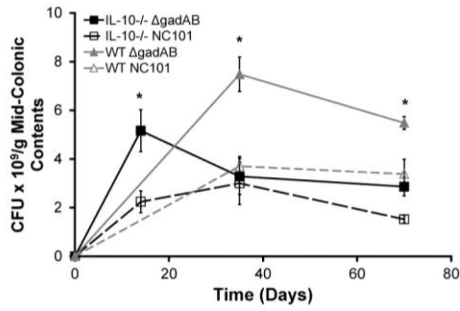
Supplementary Figures

S1



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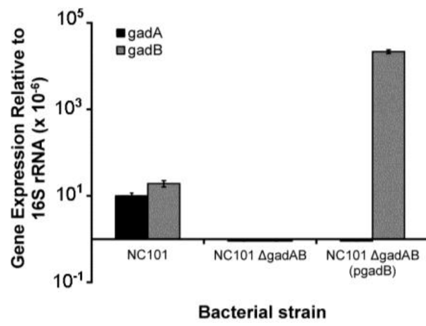

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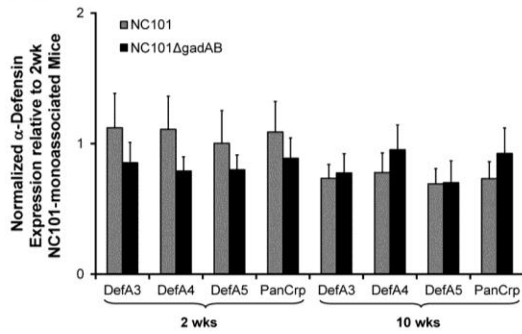

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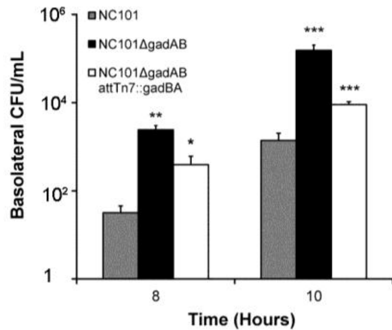

SUPPLEMENTARY FIGURE LEGENDS

Figure S1: Luminal bacterial concentrations in mid-colonic contents from monoassociated WT and *Il-10-/-* mice. Data are presented as mean ± SEM, n=5 mice/group, *p<.05 relative to mice with the same genetic background, but monoassociated with NC101.

Figure S2. *GadA* and *gadB* expression in cecal bacteria from monoassociated *Il-10-/-* mice. Real-time PCR was performed using bacterial RNA isolated from cecal contents of *Il-10-/-* mice monoassociated with the indicated bacterial strains for 5 weeks, and the specific primers gadA2 and gadB2 (Table S1). Results are normalized to 16S rRNA expression using the ΔCt method. Data are presented as mean ± SEM, n=4-6 samples per group.

Figure S3. DefA3, DefA4, DefA5 and Pan-Cryptdin expression in ileal tissues from monoassociated *Il-10-/-* mice. Real-time Taqman PCR was performed using RNA isolated from fragments of the ileum of *Il-10-/-* mice monoassociated with NC101 and NC101Δ*gadAB* for 2 and 10 weeks, and commercially-available α-defensin TaqMan primers (Life Technologies, Grand Island, NY). Results are normalized to murine β-actin expression and data are presented as mean ± SEM of fold change relative to 2 week NC101 monoassociated mice.

Figure S4. Bacterial translocation across intestinal epithelial cell monolayers. CFU/mL measured in media from the basolateral chamber of the culture at the indicated time intervals post infection with the indicated bacterial strains. Data are presented as mean ± SEM, n=5-6 samples per group. *p<.05, **p<.01 and ***p<.001 relative to NC101 infected epithelial cells.

22

23 **SUPPLEMENTARY TABLE**

24 Table S1. Sequences of oligonucleotides used in PCR reactions.

Primer Name	Organism	Sequence (5' → 3')
gadA2F	*Escherichia coli* NC101	GAAGCTGTTAACGGATTTCC
gadA2R	*Escherichia coli* NC101	GCGGAAATCGTTTTGACTCC
gadB2F	*Escherichia coli* NC101	GGTGTTCAACGTTGACTACC
gadB2R	*Escherichia coli* NC101	CCGGGCGGGAGAAGTTGA
gadAkoF	*Escherichia coli* NC101	TTTTTTATTGCCTTCAAATAAATTTAAGGAGTT CGAAATGGTGTAGGCTGGAGCTGCTTC
gadAkoR	*Escherichia coli* NC101	TTTTTACAACGTGATGTTATCAGGTGTGTTTAA AGCTGTTATGGGAATTAGCCATGGTCC
gadBkoF	*Escherichia coli* NC101	TTTTTTAATGCGATCCAATCATTTTAAGGAGTT TAAAATGGTGTAGGCTGGAGCTGCTTC
gadBkoR	*Escherichia coli* NC101	CCGTTACCGTTAAACGTTATCAGGTGTGTTTA AAGCTGTTATGGGAATTAGCCATGGTCC
gadB3Fnd	*Escherichia coli* NC101	AACATATGATCACAGCCCCGTCAACACATC
gadB3Rnh	*Escherichia coli* NC101	AAGCTAGCCCCCTAAAACGGTATTCCTGTC
gadA5Fm	*Escherichia coli* NC101	AACAATTGTTAATTTGATCGCCCGAACAG
gadA5Rn	*Escherichia coli* NC101	AAAGCGGCCGCTCAACACGATGAATAGACAG GT
gadB4Fk	*Escherichia coli* NC101	AAGGTACCATCACAGCCCCGTCAACAC
gadB5Rm	*Escherichia coli* NC101	AACAATTGCCCCTAAAACGGTATTCCTGTC
16SF	*Escherichia coli* NC101	GAATGCCACGGTGAATACGTT
16SR	*Escherichia coli* NC101	ACCCACTCCCATGGTGTGA
GAPDHF	*Mus musculus*	CCTGCTCCCCCTACACACA
GAPDHR	*Mus musculus*	CCTGTTCTTCTCGGGCAAAA

25

26

27 **SUPPLEMENTARY METHODS**

28 **Construction of NC101Δ*gadAB***

29 NC101Δ*gadAB* was constructed using the λ-*red* recombinase method as described elsewhere
30 (Datsenko and Wanner, 2000). Briefly, the FRT-flanked kanamycin cassette in pKD4 was
31 amplified using primers gadAkoF, gadAkoR, gadBkoF and gadBkoR (Table S1) each of which
32 contains 60 nucleotides that are homologous to the 5′ and 3′ regions of the *gadA* and the *gadB*
33 coding region in NC101, respectively. The PCR product was gel-purified, digested with DpnI,
34 and transformed into NC101, which had previously been transformed with pKD46. A kanamycin
35 resistant clone in which the *gadAB* genes were replaced by the kanamycin resistance cassette was
36 transformed with pCP20 in order to remove the kanamycin resistance cassette. Loss of pCP20
37 and pKD46 from the resultant colonies was confirmed by replicate plating onto kanamycin and
38 carbenicillin agar plates. The *gadAB* gene regions on the NC101 chromosome were sequenced to
39 confirm deletion of the coding region.
40
41 **Construction of NC101Δ*gadAB* (pgadB)**

42 The *gadB*-complemented strain of NC101 was constructed using standard molecular biology
43 protocols. Briefly, the NC101 *gadB* gene and its native promoter were PCR-amplified as one
44 DNA fragment using purified *E. coli* NC101 chromosomal DNA template, forward gadB3Fnd,
45 and reverse gadB3Rnh primers (Table S1), containing at the 5′ end the *NdeI* and *NheI* restriction
46 sites, respectively. Amplification was performed using Phusion DNA Polymerase (New England
47 BioLab, Ipswich, MA) according to manufacturer's instructions and using the following
48 conditions: 98ºC for 30 seconds; 30 cycles of 98ºC for 10 seconds, 67ºC for 15 seconds, 72ºC for
49 30 seconds; and 72ºC for 5 minutes. The amplicon was digested with *NdeI* and *NheI,* and

50 subcloned into the multiple cloning site of pGEN-MCS (kind gift from M. Chelsea Lane, UNC
51 Chapel Hill) to generate pGEN-gadB, which contains the *hok sok* postsegregation killing system
52 and two *par* loci to enhance plasmid stability over multiple generations (Lane et al., 2007).
53 Integrity of the *gadB* gene was confirmed by sequencing after which electro-competent
54 NC101Δ*gadAB* cells were transformed with pGEN-*gadB* using electroporation to obtain the
55 plasmid-complemented NC101Δ*gadAB* (NC101 Δ*gadAB* (pgadB)) strain. *GadB* expression was
56 confirmed by PCR.
57
58 **Construction of NC101Δ*gadAB attTn7::gadBA***
59 The chromosomal *gadAB*-complemented strain of NC101 was constructed using the mini-Tn7
60 insertion method as described elsewhere (Choi et al., 2005). Briefly, NC101 *gadA* and *gadB*
61 genes and their native promoters were PCR-amplified as one DNA fragment respectively, using
62 purified *E. coli* NC101 chromosomal DNA template and the following primer sets which contain
63 distinct restriction sites at the 5′ ends: forward gadA5Fm, reverse gadA5Rn, and forward
64 gadB4Fk and reverse gadB5Rm. Amplifications were performed using Phusion DNA
65 Polymerase (New England BioLab, Ipswich, MA) and the following conditions: 98ºC for 30
66 seconds; 35 cycles of 98ºC for 10 seconds, 65ºC for 20 seconds, 72ºC for 55 seconds; and 72ºC
67 for 5 minutes. The *gadA* amplicon was digested with *MfeI* and *NotI*; the *gadB* amplicon with
68 *KpnI* and *MfeI.* Both *gadA* and *gadB* were then subcloned into the MCL2868 plasmid (kind gift
69 from M. Chelsea Lane) to generate MCL2868-*gadA* and MCL2868-*gadB,* respectively. The
70 *gadA* fragment was digested from MCL2868-*gadA* with *MfeI* and *NotI* and subcloned into
71 MCL2868-*gadB* to generate MCL2868-*gadBA*. MCL2868-*gadBA* includes mini-Tn7 elements,
72 an ampicillin resistance gene, and pTNS2, a mobilizable helper plasmid containing specific

73 tnsABC + tnsE genes to promote transposition to *att*Tn7 sites. Electro-competent NC101Δ*gadAB*
74 cells were transformed with MCL2868-*gadBA* using electroporation and selected by replicate
75 plating onto carbenicillin agar plates to obtain the chromosomal-complemented NC101Δ*gadAB*
76 *att*Tn7::*gadBA* strain. *GadAB* expression was confirmed by PCR.
77

78 **DNA isolation from cecal tissue biopsies**

79 Approximately 50 mg of snap frozen tissue was suspended in 750 µl of sterile bacterial lysis
80 buffer (200 mM NaCl, 100 mM Tris (pH 8.0), 20 mM EDTA, and 20 mg/ml lysozyme) and
81 incubated at 37ºC for 30 min. Then 40 µl of proteinase K (20 mg/ml) and 85 µl of 10% SDS
82 were added to the mixture. After 30 min of incubation at 65ºC, 500 µL of phenol:chloroform:
83 isoamyl alcohol (25:24:1) and 300 mg of 0.1-mm zirconium beads (BioSpec Products,
84 Bartlesville, OK) were added, and the mixture was homogenized in a bead beater (Precellys 48,
85 Bertin Technologies, Rockville, MD) for 3 min. The homogenized mixture was cooled on ice
86 and then centrifuged at 14,000 rpm for 5 min. The supernatant was transferred to a new 1.5-ml
87 microfuge tube, and DNA was further extracted by phenol-chloroform-isoamyl alcohol (25:24:1)
88 and then by chloroform-isoamyl alcohol (24:1). After extraction, the supernatant was
89 precipitated with 1/10 volume of 3M sodium acetate (pH5.2) and 2.5 volumes of absolute
90 ethanol at -20ºC for 1h. The precipitated DNA was suspended in DNase-free H_2O and then
91 cleaned using the DNeasy blood and tissue extraction kit according to the manufacturer's
92 instructions.
93

94 **RNA isolation from ileal tissue**

95 Approximately 20 mg of frozen ileal tissue was thawed into 0.4mL of RLT Lysis buffer
96 containing 1% of 2-Mercaptoethanol (RNeasy Mini Kit, Qiagen, Germantown, MD), physically

97 disrupted using a T25 basic tissue homogenizer (IKA Labortechnik, Wilmington, NC) at medium

98 speed and RNA was purified using the RNeasy Mini Kit.

99

100 **SUPPLEMENTARY REFERENCES**

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