

Driving the expression of the *Salmonella enterica* sv Typhimurium flagellum using *flhDC* from *Escherichia coli* results in key regulatory and cellular differences.

Ayman Albanna, Martin Sim, Paul A. Hoskisson, Colin Gillespie, Chris V. Rao and Phillip D. Aldridge*

SUPPLEMENTARY MATERIAL

*Corresponding author. Centre for Bacterial Cell Biology, Baddiley Clark Building, Newcastle University, Richardson Road, Newcastle upon Tyne, United Kingdom, NE2 4AX. UK. Phone: +44-191-2083218. Email: phillip.aldridge@ncl.ac.uk.

Figure S1

FlhD

```
S. enterica 1 MGTMHTSELLKHIYDINLSYLLLAQRLIVQDKASAMFRLGINEEMANTLGAALTLPQMVKL
E. coli 1 ---MHTSELLKHIYDINLSYLLLAQRLIVQDKASAMFRLGINEEMATTLAALTLPQMVKL
consensus 1 ...*****.*****
```



```
S. enterica 61 AETNQLVCHFRFDDHQTITRLTQDSRVDDLQQIHTGIMLSTRLLNEVD---DTARKKRA
E. coli 58 AETNQLVCHFRFDSHQTITQLTQDSRVDDLQQIHTGIMLSTRLLNDVNQPEEALRKKRA
consensus 61 ***** ***** ***** ***** ***** * .....
```

FlhC

```
S. enterica 1 MSEKSIVQEARDIQLAMELITLGARLQMLESETQLSRGRLIIRLYKELRGSPPPKGMLPFS
E. coli 1 MSEKSIVQEARDIQLAMELITLGARLQMLESETQLSRGRLIKLYKELRGSPPPKGMLPFS
consensus 1 ***** ***** .*****
```



```
S. enterica 61 TDWFMTEQNTHASMFCAWQFLLKTGLCSGVDAVIKAYRLYLEQCPQPPLEGPLLALTRA
E. coli 61 TDWFMTEQNVHASMFCAWQFLLKTGLCNQGVDAVIKAYRLYLEQCPQAELEGPLLALTRA
consensus 61 ***** .***** ***** *****
```



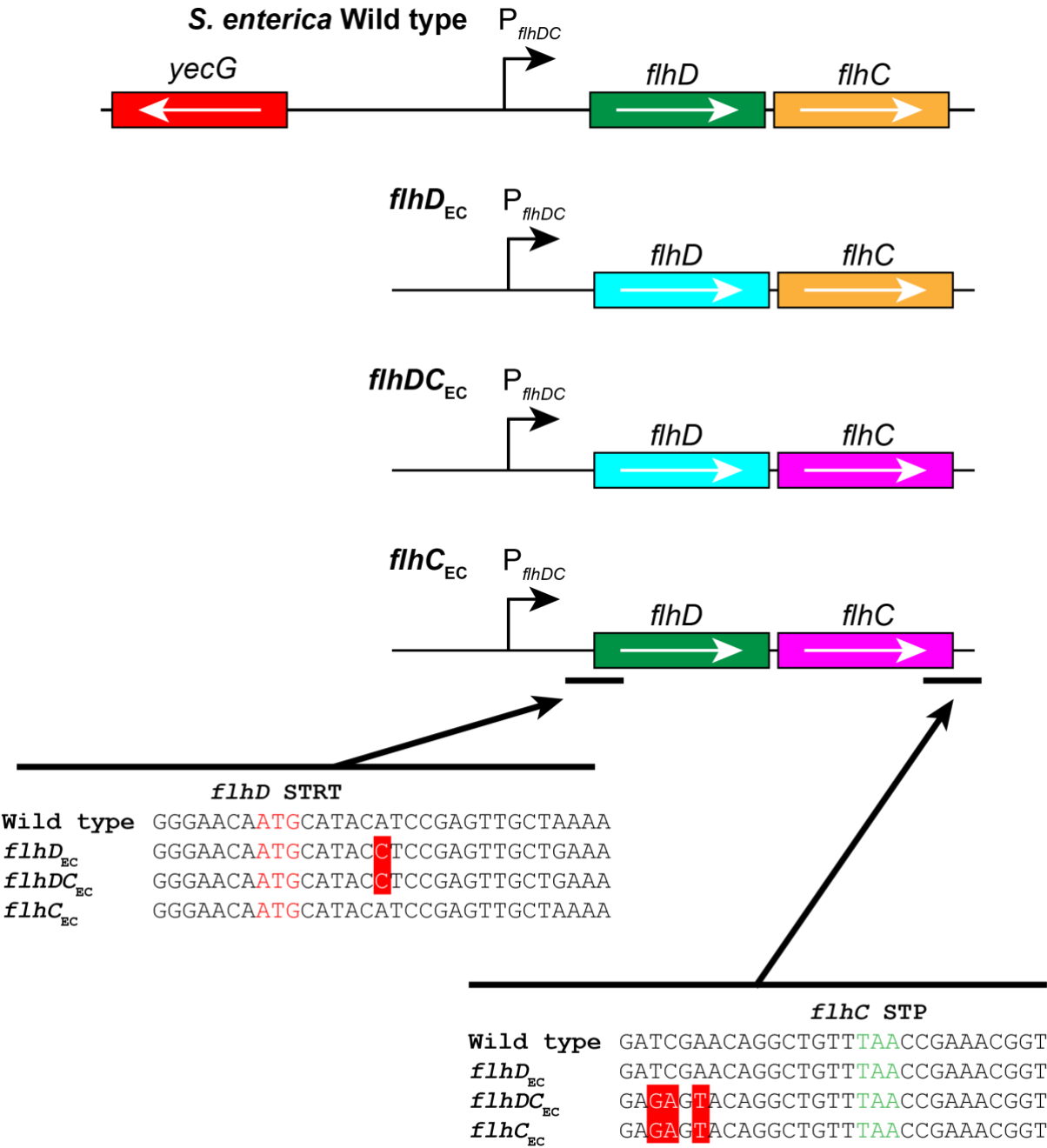
```
S. enterica 121 WTLVRFVESGLLELSSCNCCGGNFITHAQPVGSFACSLCQPPSRVKKRKLSDAADI I
E. coli 121 WTLVRFVESGLLQLSSCNCCGGNFITHAQPVGSFACSLCQPPSRVKKRKLSONPADII
consensus 121 ***** ***** *****
```



```
S. enterica 181 PQLLDEQIEQAV
E. coli 181 PQLLDEQRVQAV
consensus 181 ***** ***
```

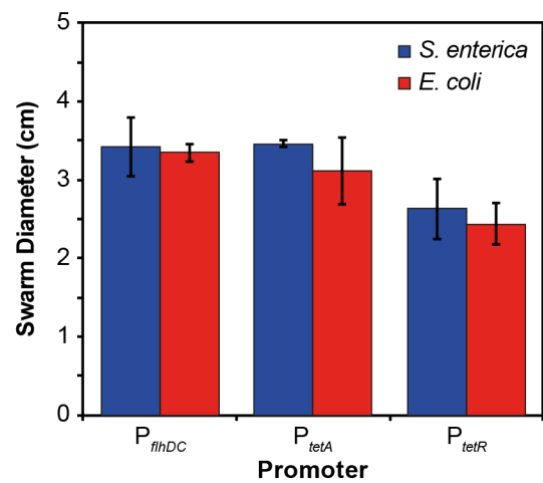
Protein alignment of FlhD and FlhC from *S. enterica* and *E. coli*. Both proteins exhibit a high level of similarity. For both proteins the greatest level of diversity occurs towards the C-termini of each protein. How these amino acid changes impact the activity of the FlhD₄C₂ complex is still unknown. However, the data presented in the current study can act as a foundation to investigate their impact.

Figure S2



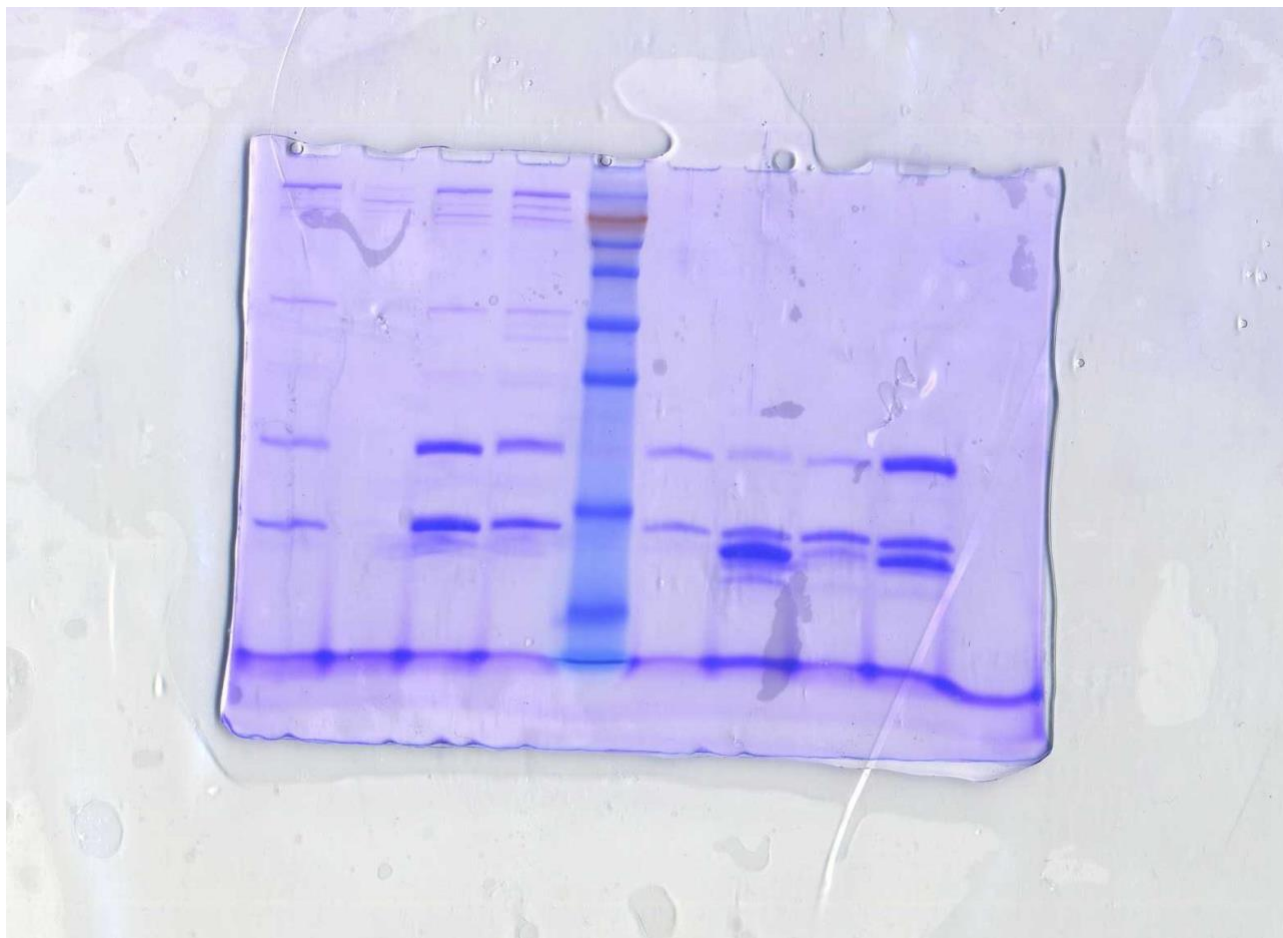
Schematic diagram detailing the 3 replacements made during this study. The *flhD* ATG start (STRT) codon boundary and the *flhC* STOP (STP) codon boundaries are shown as examples of the replacement design. Known nucleotide changes for *E. coli* in these short regions are highlighted as red boxes with white text.

Figure S3



Motility of $flhDC_{ST}$ and $flhDC_{EC}$ driven by $P_{flhDCSE}$ compared to P_{tetA} or P_{tetR} expression. Quantification of swarms produced in motility agar after 6 to 8 hours incubation. Error bars indicate calculated standard deviations.

Figure S4



Unprocessed image of Gel shown in **Figure 5A**