## SUPPLEMENTARY DATA

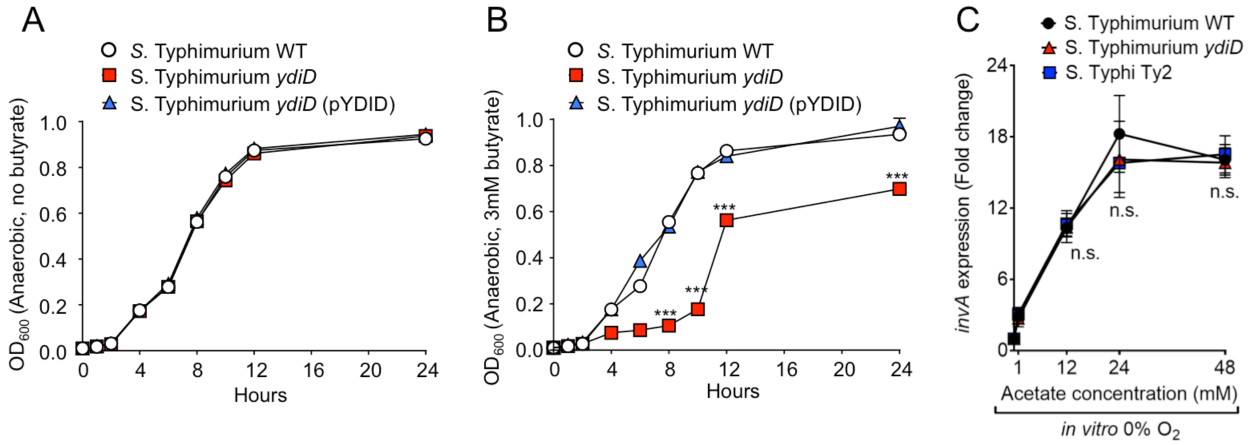

**Figure S1: Related to Figure 3; Effect of anaerobic β-oxidation on growth and gene expression *in vitro*.** (A and B) *S*. Typhimurium wild type (WT), a *S*. Typhimurium *ydiD* mutant and a *S*. Typhimurium *ydiD* mutant complemented with plasmid pYDID were grown under anaerobic conditions in rich broth in the absence (A) or presence of 3mM butyrate (B). (C) *S*. Typhimurium wild type (WT), a *S*. Typhimurium *ydiD* mutant, and *S*. Typhi strain Ty2 were grown under anaerobic conditions in rich broth supplemented with the indicated concentrations of acetate. Transcript levels of *invA* were quantified by real-time PCR, normalized to 16S rRNA levels and are shown as fold-change over transcript levels detected in bacteria grown in medium without acetate supplementation.

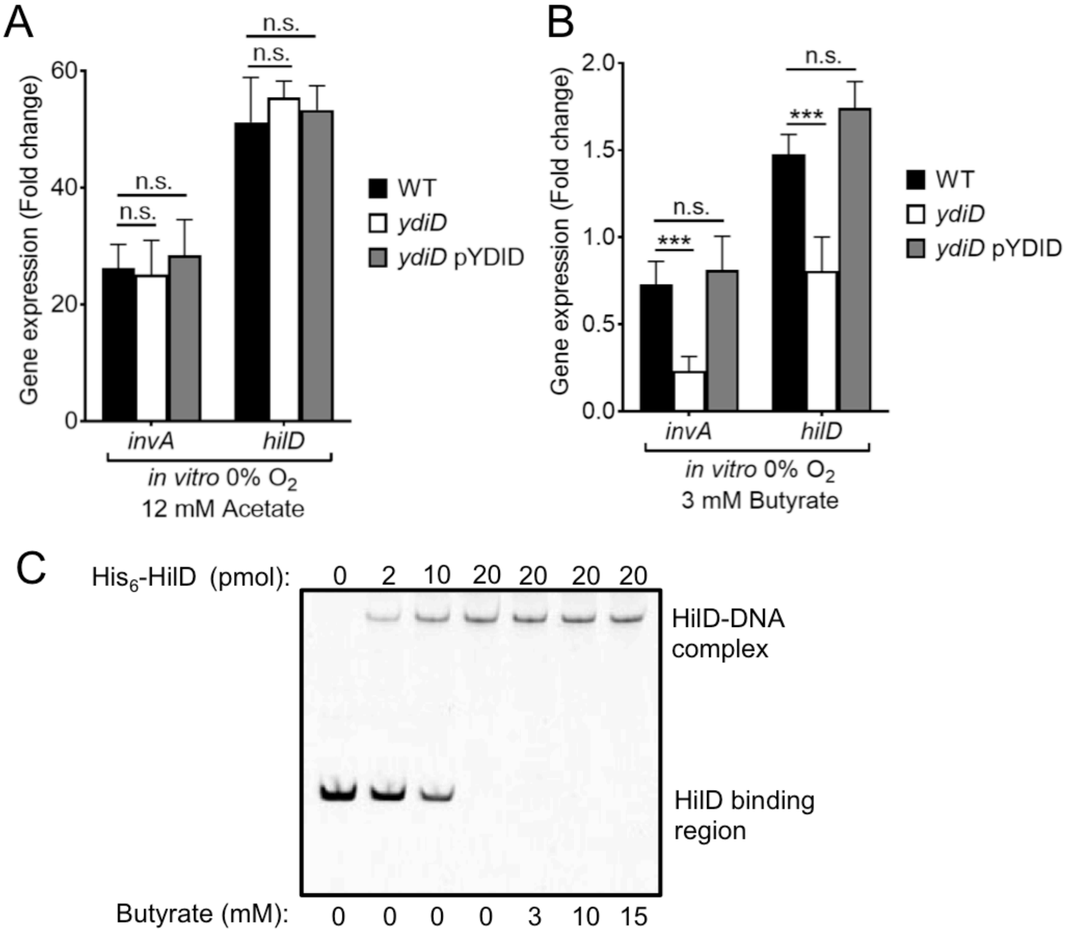

**Figure S2: Related to Figure 3; Genetic ablation of anaerobic β-oxidation reduces invasion gene expression in the presence of butyrate.** The *S*. Typhimurium wild type (WT), a *ydiD* mutant, and a complemented *ydiD* mutant (*ydiD* pYDID) were grown under anaerobic conditions in rich broth supplemented with 12 mM acetate (A) or 3 mM butyrate (B). Expression of *invA* and *hilD* was determined by real-time PCR. Gene expression is shown as fold-change over transcript levels detected in medium without SCFA supplementation. (C) Binding of affinity-purified His$_6$-HilD fusion protein to the *hilD* promoter region was determined in the presence of the indicated concentrations of butyrate using an electrophoretic mobility shift assay.

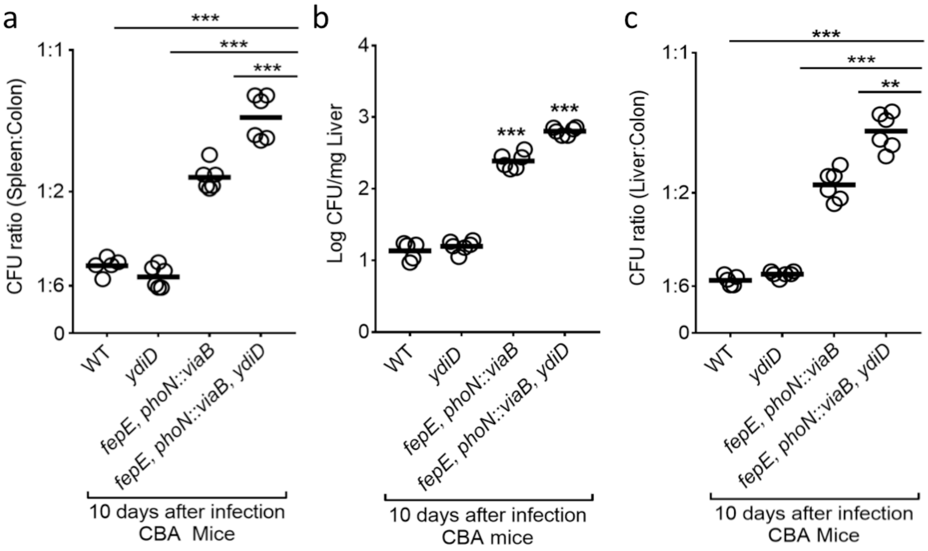

**Figure S3: Related to Figure 4; Deletion of *ydiD* cooperates with acquisition of *viaB* and pseudogene formation in *fepE* to alter bacterial tissue load.** CBA mice (n = 6) were infected with the *S.* Typhimurium wild type (WT), a *ydiD* mutant, a *fepE phoN::viaB* mutant or a *fepE phoN::viaB ydiD* mutant and bacterial numbers in organs determined 10 days after infection. (A) Ratio of bacterial numbers recovered from the colon and the spleen. (B) Bacterial numbers recovered from the liver. (C) Ratio of bacterial numbers recovered from the colon and the liver. **, $P < 0.001$; ***, $P < 0.0001$.

**Supplementary table 1:** Related to Methods; Primers used in this study

| **Primer** | **Sequence in 5' - 3'** |
|---|---|
| *Deletion of ydiD* | |
| ydiD-P1 | CACACCCGTCCTGTGCTGTTTGACGAGCGTAAC |
| ydiD-P2 | GACGCTACTTAAAACCGCCACACCTTTC |
| ydiD-P3 | CGGTTTTAAGTAGCGTCGTTATCCGGTG |
| ydiD-P4 | GCGTCCGGCGTAGAGCGATCTGTTCACCGTTGATG |
| Complementation of *ydiD* | |
| ydiDc-P1 | TCCTGCAGCCCGGGGTTATACCGCAGTGTGCTC |
| ydiDc-P2 | GAGATGCAATGAGTGTTACATTAACGTTTG |
| ydiDc-P3 | AACACTCATTGCATCTCCAGTGAGTCG |
| ydiDc-P4 | CGCTCTAGAACTAGTGCAGAAACAACATCGCACG |
| Purification of His$_6$-HilD | |
| HilD-His-F | AAA CAT ATG GAA AAT GTA ACC TTT GT |
| HilD-His-R | AAA GAA TTC TGT TAA TGC GCA GTC TGA AT |
| Electrophoretic Mobility Shift Assay (EMSA) | |
| hilD-EMSA-F | CAT TGG GGC TTA AAC TAC TG |
| hilD-EMSA-R | AGG ACG CTG ATG ACT ATT AC |
| *qPCR analysis* | |

| | |
|---|---|
| *invA* | 5’-CTCGCCTTTGCTGGTTTTAG-3’<br>5’-AGTGCCGGTTTTATCGTGAC-3’ |
| *hilD* | 5’-CCTGACTTTCCTGCCAGAAG-3’<br>5’-TGAGCCGAGCTAAGGATGAT-3’ |
| 16S rRNA gene<br>*Salmonella* | 5’-TGTTGTGGTTAATAACCGCA-3’<br>5’-GACTACCAGGGTATCTAATCC-3’ |
| *Kc*<br>*Mus musculus* | 5’-TGCACCCAAACCGAAGTCAT-3’<br>5’-TTGTCAGAAGCCAGCGTTCAC-3’ |
| *Il17a*<br>*Mus musculus* | 5’-GCTCCAGAAGGCCCTCAGA-3’<br>5’-AGCTTTCCCTCCGCATTGA-3’ |
| β-*actin*<br>*Mus musculus* | 5’-CCAGGGAGGAAGAGGATGCGG-3’<br>5’-GCTGAGAGGGAAATCGTGCGTG-3’ |
| 16S rRNA gene<br>*Clostridia* | 5’-ACTCCTACGGGAGGCAGC-3’<br>5’-GCTTCTTTAGTCAGGTACCGTCAT-3’ |