A pTTR

ttrA — subunits of the tetrathionate reductase complex

ttrC *ttrB*

ttrS — sensory histidine kinase

ttrR — response regulator

1 kb

B

Genotype: *ttrA* / *ttrA*
Plasmid: (pTTR) / (pWSK129)

C

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

I_2	−	+	−	+	+	+
$S_2O_3^{2-}$	−	−	+	+	+	+
$S_4O_6^{2-}$	+	−	−	−	−	−

NR

anaerobic — micro-aerobic — aerobic

D

CI (wild-type vs. *ttrA*)

Tetrathionate concentration (in mM): 0, 2.5, 5, 10, 15, 40

E

Bacterial load (CFU/g)

ns ns

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

colon content — spleen

F

CI

colon content / spleen

wild-type vs. *ttrA*

G

$S_4O_6^{2-}$ conc. in μM

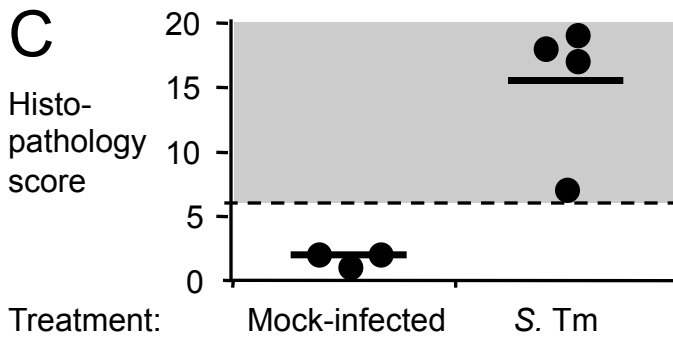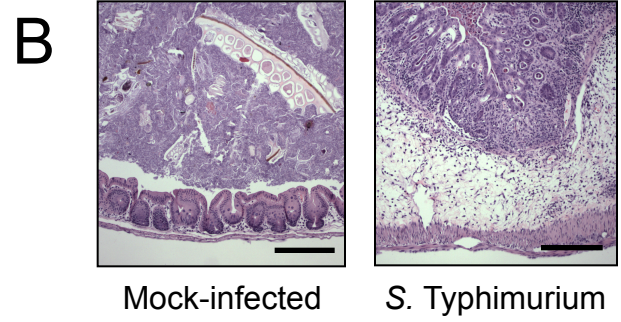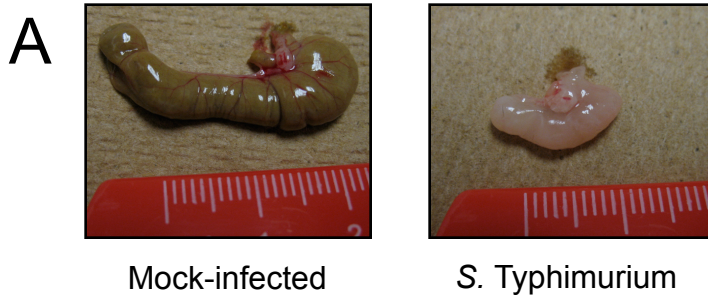| $S_2O_3^{2-}$	+	−	+
PMA	−	+	+

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 (I_2), thiosulfate ($S_2O_3^{2-}$), or tetrathionate ($S_4O_6^{2-}$). 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 ($S_4O_6^{2-}$). 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.
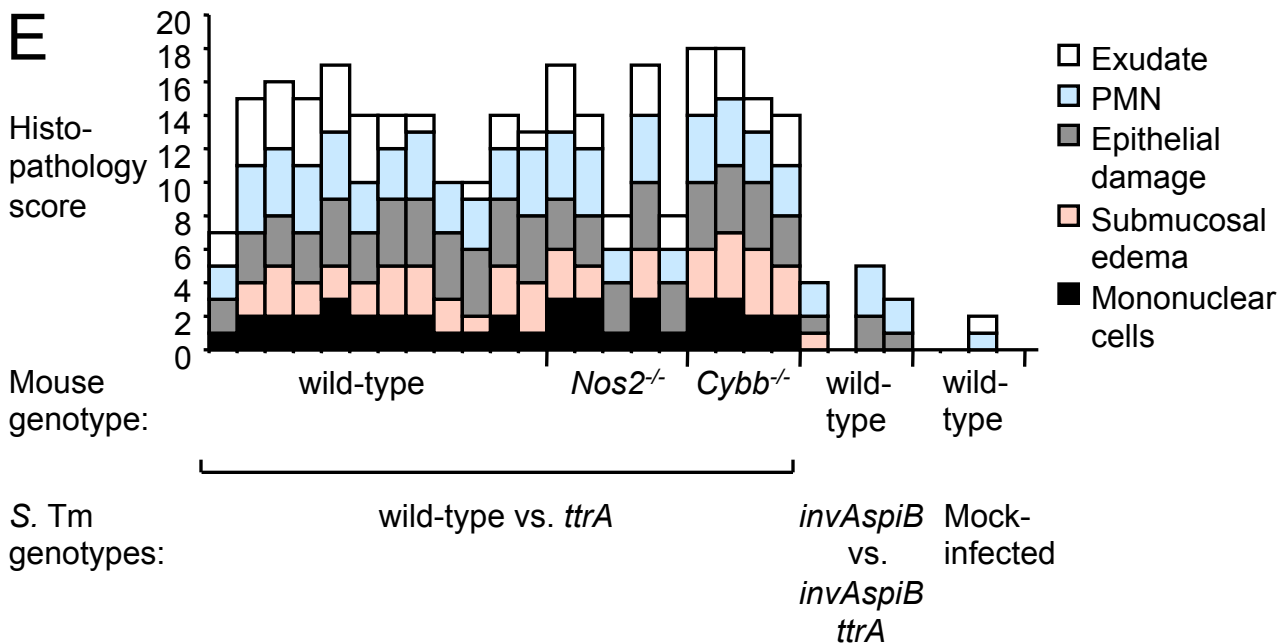
A Mock-infected   *S.* Typhimurium

B Mock-infected   *S.* Typhimurium

C

Histo-pathology score

Treatment: Mock-infected   *S.* Tm

Combined score	Description
>8	Severe inflammation
6-8	Moderate inflammation
3-5	Mild inflammation
0-2	normal

D

Score	Infiltration by PMNs*	Infiltration by mononuclear cells*	Submucosal edema	Epithithelial damage	Exudate
0	No changes (0-5)	No changes (0-5)	No changes	No changes	No changes
1	6-20	5-10	Detectable (<10%)	Desquamation	Slight accumulation
2	21-60	10-20	Mild (10%-20%)	Mild erosion	Mild accumulation
3	61-100	20-40	Moderate (20%-40%)	Marked erosion	Moderate accumulation
4	>100	>40	Marked (>40%)	Ulceration	Marked accumulation

* Number of cells per high power field (400x)

E

Histo-pathology score

Mouse genotype: wild-type   *Nos2*−/−   *Cybb*−/−   wild-type   wild-type

S. Tm genotypes: wild-type vs. *ttrA*   *invAspiB* vs. *invAspiB ttrA*   Mock-infected

Legend:
- 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.

mock-infected   wild-type   *ttrA*

S. Typhimurium
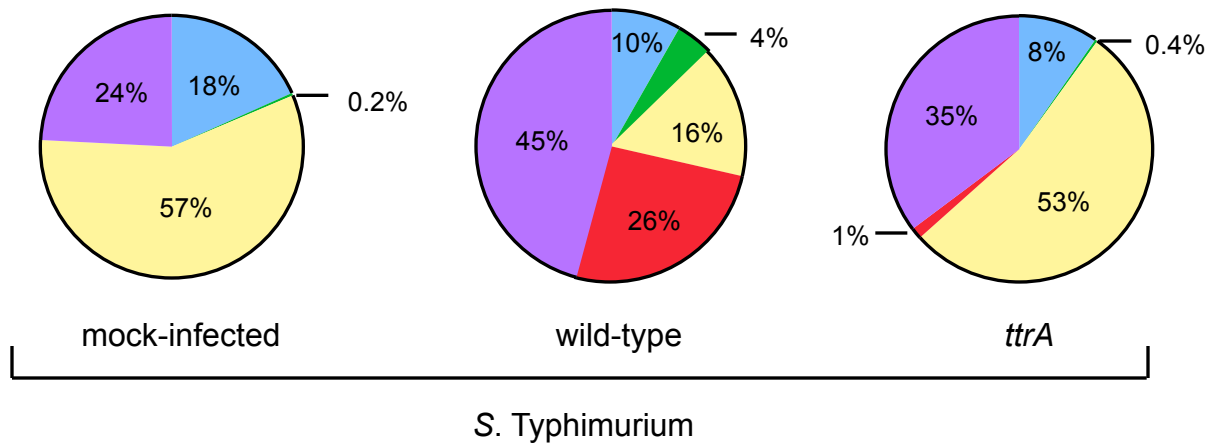
- □ *Clostridiales*
- ■ *Lactobacillales* / *Bacillales*
- □ *Bacteroidetes* / *Actinobacteria*
- ■ S*almonella*
- □ undetermined

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*).