant (Δ4). CFU in colonic contents were enumerated at 72h after infection. *S*. Typhimurium (black circles) and *E. coli* Nissle (white circles) counts are shown. Each circle represents a value from an individual mouse. A representative experiment of n=2 is shown. STM=*S*. Typhimurium; EcN=*E. coli* Nissle. (**B**) C57BL/6 mice were infected with the indicated strains. Colony forming units (CFU) in colonic contents were enumerated at 72h after infection. *S*. Typhimurium (black circles) and *E. coli* Nissle (white circles) counts are shown. Each circle represents a value from an individual mouse. A representative experiment of n=2 is shown. STM=*S*. Typhimurium; EcN=*E. coli* Nissle. (**C**) Transcript levels of *Lcn*2 were determined in the ceca of C57BL/6 mice infected with either *S*. Typhimurium *iroN* mutant (black bar) or a mixture of *S*. Typhimurium *iroN* mutant and wild-type *E. coli* Nissle (white bars) 96h post infection. Data are expressed as fold-increase over mock-infected mice. Bars represent the geometric mean ± standard deviation. Significant difference is indicated by * (*P* value ≤ 0.05) or ** (*P* value ≤ 0.01). STM=*S*. Typhimurium; EcN=*E. coli* Nissle.

Supplementary Figure 4 (Related to Figure 5)

***E. coli* Nissle 1917 ameliorates intestinal inflammation**

(**A**) Detailed histopathology scoring of cecal samples four days after infection of C57BL/6 mice administered either *E. coli* Nissle wild-type, *S*. Typhimurium, or a 1:1 mixture of *S*. Typhimurium and *E. coli* Nissle wild-type or *tonB* mutant. (**B**) Detailed histopathology scoring of cecal samples four days after infection of mice administered either *E. coli* Nissle Δ4, *S*. Typhimurium, or a 1:1 mixture of *E. coli* Nissle Δ4 and *S*. Typhimurium. Each stacked column represents an individual mouse. The light grey box indicates moderate inflammation, while the dark grey box indicates severe inflammation. STM=*S*. Typhimurium; EcN=*E. coli* Nissle. (**C**) Transcript levels of *Lcn2, Il-17a*, *Cxcl-1* and *Tnf-*α in the cecum of C57BL/6 mice administered *E.coli* Nissle or mock during DSS-induced colitis. Cecal tissue was collected from control- and EcN-administered mice 6 days following DSS administration. Transcript levels were quantified by real-time PCR analysis and were normalized over untreated mice. Values represent the geometric mean ± standard deviation for each group. Significant difference is indicated by * (*P* value $\leq$ 0.05) or ** (*P* value $\leq$ 0.01). DSS=Dextran sodium sulfate.

Supplementary Figure 5 (related to Figure 6)

Host response to *Salmonella* infection and *E. coli* Nissle administration in *Lcn2*$^{-/-}$ mice

(**A**) Detailed histopathology scoring of cecal samples four days after infection of *Lcn2*$^{-/-}$ mice administered either *E. coli* Nissle wild-type, *S*. Typhimurium, or a 1:1 mixture of *S*. Typhimurium and *E. coli* Nissle wild-type. Each stacked column represents an individual mouse. The light grey box indicates moderate inflammation, while the dark grey box indicates severe inflammation. (**B, C, D, E**) Transcript levels of *Il-17a* (**B**), *IFN-*γ (**C**), *Cxcl-1* (**D**), and *Tnf-*α (**E**) were determined in the ceca of C57BL/6 mice infected with *S*. Typhimurium or in the ceca of *Lcn2*$^{-/-}$ mice infected with either *S*. Typhimurium or a mixture 1:1 of *S*. Typhimurium and wild-type *E. coli* Nissle (white bars) 96h post-infection. Data are expressed as fold-increase over mock-infected mice. Bars represent the geometric mean ± standard deviation. Significant difference is indicated by * (*P* value $\leq$ 0.05) or ** (*P* value $\leq$ 0.01). BL/6=C57BL/6 mice; *Lcn2*$^{-/-}$ = *Lcn2*$^{-/-}$ mice; STM=*S*. Typhimurium; EcN=*E. coli* Nissle.

Table S1 (Related to Figure 1). Strains and Plasmids used in this study

Designation	Genotype	Source or Reference
STRAINS (*Escherichia coli*)		
CC118 λ_{pir}	F- *araD*139 Δ(*ara, leu*)7697 Δ*lac*X74 *phoA*Δ20 *galE galK thi rpsE rpoB argE*^{am} recA1 λ_{pir}	(Herrero et al., 1990)
DH5αMCR	F- *mcrA* Δ (*mrr-hsdRMS-mcrBC*) Φ80d*lacZ*M15 (*lacZYA-argF*)*U169 deoR recA1 endA1 phoA supE44*l- *thi-1 gyrA*96 *relA*1	Gibco BRL
One Shot® TOP10 Chemically Competent *E. coli*	F- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara, leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G l	Invitrogen
S17-1 λ_{pir}	F- *recA thi pro* rK- mK+ RP4:2-Tc::*Mu*Km Tn7 λ_{pir}	(Herrero et al., 1990)
EcN	*E. coli* Nissle 1917 wild-type	(ArdeyPharm, Germany)
ED03	EcN Nal^{R}	This study
ED50	EcN Δ*tonB*(+230 to +1033)::*cat*)	This study
ED60	ED50 (pWSK29::*tonB*(-262 to + 801), Ap^{R}, Cm^{R}	This study
MG1655	*E. coli* K-12	ATCC 700926
MPN101	ED03 Δ*iroN*(-46 to +817)::KSAC	This study
MPN104	ED03 Δ*iroN*(-46 to +817)::KSAC Δ*fyuA*(-14 to +1969)::scar Δ*iutA*(+4 to +2195)::scar	This study
MPN105	ED03 Δ*iroN*(-46 to +817)::KSAC Δ*fyuA*(-14 to +1969)::scar Δ*iutA*(+4 to +2195)::scar Δ*chuA*(+68 to +1795)::*tetRA*	This study
RJ04	ED03 Δ*iroN*(-46 to +817)::KSAC Δ*fyuA*(-14 to +1969)::scar	This study
STRAINS (*S.* Typhimurium)		
IR715	ATCC 14028 Nal^{R}	(Stojiljkovic et al., 1995)
AJB52	IR715 *iroN*::pGP704	(Bäumler et al., 1998)

PLASMIDS		
pACYomega	pHP45 omega derivative, Strep^R, Cm^R	Takeshi Haneda and Andreas Bäumler
pCR2.1	TOPO Cloning Vector (Carb^R, Kan^R)	Invitrogen
pGP704	*oriR6K* Ap^R	(Miller and Mekalanos, 1988)
pHC100	pRDH10::FR1::FR2 *chuA*	This study
pHC101	pHC100::*tetRA*	This study
pHP45omega	Strep^R, Carb^R	(Prentki and Krisch, 1984)
pKD3	Ap^R, Cm^R	(Datsenko and Wanner, 2000)
pKD46	Ap^R, temperature-sensitive, λ Red recombinase system	(Datsenko and Wanner, 2000)
pMP101	pGP704::FR2 *iroN*	This study
pMP102	pMP101::FR1 i*roN*	This study
pMP103	pMP102::KSAC	This study
pMP106	pRDH10::FR1::FR2 *iutA*	This study
pMP107	pMP106::*tetRA*	This study
pRDH10	*oriR6K*, Cm^R, Tc^R, *sacRB*	(Kingsley et al., 1999)
pRO4	pRDH10::FR1::FR2 *fyuA*	This study
pRO5	pRO4::*tetRA*	This study
pSPN23	*tetRA* cassette	(Raffatellu et al., 2009)
pUC4-KSAC	Kanamycin cassette KSAC	(Barany, 1985)
pWSK29	Ap^R, MCS *lacZ*	(Wang and Kushner, 1991)

Table S2 (Related to figure 3). Primers used in this study

Designation	**Purpose**	**Sequence (5' to 3')**
1	Flanking region 1 of *iroN* construct	AGCGTCGTCGTCGACCCTGACTTGTTG AAAGCAG
2	Flanking region 1 of *iroN* construct	TTAGATTTCTAGAGCTCACATCCTTGCC

		AG
3	Flanking region 2 of *iroN* construct	GTACTACGAGCTCGTTACCGCATCATTCTG
4	Flanking region 2 of *iroN* construct	TAGAATTCAGCGAAACCGATACCTC
5	Flanking region 1 of *iutA* construct	TATGCTGGATCCCTGTGGCTGGTAACTCAG
6	Flanking region 1 of *iutA* construct	GGCATATCTAGAATCATTATTCCTCCATACGGGCG
7	Flanking region 2 of *iutA* construct	GGCATATCTAGATGTTCTGACGGGGATGC
8	Flanking region 2 of *iutA* construct	TATGCTGGATCCTTGGGTGACCTCAAATAGAC
9	Flanking region 1 of *fyuA* construct	AGTGCAGGATCCACTTCAGAAAGGTGACGC
10	Flanking region 1 of *fyuA* construct	GGCATATCTAGATGTAAGACGGCGAAACG
11	Flanking region 2 of *fyuA* construct	GGCATATCTAGACAGGTCAATATGGGTCG
12	Flanking region 2 of *fyuA* construct	AGTGCAGGATCC GGGAGTTCTTCAGGCTG
13	Flanking region 1 of *chuA* construct	TATGGGATCCCGC TTGCTCCTGTTCAC
14	Flanking region 1 of *chuA* construct	TGCTTCTAGAGGT CGCCGCAAGGCATC
15	Flanking region 2 of *chuA* construct	GACCTCTAGAAGCAGTTTGATTTTCAGGAGC
16	Flanking region 2 of *chuA* construct	TTTAGGATCCGAGTTGTTTCCAGTAACC
H1P1truncatonBpKD3	Flanking region 1 of *tonB* construct	CGATCCCTGAACCGCCAAAAGAAGCTCCGGTGGTCATTGA GTGTAGGCTGGAGCTGCTTC
H2P2MobltonBpKD3	Flanking region 2 of *tonB* construct	GGATATTCACCACAATCCCACTGCCTGGCTTACCCGGCTC ATGGGAATTAGCCATGGTCC
Check deletion Fw	PCR confirmation 1 target sequence	GCTATCAGCTTTGTCTTGA
C1	PCR confirmation 1 target sequence	TTATACGCAAGGCGACAAGG

C2	PCR confirmation 2, 3 target sequence	GATCTTCCGTCACAGGTAGG
FR2 *tonB* Rv	PCR confirmation 2 target sequence	GCGGTTGATCCCGAAGGAA
FR2 far *tonB* Rv	PCR confirmation 3 target sequence	AGTGACCGTGCGGGTTGAA
FwSalIcheck deltonB	Forward complementation primer for *tonB*	TTGGTCGACGCTATCAGCTTTGTCTTGA
RvEcoRIFR2 tonB	Reverse complementation primer for *tonB*	TTGGAATTCGCGGTTGATCCCGAAGGAA

Table S3 (Related to Figure 5). Quantitative Real-Time PCR Primers

Species	**Target**	**Primer Pairs**
Mus musculus	*Lcn2*	5'-ACATTTGTTCCAAGCTCCAGGGC-3' 5'-CATGGCGAACTGGTTGTAGTCCG-3'
Mus musculus	*Il-17*	5'-GCTCCAGAAGGCCCTCAGA-3' 5'-AGCTTTCCCTCCGCATTGA-3'
Mus musculus	*Cxcl-1*	5'-TGCACCCAAACCGAAGTCAT-3' 5'-TTGTCAGAAGCCAGCGTTCAC-3'
Mus musculus	*β-actin*	5'-GGCTGTATTCCCCTCCATCG-3' 5'-CCAGTTGGTAACAATGCCATGT-3'
Mus musculus	*TNF-α*	5'-CATCTTCTCAAAATTCGAGTGACAA-3' 5'-TGGGAGTAGACAAGGTACAACCC-3'
Mus musculus	*IFN-γ*	5'-TCAAGTGGCATAGATGTGGAAGAA-3' 5'- TGGCTCTGCAGGATTTTCATG-3'
Mus musculus	*total hepcidin*	5'-AGAGCTGCAGCCTTTGCAC-3' 5'-GAGGTCAGGATGTGGCTCTA-3'
Mus musculus	*ferroportin 1*	5'-TGGATGGGTCCTTACTGTCTGCTAC-3' 5'-TGCTAATCTGCTCCTGTTTTCTCC-3'

Supplemental Experimental Procedures

Bacterial Strains and Culture Conditions

IR715 is a fully virulent, nalidixic acid-resistant derivative of *S*. Typhimurium wild-type isolate ATCC 14028 (Stojiljkovic et al., 1995). *Escherichia coli* Nissle 1917 (also *E. coli* Nissle or EcN) was kindly provided by Ardeypharm (Germany). Construction of *E. coli* Nissle derivatives carrying mutations in *tonB,* or in *iroN iutA fyuA chuA*, and the complemented *tonB* strain is described below in detail. A spontaneous nalidixic acid-resistant (Nal^R) strain was used to construct the *E. coli* Nissle strain lacking the four iron-acquisition system receptors IroN, IutA, FyuA and ChuA. A complete list of strains, plasmids, and primers used for cloning and strain construction is provided in **Tables S1 and S2**. All strains were grown aerobically at 37°C in LB broth, unless otherwise noted.

Allelic Exchange Deletion of *iroN in E. coli* Nissle

To construct a strain carrying a deletion of *iroN,* DNA regions flanking upstream the *iroN* gene (flanking region 1) were amplified with primers 1 and 2 of ED03 (**Table S2**). Primers 3 and 4 were used to PCR amplify the region downstream of *iroN* (flanking region 2). Reaction products of the predicted size were ligated using the rapid DNA ligation kit (Roche) and cloned into pCR2.1 vector (TOPO TA cloning kit; Invitrogen), into *E. coli* TOP10 and plated on LB+carbenicillin (Carb) + Xgal. White colonies were screened by *EcoR*I digestion for the appropriate length of linearized plasmid construct. Positive clones were sequence confirmed by using M13 forward and M13 reverse universal primers. Flanking region 2 cassette was digested out using *SacI* and *EcoRI*

double-digestion, ligated into *SacI* and *EcoRI* double-digested pGP704 (Miller and Mekalanos, 1988), heat shocked into *E. coli* CC118 λ_{pir}, and plated on LB+Carb, generating the plasmid pMP101. Flanking region 1 cassette was digested out of pCR2.1 using *Sal*I and *Xba*I double-digestion, ligated into *Sal*I and *Xba*I double-digested pMP101, heat shocked into *E. coli* CC118 λ_{pir}, and plated on LB+ Carb, generating pMP102. The KSAC cassette from pUC4-KSAC (Barany, 1985) was excised using *Sac*I digestion and ligated into the compatible *Sac*I site of pPM102, to give rise to plasmid pMP103, which was then purified and heat shocked into *E. coli* S17-1 λ_{pir}. Plasmid pMP103 was maintained in *E. coli* strain S17-1 λ_{pir} and then introduced by conjugal transfer into ED03. Transconjugants were selected based on their growth in kanamycin (Kan) and colonies with double crossover events were screened for by loss of Carb resistance. The chromosomal construct was confirmed using Southern blot analysis amplifying a probe designed to flanking region 1 on MP102. The PCR product was ligated into pCR2.1 digested with *EcoR*I and used to confirm the deletion of *iroN* (data not shown). The resulting strain (*E. coli* Nissle Nal^R, Δ*iroN*(-46 to +817)::KSAC) was termed MPN101.

Allelic Exchange Deletion of *fyuA* in MPN101

Primers 9 and 10 were used to PCR amplify the region upstream of *fyuA* (flanking region 1) of *E. coli* Nissle. Primers 11 and 12 were used to PCR amplify the region downstream of *fyuA* (flanking region 2). The PCR products were ligated into pCR2.1 using the TOPO TA cloning kit (Invitrogen), heat shocked into *E. coli* TOP10 and plated on LB+Carb+Xgal. White colonies were screened by *EcoR*I digestion for the appropriate

length of linearized plasmid construct. Positive clones were sequence confirmed by using M13 forward and M13 reverse universal primers. Flanking region 1 and 2 cassette was digested out using *BamHI* and *XbaI* double-digestion, ligated into *BamH*I digested pRDH10 suicide vector (Kingsley et al., 1999), generating the pRO4, heat shocked into *E. coli* CC118 λ_{pir}, and plated on LB+Cm. The Tetracycline cassette *tetRA* was inserted into the compatible *Xba*I site of pRO4, generating pRO5 plasmid (**Table S1**). pRO5 was purified and heat shocked into *E. coli* S17 λ_{pir}, then conjugated into MPN101. Transconjugants and double crossover events were selected and screened. Confirmation of mutant was performed using Southern blot analysis, as described before. The resulting strain (*E. coli* Nissle Nal^R, Δ*iroN*(-46 to +817)::KSAC, Δ*fyuA*(-14 to +1969)::*tetRA*), was termed RJ02. Tetracycline-sensitive colonies of RJ02 strain were screened using the sucrose selection to make clean deletion of the *tetRA* cassette, giving rise to the strain RJ04 (*E. coli* Nissle Nal^R, Δ*iroN*(-46 to +817)::KSAC, Δ*fyuA*(-14 to +1969):: scar).

Allelic Exchange Deletion of *iutA in* RJ04

Primers used are detailed in **Table S2**. Primers 5 and 6 were used to PCR amplify the region upstream of *iutA* (flanking region 1) of *E. coli* Nissle. Primers 7 and 8 were used to PCR amplify the region downstream of *iutA* (flanking region 2). The PCR products were ligated into pCR2.1 using the TOPO TA cloning kit (Invitrogen), heat shocked into *E. coli* TOP10 and plated on LB+Carb+Xgal. White colonies were screened by *EcoRI* digestion for the appropriate length of linearized plasmid construct. Positive clones were sequence confirmed by using M13 forward and M13 reverse universal primers. Flanking

region 1 and 2 cassette was digested out using *BamH*I and *Xba*I double-digestion, ligated into *BamHI* digested pRDH10 generating the pMP106, heat shocked into *E. coli* CC118 λ_{pir}, and plated on LB+chloramphenicol (Cm). The Tetracycline cassette *tetRA* was cloned from pSPN23 and was inserted into the compatible *Xba*I site of pPM106, generating pMP107 (**Table S1**). pMP107 was purified and heat shocked into *E. coli* S17 λ_{pir}, and plated on LB-Cm, then conjugated into RJ04. Exconjugant colonies that were resistant to kanamycin but sensitive to chloramphenicol were identified, and the deletion of the *iutA* gene was confirmed by Southern hybridization by using a specific probe derived from the FR1 of *iutA* construct pMP106. The resulting strain (*E. coli* Nissle Nal^R, Δ*iroN*(-46 to +817)::KSAC, Δ*fyuA*(-14 to +1969):: scar, Δ*iutA*(+4 to +2195)::*tetRA*) was termed MPN103. Tetracycline-sensitive colonies of MPN103 strain were screened using the sucrose selection to make clean deletion of the *tetRA* cassette, giving rise to the strain MPN104 (*E. coli* Nissle Nal^R, Δ*iroN*(-46 to +817)::KSAC, Δ*fyuA*(-14 to +1969)::scar, Δ*iutA*(+4 to +2195)::scar).

Allelic Exchange Deletion of *chuA* in MPN104

To clone the flanking regions of *chuA*, three rounds of PCR were performed: first round PCR amplified flanking region 1 of *chuA*; second round PCR amplified flanking region two of *chuA* gene; third round PCR amplified, in tandem, both flanking regions with an engineered *XbaI* site in the middle and two *BamHI* sites flanking 5' and 3' end of the tandem flanking regions. PCR product was ligated and transformed into pCR2.1-TOPO, colonies were selected via blue-white screening method, and white colonies were sequenced with M13 universal primers. Plasmids with the correct sequences

were isolated and digested to release then entire in-tandem flanking region to later be ligated into the *BamHI* site of pRDH10. Once the in-tandem flanking region was ligated into pRDH10 giving rise to the pHC100 constructs were transformed into *E. coli* CC118 λ_{pir} and stocked. Finally, the *tetRA* cassette was cloned into the XbaI site of pRDH10 found in between the *chuA* flanking regions generating the pHC101 plasmid. pHC101 was then transformed into *E. coli* S17 λ_{pir}, giving rise to the strain HC012, which was and coniugated with MPN104. A 544 base pair region upstream of the deleted *chuA* gene will be used to perform Southern blot confirmation of putative Δ*chuA* mutants (data not shown). The resulting strain (*E. coli* Nissle Nal^R, Δ*iroN*(-46 to +817)::KSAC, Δ*fyuA*(-14 to +1969)::scar, Δ*iutA*(+4 to +2195)::scar*),* Δ*chuA*(+68 to +1795)::*tetRA*) was termed MPN105 (*E. coli* Nissle Δ4).

Construction of *E. coli* Nissle *tonB* mutant

Isogenic mutant in *E. coli* Nissle carrying a deletion in *tonB* was constructed using the lambda red recombinase system (Datsenko and Wanner, 2000). Briefly, primers (H1P1tructonBpKD3 and H2P2MobltonBpKD3) homologous to sequences within the 5′ and 3′ ends of the target regions were designed (H1 and H2 primers, respectively; **Table S2**) and were used to replace the C-terminal region (+230 to +1033) with a nonpolar chloramphenicol resistance cassette derived from plasmid pKD3, respectively (Datsenko and Wanner, 2000). Chloramphenicol was used for selection of the deletion construct. The resulting strain was termed ED50 (*E. coli* Nissle *tonB*). To verify whether the chloramphenicol resistance cassette recombined within the target gene site, primers that flank target sequence were designed (**Table S2**). Both wild-type and mutant gene

sequences were amplified with genomic confirmation primers by PCR using *Phusion High Fidelity* DNA polymerase (New England Biolabs). Three PCRs were used to show that all mutants have the correct structure. Three reactions were done by using nearby locus-specific primers with the respective common test primer (C1, C2) to test for both new junction fragments (**Table S2**).

Complementation of *E. coli* Nissle *tonB* mutant

Polymerase chain reaction (PCR) was performed on the *tonB* gene intended for complementation using *Phusion High Fidelity* DNA polymerase. A region of 1063bp was amplified to be sure that all the regulatory elements were included. The primers FwSalIcheckdeltonB and RvEcoRIFR2tonB used are listed in **Table S2**. The PCR fragment was cloned into high copy number pCR-Blunt II using Invitrogen Zero Blunt TOPO PCR Cloning Kit's provided protocol. The plasmid vectors were subsequently transformed into One Shot® TOP10 Chemically Competent *E. coli* via heat-shock, according to the manufacturer's instructions. The transformations were plated onto Luria Bertani (LB) agar with kanamycin (50 µg/mL) to select for successful transformations. Individual colonies were selected and grown overnight at 37°C in LB- Kan 50. Individual pCR-BluntII TOPO vectors were then isolated using a QIAGEN QIAprep Spin Miniprep Kit according to the provided protocol. The plasmids were digested using endonucleases *SalI* HF and *EcoRI* HF, (New England Biolabs) which cut solely in the primer region, ensuring the restriction enzymes did not cut within the gene and disrupt functionality. The freshly cut fragments of interest were ligated into the multiple cloning site (MCS) of low copy number plasmid pWSK29 (Wang and Kushner, 1991) that has

also been double-digested with the corresponding restriction enzymes after treatment with Antarctic Phosphatase (New England Biolabs) to prevent pWSK29 from self-ligation. Ligation reactions were then transformed into One Shot® TOP10 Chemically Competent *E. coli*. The recombinant plasmid was submitted for sequencing using PCR primer M13F which binds near the MCS of pWSK29 to verify the gene of interest was present and in the correct orientation. The plasmid was extracted from One Shot® TOP10 *E. coli* and transformed by electroporation according to established protocol into ED50 mutant of interest, effectively restoring wild type like gene function. As a control, self-ligated pWSK29 vectors were similarly electroporated into the mutants.

Measurement of Iron in Fecal Samples by ICP-MS

C57BL/6 mice were treated with streptomycin and infected with *S*. Typhimurium wild-type or mock as described. At 96 hours post-infection, fecal pellets were collected with plastic forceps and placed in glass containers that were previously cleaned with nitric acid to remove metal contamination. The fecal pellets were then autoclaved to kill all bacteria. Sample analysis was performed by Applied Speciation (Bothell, WA) as previously described (Corbin et al., 2008; Liu et al.). Briefly, the fecal samples from 4 infected and 4 uninfected mice were digested by boiling in nitric acid and hydrochloric acid. The samples were then resuspended in water and analyzed by inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS). Aliquots of each sample are introduced into a radio frequency (RF) plasma where energy-transfer processes cause desolvation, atomization, and ionization. The ions were extracted from the plasma through a differentially-pumped vacuum interface and traveled through a

pressurized chamber (DRC) containing a specific reactive gas, which preferentially reacts with interfering ions of the same target mass to charge ratios (m/z). A solid-state detector detected ions transmitted through the mass analyzer, on the basis of their mass-to-charge ratio (m/z), and the resulting current was processed by a data handling system. The results were reported as mg of iron per kg of dry weight.

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