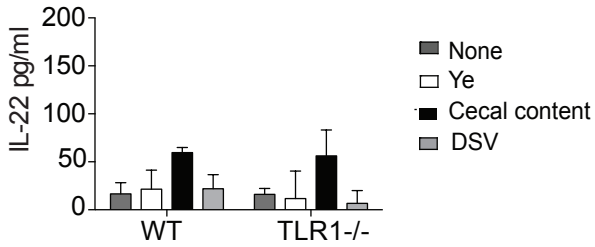
Supplemental Figure Legends

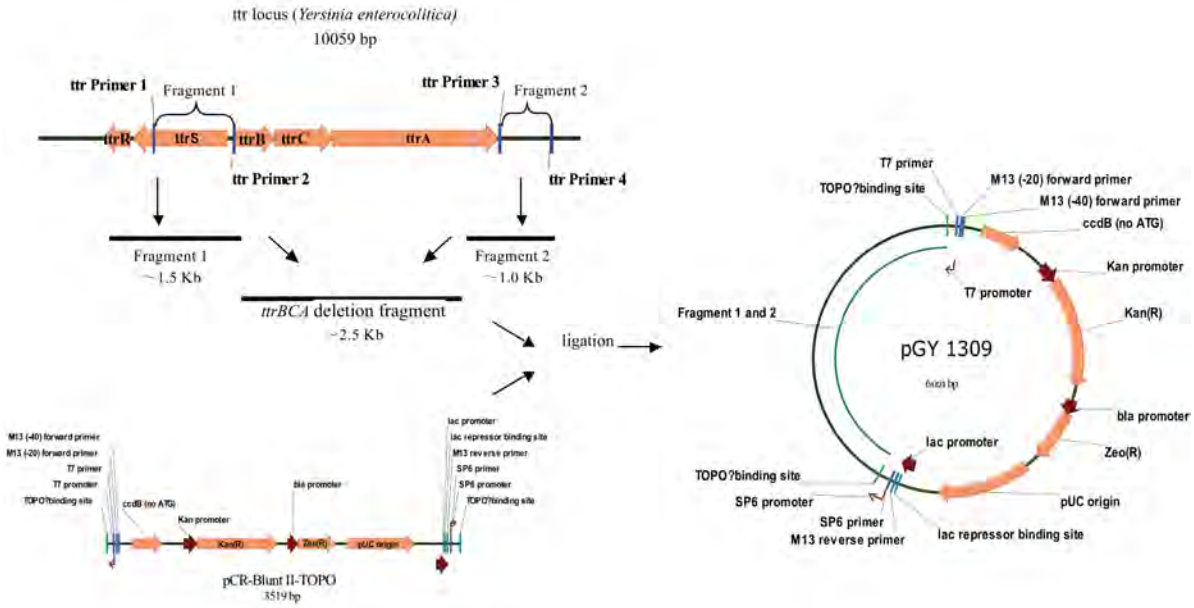
Figure S1. Related to Figure 1; No difference in IL-22 production from re-stimulated colonic LPL. Mean concentration of IL-22 from colonic lamina propria lymphocytes (LPC) harvested 70 days after infection by *Y. enterocolitica* and re-stimulated with cecal lysate, *Y. enterocolitica* (Ye) or *D. desulfuricans* (DSV) from WT and $TLR1^{-/-}$ mice. Data is the mean ± SEM from two independent experiments (n=5-6 mice/group).

Figure S2. Related to Figure 5; Generation and validation of a tetrathionate respiration mutant in *Y. enterocolitica*. (A) Deletion fragment of *ttrBCA* gene was ligated to produce plasmid pGY1309 Fusion PCR was carried out using primers to amplify different portions of the *ttrBCA* and ligated with pCR™II-Blunt-TOPO® to produce plasmid pGY1309. **(B)** The fragment containing the *ttrBCA* deletion fragment was isolated from plasmid pGY1309 and ligated into the *Bam*H I site of pSR47S. **(C)** Colony PCR using ttr primers 1 and 4 on kanamycin-sensitive and sucrose-resistant colonies. L= ladder, 1-6 individual colonies. **(D)** Tetrathionate agar plate streaked with a 8081Δ*ttrBCA* candidate and wild type 8081.

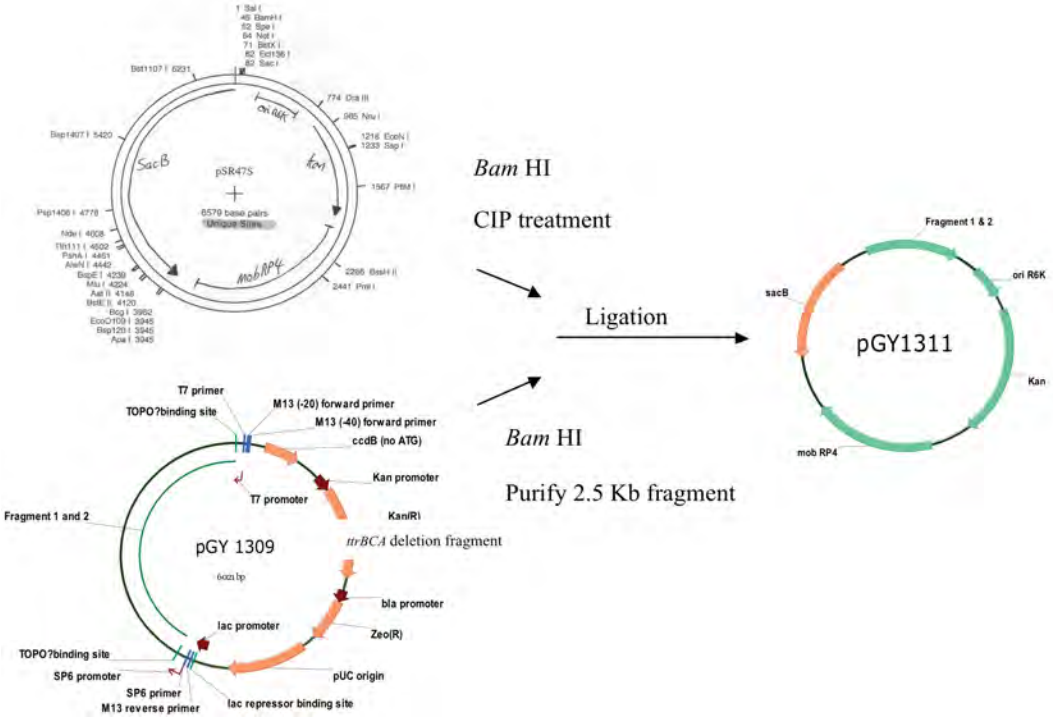
Figure S3. Related to Figure 6; Specificity of Lactobacillus treatment. **(A)** Percent of Lactobacillus species to total 16S DNA over the 70 days following infection in WT (left) and $TLR1^{-/-}$ (right) mice. Data is the mean ± SEM of n=4-6 mice/group. **(B)** The percent change in weight of antibiotic treated mice reconstituted with cecal contents of L. reuteri treated or control infected mice as indicated. Data is the average of n=6 mice collected from two separate experiments. *, $p < 0.05$ Student's paired t-test. **(C)** Mean IL-22 levels from colonic LP at indicated time-points from mice treated with *L. reuteri*. Data is the mean ± SEM of n=4-6 mice/group.


200
150
100
50
0
IL-22 pg/ml
WT
TLR1-/-
None
Ye
Cecal content
DSV

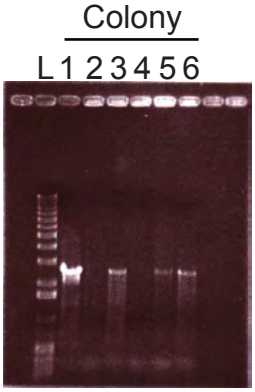
A


ttr locus (Yersinia enterocolitica)
10059 bp
ttr Primer 1
Fragment 1
ttr Primer 3
Fragment 2
ttrR
ttrS
ttrB
ttrC
ttrA
ttr Primer 2
ttr Primer 4
Fragment 1
~1.5 Kb
Fragment 2
~1.0 Kb
ttrBCA deletion fragment
~2.5 Kb
ligation
pCR-Blunt II-TOPO
3519 bp
T7 primer
TOPO?binding site
M13 (-20) forward primer
M13 (-40) forward primer
ccdB (no ATG)
Kan promoter
T7 promoter
Kan(R)
Fragment 1 and 2
pGY 1309
bla promoter
lac promoter
Zeo(R)
TOPO?binding site
SP6 promoter
pUC origin
SP6 primer
M13 reverse primer
lac repressor binding site

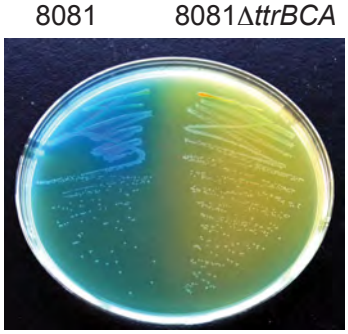
B


pSR47S
8579 base pairs
sacB
kan
mobRP4
Bam HI
CIP treatment
Ligation
pGY1311
Fragment 1 & 2
ori R6K
sacB
Kan
mob RP4
Bam HI
Purify 2.5 Kb fragment
pGY 1309
ttrBCA deletion fragment

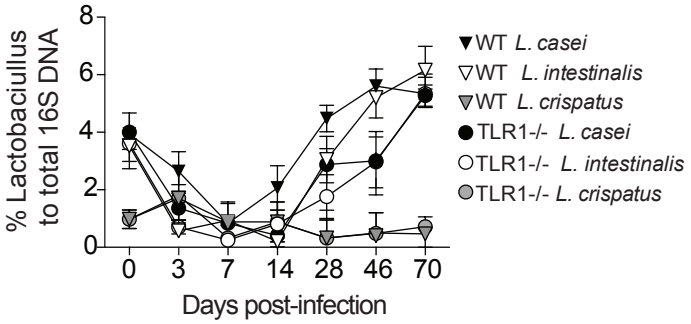
C


Colony
L 1 2 3 4 5 6

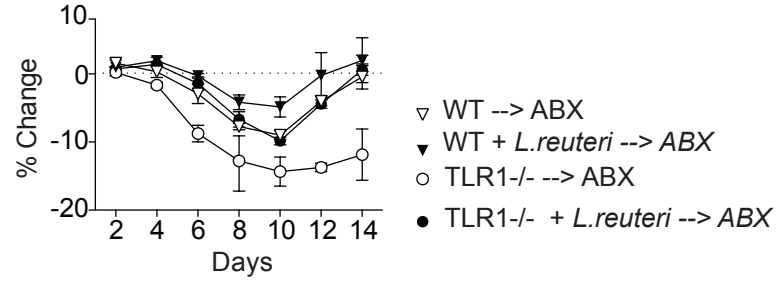
D


8081
8081ΔttrBCA

A


% Lactobaciullus to total 16S DNA
8
6
4
2
0
0 3 7 14 28 46 70
Days post-infection
WT L. casei
WT L. intestinalis
WT L. crispatus
TLR1-/- L. casei
TLR1-/- L. intestinalis
TLR1-/- L. crispatus

B


% Change
10
0
-10
-20
2 4 6 8 10 12 14
Days
WT --> ABX
WT + L.reuteri --> ABX
TLR1-/- --> ABX
TLR1-/- + L.reuteri --> ABX

C

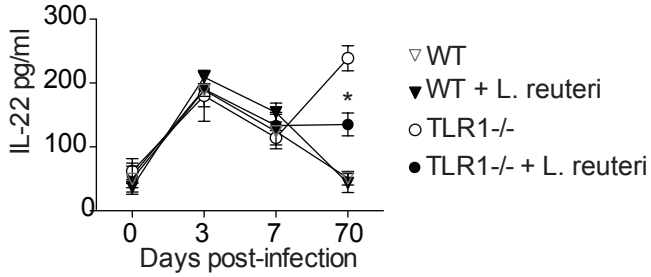

IL-22 pg/ml
300
200
100
0
0 3 7 70
Days post-infection
*
WT
WT + L. reuteri
TLR1-/-
TLR1-/- + L. reuteri

Table S1. Related to Experimental Procedures; Strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Source or Ref.
Yersinia enterocolitica 8081	Serogroup O:8, ΔyenR ($R^{-}M^{+}$), Nal^{r}	Kinder et al. (1993)
Yersinia enterocolitica 8081Δ*ttrBCA*	Derivative of *Y. enterocolitica* 8081, Δ*ttrBCA*	This study
Escherichia coli S17-1 λpir	*thi pro hsdR*$^{-}$ *hsd*M^{+} *recA* RP4-2(Tc::Mu-1, Km::Tn*7)* (Tp^{R} Sm^{R}) λ-pir lysogen	Simon et al. (1983)
pCR™II-Blunt-TOPO®	*pUC origin, lacZ*α *,* Km^{R}	Invitrogen
pSR47S	R6K, *sacB*, Km^{R}, suicide vector	Merriam et al (1997)
pGY1309	Derived from pCR™II-Blunt-TOPO®, harboring Δ*ttrBCA* fragment	This study
pGY1311	Derived from pSR47S, harboring Δ*ttrBCA* fragment, for allelic exchange	This study

Table S2. Related to Experimental Procedures; List of primers used in this study

Primer	Sequence
Angiogenin-4 (Ang4-F) Angiogenin-4 (Ang4-R)	5'-GAAATCTTTAAAGGCTCGGTACCC-3' 5'-CTCTGGCTCAGAATGTAAGGTACGA-3'
RegIIIg-F RegIIIg-R	5'-ATGCTTCCCCGTATAACCATCA-3' 5'-TACGTGAAGATGGGGCATCT-3'
GAPDH-F GAPDH-R	5'-AGGTCGGTGTGAACGGATTTG-3' 5'-TGTAGACCATGTAGTTGAGGTCA-3'
ttr Primer 1	GGATCCAATATCGGTATGTAGCTGGC
ttr Primer 2	TCTGCAAGTGAAAGGCTCACCCTCTTCTTG
ttr Primer 3	CCTTTCACTTGCAGAGGGAT
ttr Primer 4	GGATCCGTTGTTCGCGGAAAGTATCC
L. crispatus	AGCGAGCGGAACTAACAGATTTAC AGCTGATCATGCGATCTGCTT
L. casei	ACAGTTGCTTCTGGCCGTAT GCTTCACCAAGATCCAAGGT
L. reuteri	GCTTCACCAAGATCCAAGGT TTGTGAGTTTGGATTGAACC
L. johnsonii	CCTTTATTTGACGGTAATTACT CTCTTCTGCACTCAAGTTC
D. desulfuricans	GGCATCTATAGACCTCCTTGTAGAC TGTAGATCGTAGGTAGCAAATGTCG

Supplemental Procedures

Yersinia enterocolitica enumeration. Bacterial burden was determined by serially diluting mesenteric lymph node (MLN), luminal contents and isolated lamina propria in sterile PBS onto Trypic Soy Agar plates (BD, Biosciences) and grown for 48 hours at 26^{o}C.

Full-length SSU library generation and analysis. Cecal contents were removed from WT and TLR1$^{-/-}$ mice and suspended in 500 µL of MagNA Pure Bacteria Lysis Buffer (Roche Applied Science, Indianapolis, IN) prior to addition of 40 µL of 20 mg/mL proteinase K (Qiagen, Valencia, CA) followed by 1 minute of bead-beating (maximum speed using Mini-Beadbeater-16 from BioSpec Products, Inc. Bartlesville, OK). Following centrifugation, the supernatant was transferred to a clean tube and the DNA was extracted using the preset "Tissue Protocol" in conjunction with the Nucleic Isolation Kit I on the MagNA Pure Compact Instrument (Roche Applied Science). Construction of 16S rRNA-encoding gene clone libraries was performed as previously described (Young and Schmidt, 2004). Briefly, DNA extracted from cecal lysates were subjected to PCR amplification (illustra PuReTaq™ Ready-To-Go™ PCR beads (GE Healthcare) using 20 cycles of 30 seconds at 94^{o}C, 45 seconds at 58^{o}C, and 1.5 minutes at 72^{o}C) with broad-range primers targeting conserved regions of the 16S rRNA-encoding gene (1 pmol/µL final concentration of each: 8F, 5'-AGAGTTTGATCCTGGCTCAG-3'; 1492R, 5'-GGTTACCTTGTTACGACTT-3'; (Schmidt and Relman, 1994). PCR amplifications were purified, ligated to a plasmid vector (pCR®4-TOPO®; Invitrogen, Waltham, MA) and used to transform *Escherichia coli* competent cells. PCR amplification of cloned inserts from randomly selected colonies was then used to generate DNA sequencing template. Each cloned insert sequenced using the 8F primer would act as a proxy for the microbial community members in the splenic abscess samples. DNA sequencing was performed at the University of Chicago's Cancer Research Center DNA Sequencing Facility using the Applied Biosystems 3730xl DNA Analyzer. Raw sequence data were processed and trimmed according to quality scores through an automated workflow (RDP Pipeline Tool via myRDP) available from the Ribosomal Database Project (RDP) II website (http://rdp.cme.msu.edu/; (Cole et al., 2009). All sequences from the experiment were then pooled prior to trimming to conserved sites across them. Subsequently, sequences were grouped by mouse, aligned and chimeras were removed using Mallard (http://www.bioinformatics-toolkit.org/Mallard/;(Ashelford et al.,

2006). Taxonomic classification of the sequences was performed with the RDP Classifier and SeqMatch (Cole et al., 2009).

Lamina propria isolation. Terminal small intestine or proximal colon was removed, flushed with ice cold PBS and 1 mM DTT, cut longitudinally and then in 1 mm pieces. To remove intestinal epithelial cells the tissues were shaken at 37°C in HBSS with 2 mM EDTA (Sigma-Aldrich, St. Louis, MO) and 2 mM DTT (Sigma-Aldrich) for 10 minutes; this was repeated twice. The remaining tissue was digested with collagenase type IV in HBSS with 20% FBS while shaking at 37^{o}C for 10 minutes. The supernatant was removed and placed on ice and this process was repeated one more time and subsequent supernatants were pooled together, passed through 70 uM mesh filters (BD Biosciences, San Jose, CA) and washed twice in PBS.

Bacterial and cecal lysates. Bacterial cultures and colonic contents were lysed in 1 mL PBS using a MiniBeadbeater (BioSpec Products) and 0.1 mm diameter zirconia/silica beads (BioSpec Products) or 0.7 mm garnet beads (Mo Bio Laboratories, Carlsbad, CA), respectively. Protein concentration of lysates was quantified using Quick Start™ Bradford Protein Assay (Bio-Rad Laboratories).

Anti-bacterial and commensal T cell and antibody responses. Lamina propria lymphocytes were washed and resuspended at 1x10^{6} cells/mL and 100 µL were plated in a 96 well plate with 5x10^{4} syngeneic antigen presenting cells (CD3 and CD19-depeleted splenocytes) that had been pulsed with 10 µg/mL bacterial lysate made from cultures of *Y. enterocolitica*, *D. desulfuricans* or naïve cecal contents for 48 hours at 37^{o}C. Supernatants were assessed for cytokines by ELISA. For antibody levels, 96 well plates were coated overnight at 4^{o}C with 10 µg/mL cecal lysate. Serum was prepared via terminal bleed of the retro-orbital plexus. Serum (diluted 1:2) was added to bacteria coated plates for 2 hours at room temperature. Plates were washed and then incubated with HRP-conjugated anti-mouse IgA (Santa Cruz Biotechnology Inc, Santa Cruz, CA), or rabbit anti-mouse IgG1, rabbit anti-mouse IgG2a, and rabbit anti-mouse IgM followed by anti-Rabbit HRP (BD Biosciences). TMB substrate (Dako, Carpinteria, CA) was used for detection and absorbance was read at 495 nm OD.

Cytokine evaluation of lamina propria. After isolation of the lamina propria, the pellet was homogenized in 2 mL of PBS and protein was quantified using Quick Start™ Bradford Protein Assay (Bio-Rad

Laboratories, Irvine, CA). Cytokine levels in 50 µg/mL of protein was determined using ELISA assays for IL-6 (BD), IL-23 (BD), IL-17 (R&D,Minneapolis, MN), and IL-10 (BD).

Concentration of antibiotics. Ampicillin (A; 1 g/L; Sigma), vancomycin (V; 500 mg/L; Sigma), neomycin sulfate (N; 1 g/L; Sigma), metronidazole (M; 1 g/L; Sigma) and gentamicin (G; 1 g/L; Sigma)

Antibodies and flow cytometry. The following conjugated antibodies were purchased from eBioscience: CD3e (17A2), Ly6G (1A8-Ly6g), CD11b (M1/70), and CD45 (30-F11). Flow cytometry analysis was performed with a FACS Canto (BD Biosciences) and analyzed using FlowJo software (2011).

Real time PCR. RNA was extracted from mucosal scrapings or from isolated lamina propria using ISOLATE II RNA Mini Kit (Bioline, Boston, MA). RNA was reverse-transcribed into cDNA with SensiFAST™ cDNA Synthesis Kit (Bioline) and diluted 1:2. qPCR was performed on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories) using a SensiFAST™ SYBR® No-ROX Kit (Bioline). Quantitative real-time RT-PCR was performed using the iCycler iQ real-time PCR detection system (Bio-Rad Laboratories) and a SYBR green amplification kit (Bioline). Each PCR reaction was performed and normalized using primers for GAPDH. In other experiments the lamina propria cell pellet is washed twice and filtered through 70 µM mesh and CD4+ cells were isolated via positive selection using magnetic beads (Miltenyi, San Diego, CA).

Plasmid and Y. enterocolitica 8081Δ*ttrBCA constructions.* For PCR production of a *ttrBCA* deletion fragment, fusion PCR was carried out as follows (Fig. 5A-B): PCR 1, *ttr* Primer 1 and *ttr* Primer 2 were used to amplify the *ttrB* upstream sequence with chromosomal DNA as the template; PCR 2, *ttr* Primer 3 and *ttr* Primer 4 were used to amplify the *ttrA* downstream sequence with chromosomal DNA as the template; PCR 3, *ttr* Primer 1 and *ttr* Primer 4 were used to amplify a 2.5 kb fusion fragment by mixing the PCR 1 and PCR 2 products as templates. *PfuTurbo*® DNA Polymerase (Stratagene, La Jolla, CA, USA) was used according to the manufacturer's instructions. For PCR 1 and PCR 2, PCR amplification was initiated at 95°C for 5 min, then carried out using 25 cycles of 95°C for 40 seconds (s), 51°C for 40 s and 72°C for 2 minutes (min) with a final extension step for 10 min at 72°C. For PCR 3, PCR amplification was performed without primers firstly (being initiated at 95°C for 5 min, then carried out using 15 cycles of 95°C for 40 s, 34°C for 1 min and 72°C for 3 min with a final extension step for 10 min at 72°C). After

adding primers, the PCR was continued using 25 cycles of 95°C for 40 s, 50°C for 1 min and 72°C for 3 min with a final extension step for 10 min at 72°C. After the *ttrBCA* deletion fragment was PCR amplified, it was ligated with pCR^{TM}II-Blunt-TOPO$^{®}$ to produce plasmid pGY1309 (Fig. 5A). To construct plasmid pGY1311, a 2.5-kb *Bam*H I fragment containing the *ttrBCA* deletion fragment was isolated from plasmid pGY1309 and ligated into the *Bam*H I site of pSR47S (Fig. 5B). All molecular manipulations were carried out by standard procedures. Plasmid pGY1311 was introduced into *Y. enterocolitica* 8081 by conjugation using *Escherichia coli* S17-1 λpir as donor. The *ttrBCA* gene was deleted by integration of the *ttrBCA* deletion fragment on plasmid pGY1311 into the *ttr* locus by homologous recombination. In detail, *Escherichia coli* S17-1 λpir was mated with *Y. enterocolitica* 8081 on Luria-Bertani (LB) agar at 26°C for 6 hours (h). Cells on the plate were collected, resuspended, serially diluted and plated on LB agar containing 20 μg/mL nalidixic acid and 50 μg/ml kanamycin at 26°C for 2 days (d) to select for transconjugants in which the plasmid had integrated into the chromosome by a single crossover. Single colonies were isolated and restreaked on LB agar containing 20 μg/mL nalidixic acid and 50 μg/mL kanamycin at 26°C for 2 d to obtain pure clonal colonies. Then, the isolated colonies were inoculated into 5 mL TYE medium and incubated at 26°C for 6 h to allow second crossover. After that, the broth was serially diluted and plated on Tryptone-Yeast Extract (TYE) agar (1% tryptone, 0.5% yeast extract and 2% agar) containing 20 μg/ml nalidixic acid and 7.5% sucrose to select for excision and loss of the plasmid by a second recombination event. Individual colonies were isolated, screened for sensitivity to kanamycin and resistance to sucrose, and analyzed by colony PCR using *ttr* Primer 1 and *ttr* Primer 4.

Tetrathionate utilization test. On tetrathionate agar plate, *Y. enterocolitica* 8081 can develop a yellow color under anaerobic conditions which indicates a decrease in pH due to tetrathionate respiration, while *Y. enterocolitica* 8081Δ*ttrBCA* cannot change the color of the plate since the *ttrBCA* gene was deleted. Tetrathionate agar plate contains 10 g/L tryptone, 5 g/L sodium chloride, 15 g/L agar, 5 g/L sodium tetrathionate, and 50 mg/L bromthymol blue.

Analysis of pediatric IBD patients. The retrospective studies involving human subjects were performed in compliance with the Institutional Review Boards of The University of Chicago and The university of Southern California. Participants were consented at The University of Chicago. Saliva samples were collected with Oragene® OG-500 kits. Specimen labels were provided to the clinical centers by the

PediIBD Coordinating Center and had a unique specimen identification number that is pre-printed. The date the sample was obtained will be completed by the clinical center at the time the specimen is obtained. No other markings will be placed on the specimens that contain personally identifying information. All samples were stored at -80C. DNA extraction was performed by placing samples into a 50C water bath for 2 hours and gently inverted to homogenize solution. A 0.5 mL aliquot was removed and transferred to a 1.5mL microcentrifuge tube with 20 ul of prepIT®•L2P reagent and incubated on ice for 10 minutes. After incubation, samples were centrifuged at 15,000g and the clear supernatant separated from pellet and transferred to a fresh 1.5mL microcentrifuge tube containing 0.5 mL of 100% ethanol. Samples were then centrifuged for 2 minutes at 15,000g and the pellet is gently washed with 0.25 mL 70% ethanol in DI H_2O. 100 microliters of TE solution (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) was added after ethanol solution removed. Samples were quantified by nanodrop and TLR1 I602S custom probes from Life Technologies were used to detect the SNPs using VIC and FAM dyes on a BioRad cycler.