

**Colonic mucosa-associated Diffusely-Adherent *afaC*<sup>+</sup> *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 S4**

**Table S4: *E. coli* uptake and replication of fosmid library clones within J774-A1 murine macrophages**

Strain	Uptake of bacteria <sup>a</sup> CFU/wells x10 <sup>4</sup>	Fold replication 6h/3h <sup>b</sup>	Fold replication 24h/3h <sup>b</sup>
<b>HM358</b>	5.36± 1.1	<b>17.66± 3.80<sup>c</sup></b>	<b>11.26± 2.67<sup>c</sup></b>
<b>HA+ (<i>afaC</i><sup>+</sup>)</b>			
1a12	5.14± 2.3	2.37± 0.51	0.77± 0.19
2f4	5.52± 3.8	1.94± 0.40	0.19± 0.03
2f9	6.43± 2.7	1.84± 0.18	0.23± 0.05
3b9	3.99± 2.3	1.53± 0.28	0.20± 0.01
4b6	6.26± 2.8	1.89± 0.09	0.49± 0.06
4g9	10.86± 1.5	2.29± 0.51	1.54± 0.25
8h8	5.08± 2.7	1.19± 0.08	0.51± 0.28
9b12	7.75± 3.4	1.62± 0.05	0.14± 0.01
<b>HA- (<i>afaC</i><sup>-</sup>)</b>			
1c2	7.75± 3.4	1.81± 0.15	0.92± 0.10
3h5	2.38± 1.0	2.3± 0.48	1.18± 0.01
4c11	6.45± 2.7	2.15± 0.35	2.63± 0.60
5b9	5.62± 4.1	1.69± 0.31	0.18± 0.008
7c11	7.76± 6.6	1.66± 0.39	0.24± 0.05
8d8	6.29± 3.09	2.19± 0.16	0.44± 0.11
9b7	4.73± 2.0	1.61± 0.26	0.23± 0.06

**Haemagglutinin positive (HA+) and negative (HA-) library clones**

**Data expressed as means ± SEM, determined from N=2-7 independent experiments, with each experiment performed with n=2-3 replicates.**

<sup>a</sup> CFU recovered from lysed macrophages after 3h (2h infection followed by 1h gentamicin treatment)

<sup>b</sup> recovered intracellular bacteria from lysed macrophages after 6h or 24h, relative to intracellular numbers at 3h

**Statistical analysis was performed using Mann-Whitney U with Bonferroni correction.**

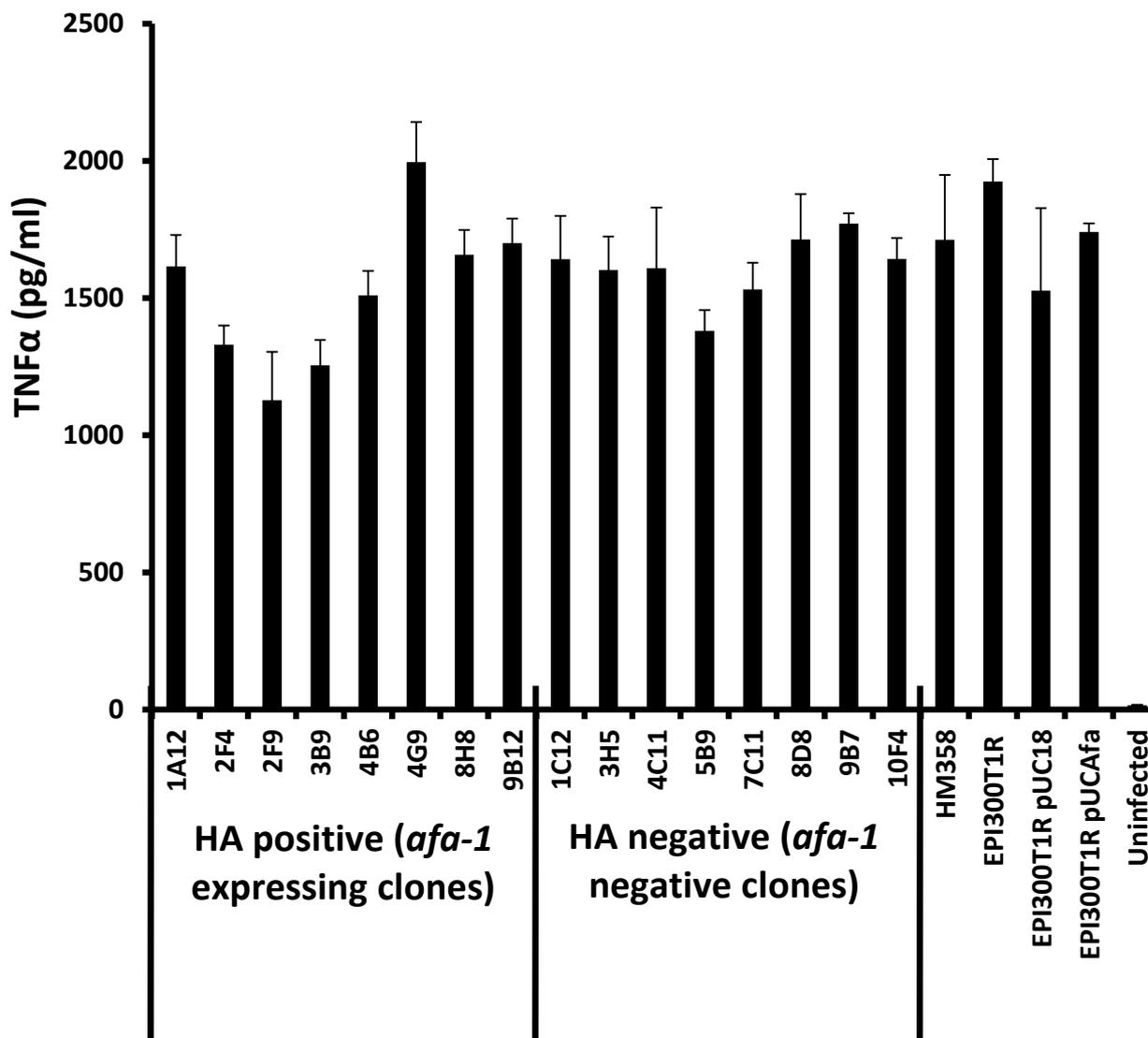
<sup>c</sup> significantly different from EPI300<sup>TM</sup>-T1<sup>R</sup>/pCC1FOS<sup>TM</sup>; p<0.0001.

**Colonic mucosa-associated Diffusely-Adherent *afaC*<sup>+</sup> *Escherichia coli* expressing *lpfA* and *pks* are increased in inflammatory bowel disease and colon cancer.**

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**Methods S4: Assessment of TNF $\alpha$  release from macrophages.**

Macrophages were infected with *E. coli*, library clones or *E. coli* constructs in antibiotic-free media for 2h, at MOI 10. Media was removed, macrophages washed and replaced with media containing 20 $\mu$ g/mL gentamicin. Followed 4h incubation, supernatant was collected and assayed using a commercial mouse TNF $\alpha$  Quantikine<sup>®</sup> ELISA (SMTA00: R&D systems, Abingdon; UK).



**Figure S4: TNF $\alpha$  release from macrophages by *afa-1* positive clones, wild type *E. coli* HM358 and *E. coli* expressing the full *afa-1* operon.** Murine J774.A1 macrophages were infected with *E. coli* for 2h, at MOI 10. The 8 HA positive fosmid library clones possessing the *afa-1* gene cluster, exhibiting no significant ability above HA negative clones to release TNF $\alpha$  to culture supernatant (6h post-infection) as assessed by ELISA. Similar TNF $\alpha$  levels were released by the wild-type strain HM358 and *E. coli* EPI300<sup>TM</sup>-T1<sup>R</sup>/pCC1FOS<sup>TM</sup> expressing Afa-1 from pUCAfa. Results are presented as mean  $\pm$  SEM (n=4 for clones; n=3 for other *E. coli*).