**Genotype:**

- **Plasmid:** (pTTR) (pWSK129)

---

**Bacterial load (CFU/g)**

**Genotype:**
- wild-type
- ttrA

**Colon content**
- wild-type
- ttrA

**Spleen**
- wild-type
- ttrA

---

**CI in tetrathionate broth (wild-type / ttrA)**

- Tetrathionate concentration (in mM)
- CI in tetrathionate broth (wild-type / ttrA)

---

**Cl in tetrathionate broth (wild-type / ttrA)**

- Tetrathionate concentration (in mM)
- CI in tetrathionate broth (wild-type / ttrA)

---

**CI in tetrathionate broth (wild-type / ttrA)**

- Tetrathionate concentration (in mM)
- CI in tetrathionate broth (wild-type / ttrA)

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Figure S1: (A) Schematic drawing of the ttr locus in S. Typhimurium. Location and orientation of genes (solid arrows) and promoters (arrow heads) are indicated. The DNA region carried by plasmid pTTR is indicated above the graph. Functions of genes are indicated below the graph. (B) Growth of the S. Typhimurium ttrA mutant (SW661) carrying a cloning vector (pWSK129) or the cloned ttrBCA genes (pTTR) on tetrathionate agar plates. Development of a yellow color indicates a decrease in pH due to tetrathionate respiration, which was observed for the ttrA mutant complemented with pTTR. (C) Competitive growth of the S. Typhimurium wild-type (IR715) and a ttrA mutant (SW661) in the presence (+) or absence (-) of iodine (I₂), thiosulfate (S₂O₃²⁻), or tetrathionate (S₄O₆²⁻). Growth conditions (aerobic, microaerobic, or anaerobic) are indicated. Bars represent geometric means (n=3) of competitive indices (CI) ± standard error. NR: no bacteria recovered. (D) Competitive growth of the S. Typhimurium wild-type (IR715) and a ttrA mutant (SW661) under anaerobic conditions in the presence of the indicated concentrations of tetrathionate (S₄O₆²⁻). Bars represent geometric means (n=3) of competitive indices (CI) ± standard error. (E and F) Contribution of tetrathionate respiration to S. Typhimurium virulence in the mouse model of typhoid fever in which intestinal inflammatory infiltrates are dominated by mononuclear cells while neutrophils are scars (data not shown). (E) Bacterial numbers recovered from the spleen and colon contents of groups of mice (C57BL/6) 4 days after infection with the S. Typhimurium wild-type strain (IR715; n=3) or a ttrA mutant (SW661; n=4). ns, not significant. (F) Recovery of bacteria from the spleen and colon contents of groups of mice (C57BL/6, n=3) four days after infection with a 1:1 mixture of the S. Typhimurium wild type (IR715) and a ttrA mutant (SW661). Bars represent geometric means of competitive indices (CI) ± standard error. (G) Oxidation of thiosulfate to tetrathionate by PMA-treated human blood leukocytes. Bars represent geometric means (n=3) ± standard error.
A
Mock-infected  S. Typhimurium

B
Mock-infected  S. Typhimurium

C

Histopathology score

Treatment:  Mock-infected  S. Tm

D

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<th>Infiltration by mononuclear cells*</th>
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<th>Epithelial damage</th>
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<td>Marked (&gt;40%)</td>
<td>Ulceration</td>
<td>Marked accumulation</td>
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* Number of cells per high power field (400x)

E

Histopathology score

Mouse genotype:

wild-type  Nos2<sup>-/-</sup>  Cybb<sup>-/-</sup>  wild-type  wild-type

S. Tm genotypes:

wild-type vs. ttrA  invAspiB vs. invAspiB<sup>ttrA</sup>  Mock-infected

Exudate  PMN  Epithelial damage  Submucosal edema  Mononuclear cells
Figure S2: Evaluation of cecal inflammation in the mouse colitis model 4 days after S. Typhimurium infection. Gross pathological (A) and histopathological (B) appearance of the cecum from mock-infected (left panels) or S. Typhimurium (S. Tm)-infected mice (right panels). Scale bars represents 400 μm (C) Blinded histopathology scoring of mice used for experiments shown in Figure 1B, C and D. Data are shown as averages for each group (bars) and as scores for each individual animal (filled circles). (D) Chart indicating scoring criteria for blinded examination of H&E stained sections from the cecum. (E) Detailed view of the histopathological scoring for each individual animal shown in Figure 2B.
Supplementary Figure S3: Detailed analysis of bacterial composition of the cecal microbiota in the mouse colitis model 4 days after S. Typhimurium infection. 16S gene copy number in the cecum of animals depicted in Figure 3E was determined by real-time PCR. Animals were mock-infected (n=4), or infected with the wild-type (IR715; n=6) or the ttrA mutant (SW661; n=6) Data shown are the geometric mean of the fraction of each population in the entire bacterial microbiota (Eubacteria).