Supplementary Information

The role of H4 flagella in *Escherichia coli* ST131 virulence

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Supplementary Information

Supplementary Figures S1-S9

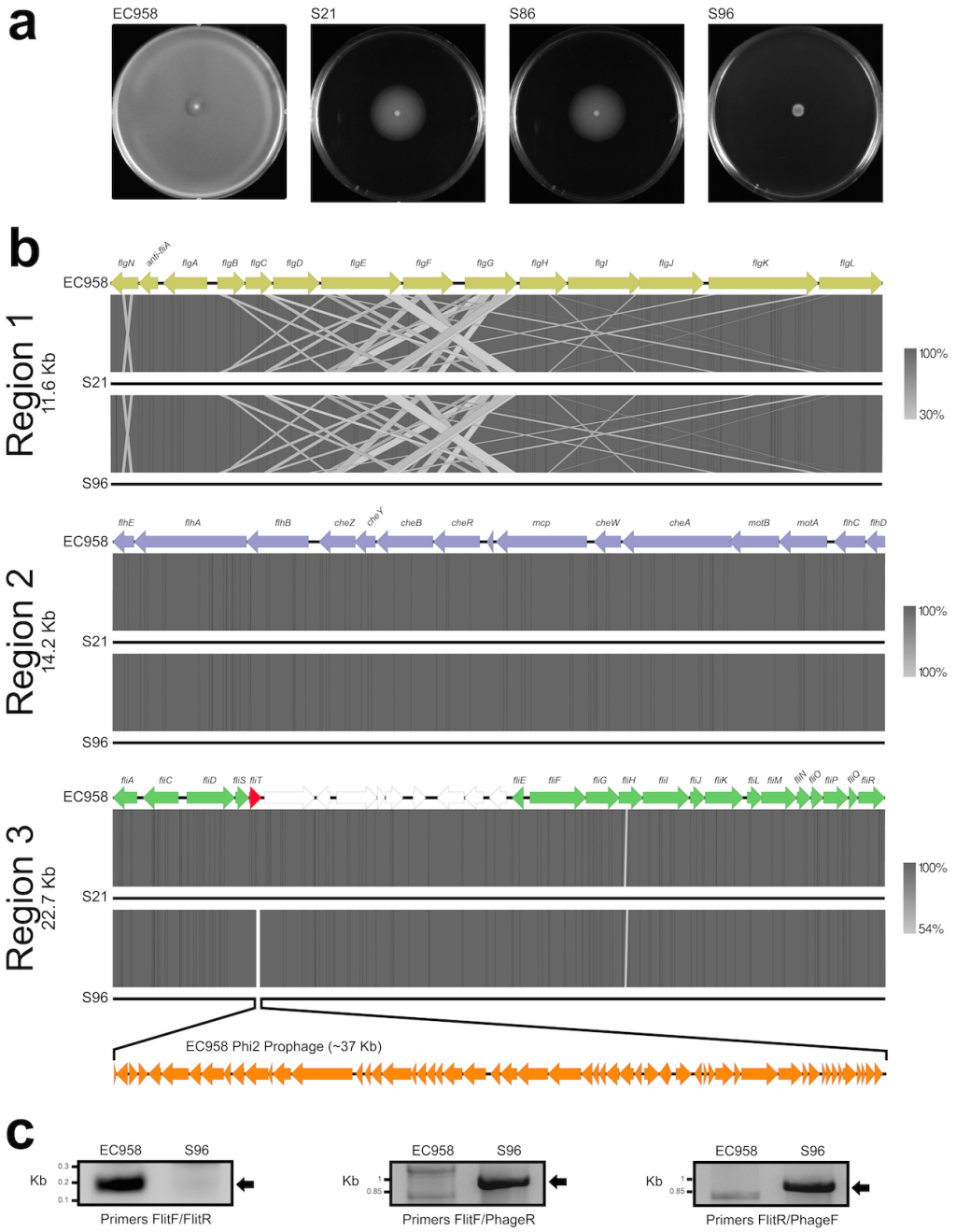

Fig. S1 Comparative genome analysis of flagellar regions of EC958 (motile), S21, S86 (strains displaying delayed motility) and S96 (non-motile strain). (a). Swimming phenotype of EC958, S21, S86 and S96 after 24 hours of incubation at 37^{o}C. (b). Bioinformatic analysis of the three flagellar regions of EC958 compared to the corresponding region from S21 and S96 (prepared using Easyfig). Region 1 (yellow) and 2 (purple) are highly conserved among the three strains, while Region 3 (green) contains an insertion of the Phi2 prophage in *fliT* (red). In EC958, the Phi2 prophage (~37 kb) is present at a different genomic location. Genes depicted in white are not associated with flagella biosynthesis according to the literature. The regions are not drawn to scale. (c) PCRs to confirm the disruption of *fliT* by Phi2 prophage in S96, using *fliT* and Phi2 specific primers, and EC958 as a control.

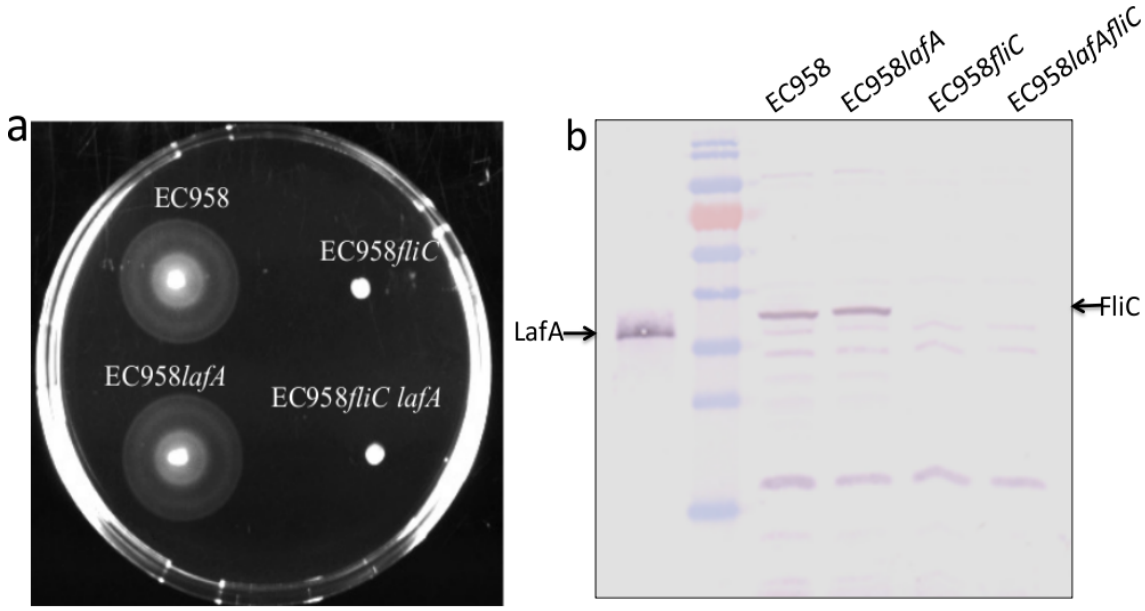

Fig. S2. The Flag-2 system of EC958 is non functional. (a). Motility of WT EC958, as well as EC958*fliC*, EC958*lafA* and EC958*fliC lafA* mutants in 0.25% LB agar. A volume of 5 µL of each bacterial strain grown in LB broth under shaking conditions was used as an inoculum. Shown is the motility of each strain after 6 hours of incubation at 37^{o}C. (b). Western blot analysis using a LafA antibody in combination with whole cell lysates prepared from same strains cultured under shaking conditions at 37^{o}C in LB broth. Lane 1, purified LafA protein (positive control), Lane 2, molecular weight markers, additional lanes represent strains as indicated. The LafA antibody was observed to cross-react with FliC; no cross-reacting band was detected in the two *fliC* mutant strains.

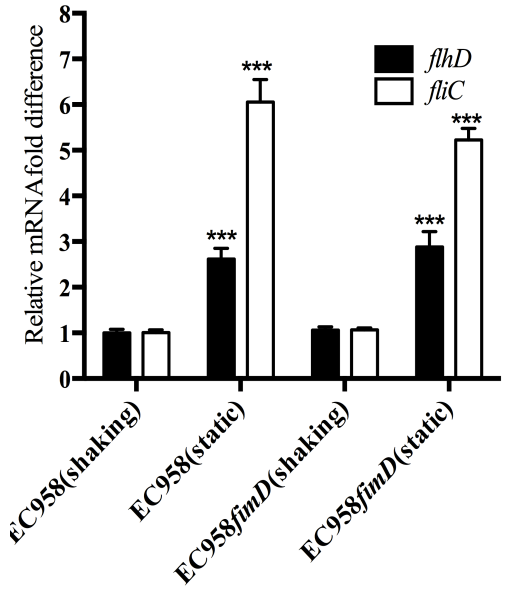

Fig. S3. Enhanced flagella expression by static growth is independent of type-1 fimbriae. Relative fold-difference of *flhD and fliC* transcript levels for EC958 and EC958*fimD* during growth in shaking and static conditions, determined by qRT-PCR. The data was obtained from three independent experiments; error bars indicate standard deviation. (***p<0.001)

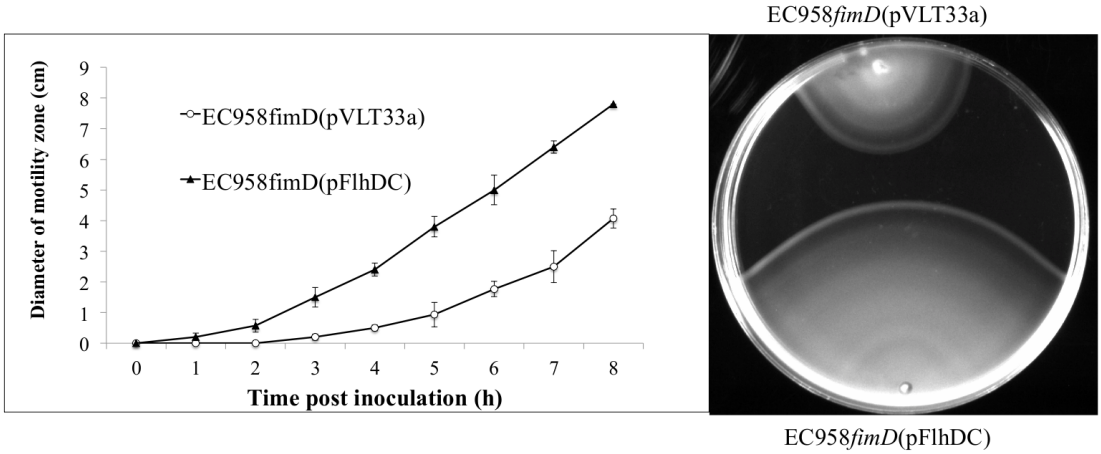

Fig. S4. FlhDC over-expression (via plasmid pFlhDC) leads to enhanced motility in EC958*fimD*. Graph demonstrating the rate of motility of EC958*fimD*(pVLT33a) and EC958*fimD*(pFlhDC) in 0.25% LB agar. Strains were grown overnight with 1mM IPTG induction in shaking LB broth cultures (left). Comparative motility of the same EC958 cultures in 0.25% agar at 6 hours post inoculation (right).

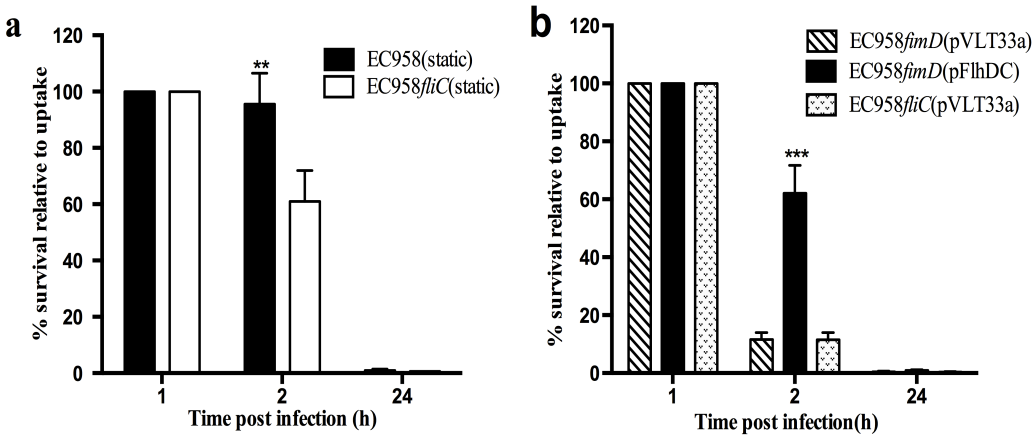

Fig. S5. Relative intramacrophage survival of (a) EC958 and EC958*fliC* grown under static conditions and (b) EC958*fimD*(pVLT33a), EC958*fimD*(pFlhDC) and EC958*fliC*(pVLT33a) grown under shaking condition Triplicate monolayers of BMM were infected at a MOI of 10. Intracellular bacterial loads were determined at 1, 2 and 24 hpi. Shown is the percentage bacterial survival relative to 100% uptake from three independent experiments ± standard deviation. (*** $p<0.001$; ** $p<0.01$)

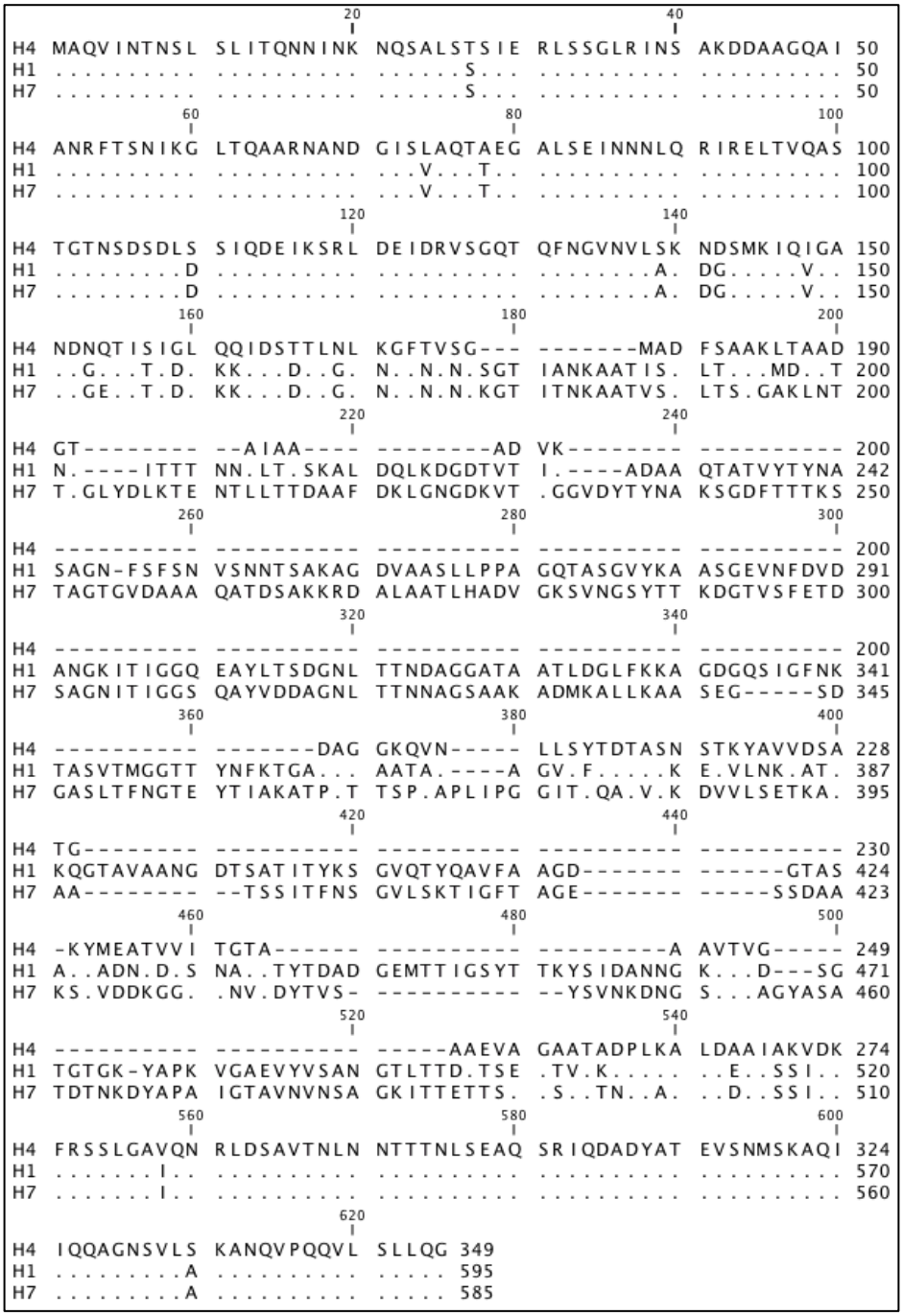

Fig. S6. Amino acid alignment of the H4 (EC958), H1 (CFT073) and H7 (UTI89) flagellins. Sequence alignments were performed using CLC main workbench 7.0.2. Residues identical to those of the H4 flagellin are indicated by dots.

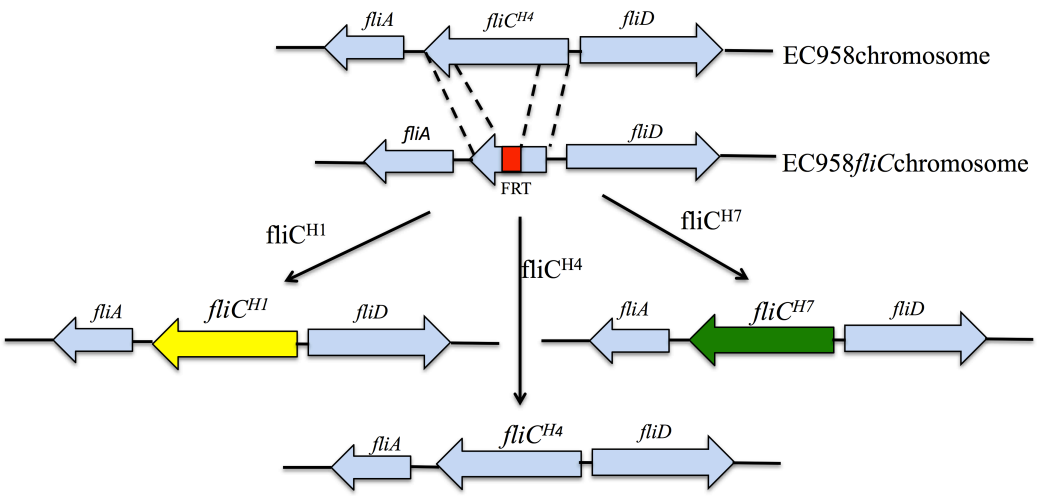

Fig. S7. Schematic representation of the method used for markerless replacement of the *fliC* allele in the EC958 chromosome. In the first step, the *fliC* gene was mutated by insertion of a chloramphenicol gene cassette using λ-Red recombination. The chloramphenicol cassette was subsequently removed by the introduction of plasmid pCP20, resulting in a large deletion in *fliC*. Next, the *fliC* gene from CFT073 (H1) and UTI89 (H7) and EC958 (H4; control) was amplified with 500bp homologous arms matching the EC958 *fliC* sequence; colour-coded as yellow, green and blue. These cassettes were then transformed into EC958*fliC* by electroporation and the transformation mix was spotted onto the centre of a 0.25% LB agar plate. Isogenic strains containing a reconstructed *fliC* allele were selected by their ability to swim through the agar.

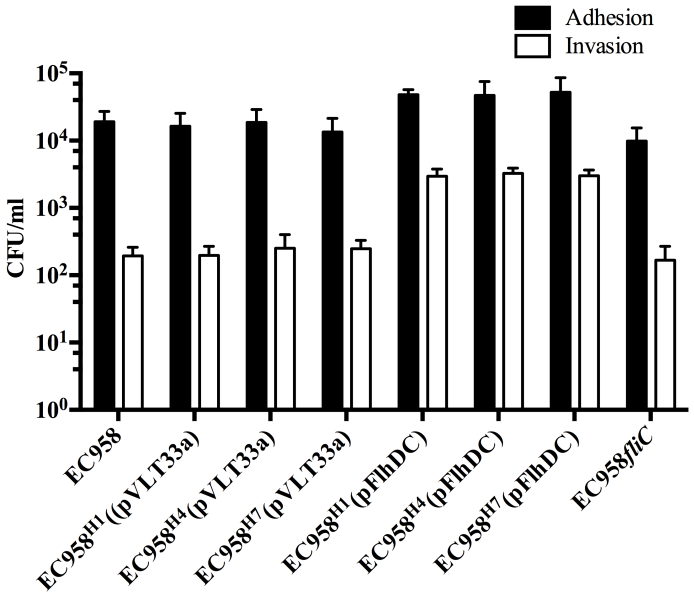

Fig. S8. Isogenic EC958 flagella variants show similar level of adhesion and invasion. Adhesion to and invasion of T24 bladder epithelial cells by EC958, EC958*fliC* (controls), EC958^{H1} (+/- pFlhDC) EC958^{H7} (+/- pFlhDC) and EC958^{H4} (+/- pFlhDC). Bacterial strains were grown under shaking conditions. Monolayers were infected in triplicate, and data represents the mean CFU/ml from three independent experiments ± standard deviation.

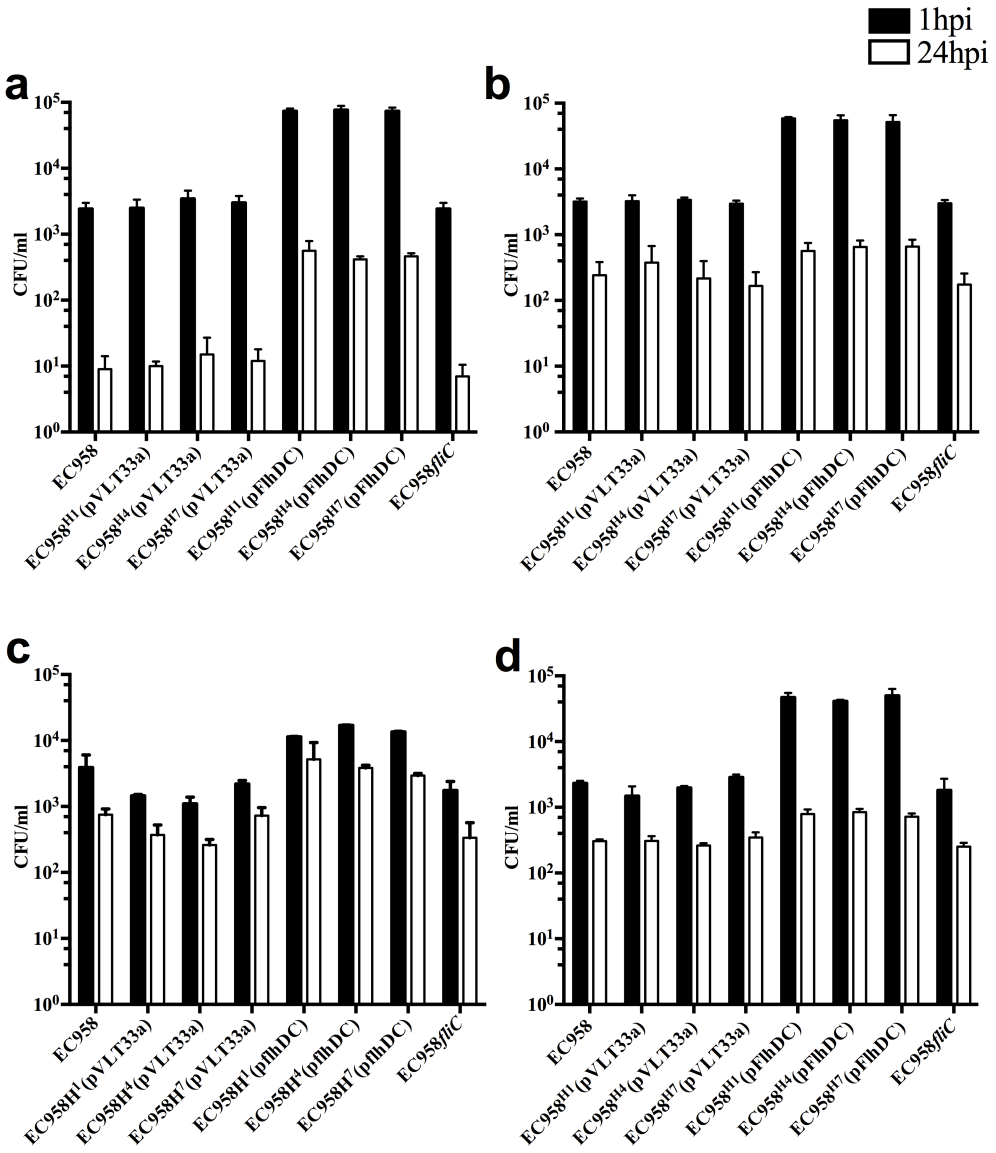

Fig. S9. Isogenic EC958 flagella variants show similar intramacrophage survival. Intracellular survival of EC958, EC958*fliC* (controls), EC958^{H1} (+/- pFlhDC) EC958^{H7} (+/- pFlhDC) and EC958^{H4} (+/- pFlhDC) in (a) BMM (b) HMDM (c) THP-1 cells and (d) U937 cells. Bacterial strains were grown under shaking conditions. Cells were infected at an MOI of 10 and intracellular survival was measured at 1 (black bar) and 24 (white bar) hpi. Shown is the mean CFU/ml from three independent experiments ± standard deviation.