Typhimurium with coincident LAMP1 staining cell was scored visually by fluorescence microscopy. Shown are means ± SD from three independent experiments. *= $P<0.001$, Bonferroni's post-hoc test.

Figure 7. Intracellular virulence gene expression can be influenced by pre-invasion growth conditions. (a) *gfp*[LVA] transcriptional fusions were used to assess promoter activity for *prgH*, *sopB*, *ssaG*, *pipB* and *fliC* following invasion of HeLa cells. GFP-positive "induced" bacteria were scored visually by fluorescence microscopy. Extracellular *S.* Typhimurium were stained with anti-LPS mAb before permeabilisation and excluded from analysis. Shown are means ± SD from at least three separate experiments. *= $P<0.05$, two-way ANOVA and Bonferroni's post-hoc analysis. (b) QuantiGene detection of *prgH*, *sopB*, *ssaJ*, *pipB* and *fliC* gene expression. The expression of each gene was normalized to *nusG* as an internal control and then compared to the value for aer-LL bacteria at 30 min. Each symbol represents the mean from one experiment in duplicate. The statistical means from three (*prgH*, *sopB* and *fliC*) or four (*ssaJ* and *pipB*) experiments are indicated by horizontal lines.

Supplementary Material

Figure S1. Distribution of *S.* Typhimurium in infected HeLa cells. To obtain comparable invasion cells were infected with an m.o.i. of ~50-60 for aer-LL and ~150-180 for μaer-ST. Extracellular *S.* Typhimurium were stained with anti-LPS mAb before permeabilisation and excluded from analysis. After permeabilisation, all bacteria were stained with rabbit anti-LPS antibodies followed by AlexaFluor 568-conjugated secondary antibodies. The number of

intracellular *S.* Typhimurium per infected cell was scored visually. Shown are means ± SD from three independent experiments (*n* =100).

Figure S2. Plasmids are retained in *S.* Typhimurium. Plasmids stabilities were determined by culturing bacteria in either μaer-ST (a) or aer-LL (b) conditions without antibiotic selection, thereafter bacteria were plated on LB-Miller agar with or without carbenicillin. Shown are means ± SD from three independent experiments.

Figure S3. Motility of *S.* Typhimurium strains. Flagellar function was determined by rate of spread, after 6 h incubation at 37 °C in semisolid agar. Strains were WT *S.* Typhimurium (WT), *fliC*::Tn10 (fliC), *fljB*::Mud-Cm mutant (fljB), *fliC*::Tn10 *fljB*::Mud-Cm mutant (fliC/fljB), WT *S.* Typhimurium bearing pFPV25.1 (pFPV25.1) or P*prgH-gfp*[LVA] (P*prgH-gfp*[LVA]). Shown are means ± SD, from three independent experiments.

Figure S4. Electron micrographs showing flagella (black arrows) on the surface of aer-LL and μaer-ST bacteria, but fimbriae (white arrows) only on the surface of μaer-ST bacteria. Scale bars are 500 nm (left panels) or 100 nm (right panels).

Table S1. Genome wide-expression changes for aer-LL compared to μaer-ST *S.* Typhimurium SL1344. Probe-set identification (ID) for *S.* Typhimurium SL1344 is shown and the corresponding *S.* Typhimurium LT2 gene or synonym is denoted where identified by BLAST analysis. Check marks show genes where both a 2-fold (aer-LL/μaer-ST fold Δ) change and *P*-value passing the false discovery rate at a significant level was obtained (2X and *P*-value Sig.).