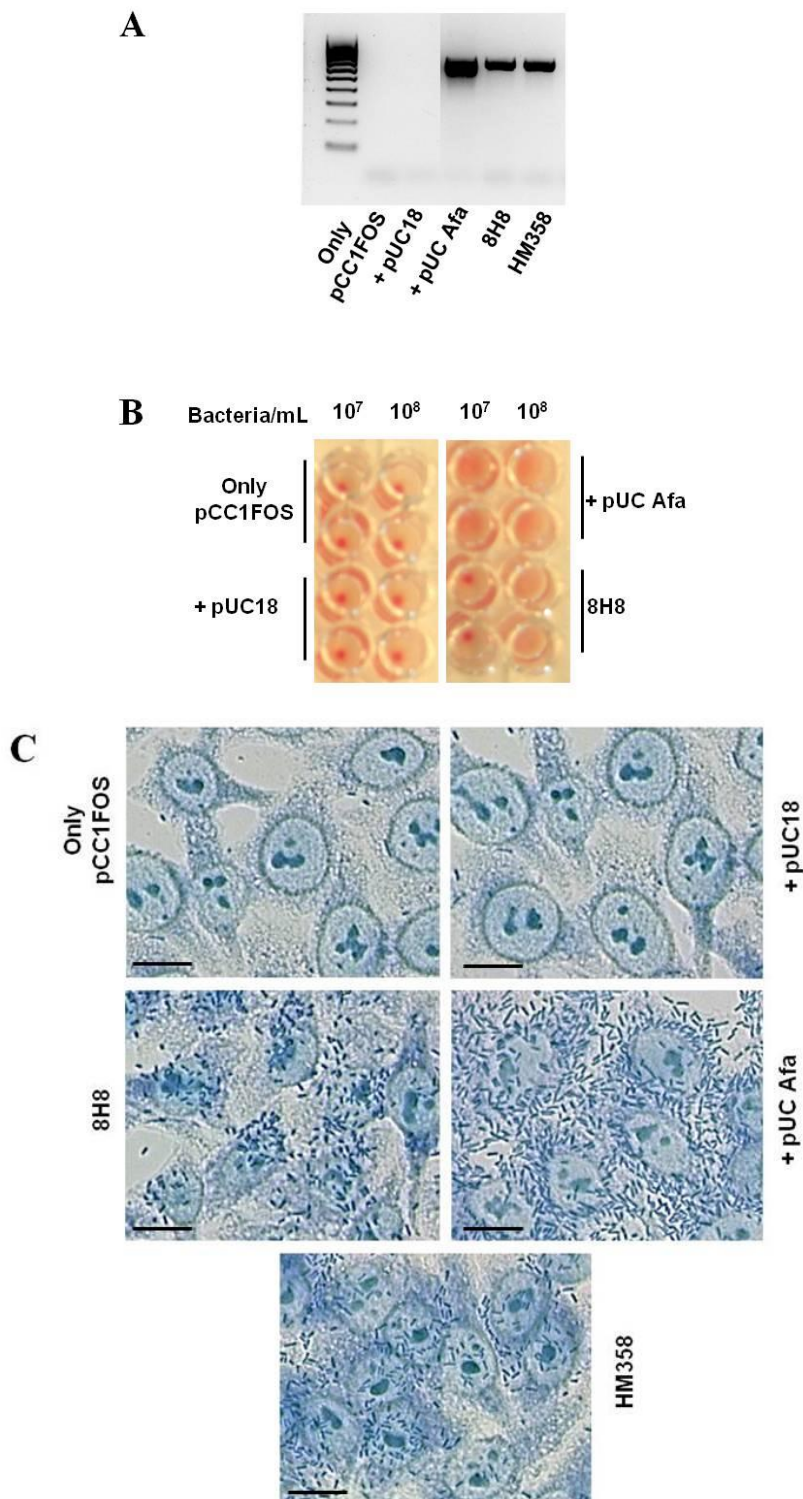


**Colonic mucosa-associated Diffusely-Adherent *afaC*+ *Escherichia coli* expressing *lpfA* and *pks* are increased in inflammatory bowel disease and colon cancer.** Maelle Prorok-Hamon, Melissa K. Friswell, Abdullah Alswied, Fei Song, Carol L. Roberts, Paul Flanagan, Paul Knight, Caroline Codling, Julian R. Marchesi, Craig Winstanley, Neil Hall, Jonathan M. Rhodes and Barry J. Campbell.

**Supplementary file S2**

**Figure S2(A): Phenotype characterisation of the full *afa-1* operon within *E. coli* EPI300™-T1<sup>R</sup>/pCC1FOS™ containing pUCAfa.** A) PCR for *afaC* (652bp amplicon). Hyperladder™ IV B) Haemagglutination assay and, C) Giemsa staining of *E. coli*-HEp-2 cell adhesion. Bar = 25 µm.



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**Table S2 - Oligonucleotide primers used to confirm orientation of the *afa-1* operon within *E. coli* EPI300<sup>TM</sup>-T1<sup>R</sup>/pCC1FOS<sup>TM</sup> containing pUCAfa.**

Oligonucleotide sequence (5'-3')	PCR product size (bp)
<p><b>pUC18 forward sequencing primer:</b> GTAAAACGACGGCCAGTG</p> <p><b><i>afa-1</i> reverse primer:</b> CCAATAATTACGCGACTCCC</p>	652
<p><b><i>afa-1</i> forward primer:</b> GCGGGTTCTGGACAAAATAA</p> <p><b>pUC18 reverse sequencing primer:</b> CAGGAAACAGCTATGACCAT</p>	759

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**Figure S2(B): Characterisation of the orientation of the full *afa-1* operon within *E. coli* EPI300<sup>TM</sup>-T1<sup>R</sup>/pCC1FOS<sup>TM</sup> containing pUCAfa.** A) PCR using pUC18 forward sequencing and *afa-1* reverse primer pair (expected amplicon of 652bp). B) PCR using *afa-1* forward primer and pUC18 reverse sequencing primer pair (expected amplicon of 759bp). Primer sequences detailed in Supplementary Table S2. PCR conditions as per *afaC* but with an annealing temperature of 55°C. Markers are 100bp Hyperladder<sup>TM</sup> IV.

