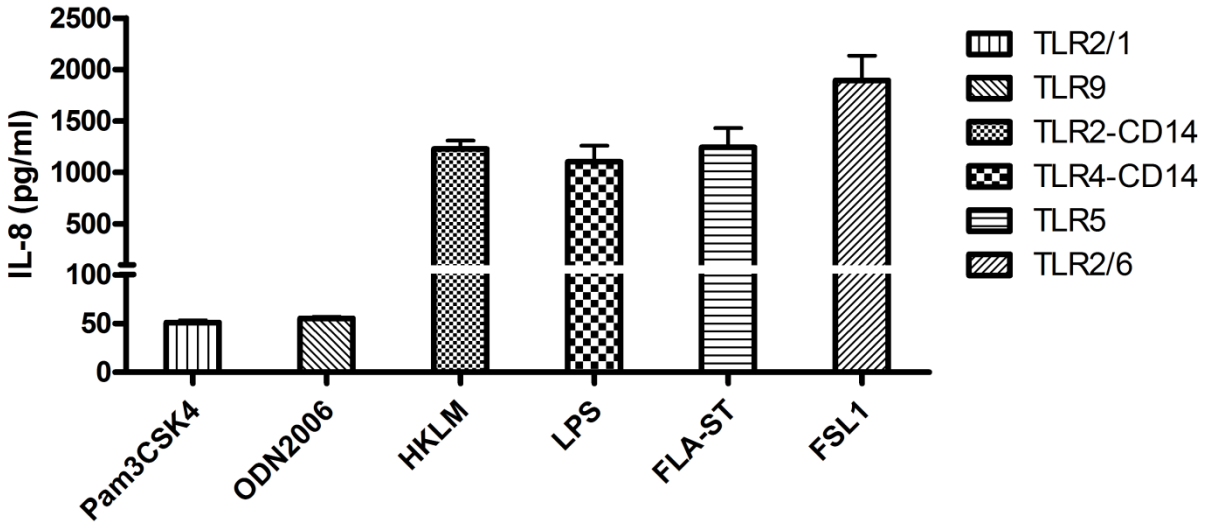


FIG S1. IL-8 response to TLR ligands.

Stably transfected HEK293 cells with one or two TLR genes were stimulated with specific ligands known to activate each TLR for 8h. Data represent mean ± SEM of three independent assays.

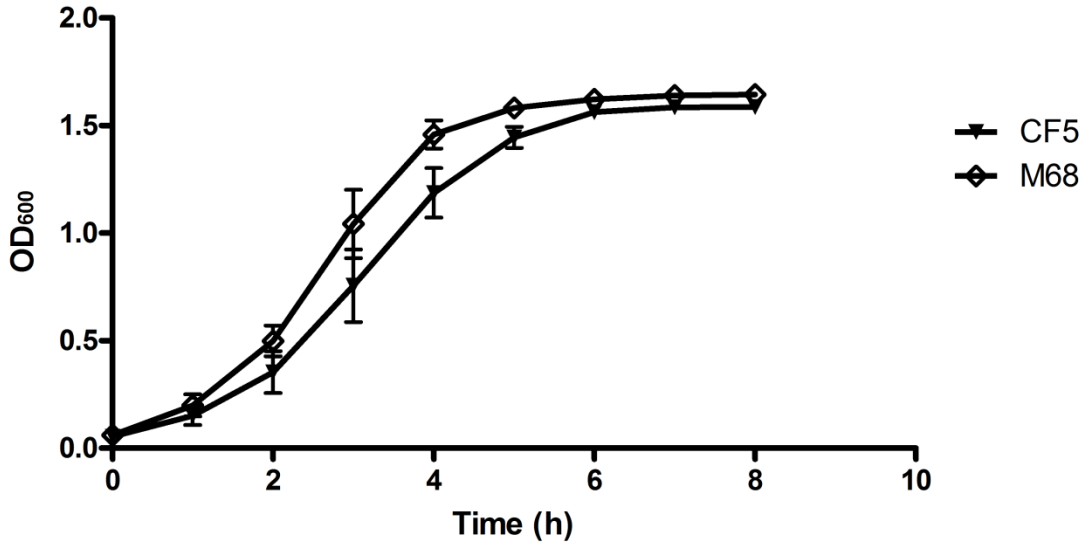


FIG S2. CF5 and M68 exhibit similar growth kinetics.

C. difficile strains were inoculated into BHI broth and growth rates monitored by optical density at 600nm. Statistically, there was no significant difference between the growth rates of CF5 and M68. Statistical significance was determined by ANOVA followed by Bonferroni's test. Data presented as mean ± SEM of three independent experiments.

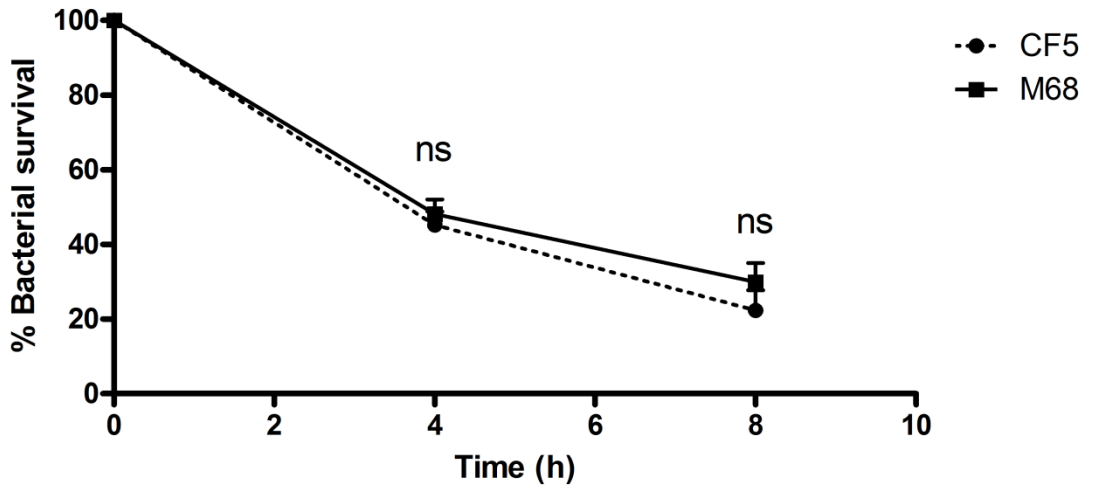


FIG S3. Viability status of *C. difficile* RT017 CF5 and M68 in aerobic conditions. Stationary phase bacterial cultures were exposed to aerobic conditions and cell survival determined by enumeration of colony formation units (cfu). Data represent mean ± SEM from four independent experiments. Statistical significance was determined by ANOVA followed by Bonferroni post-test. ns, not significant.

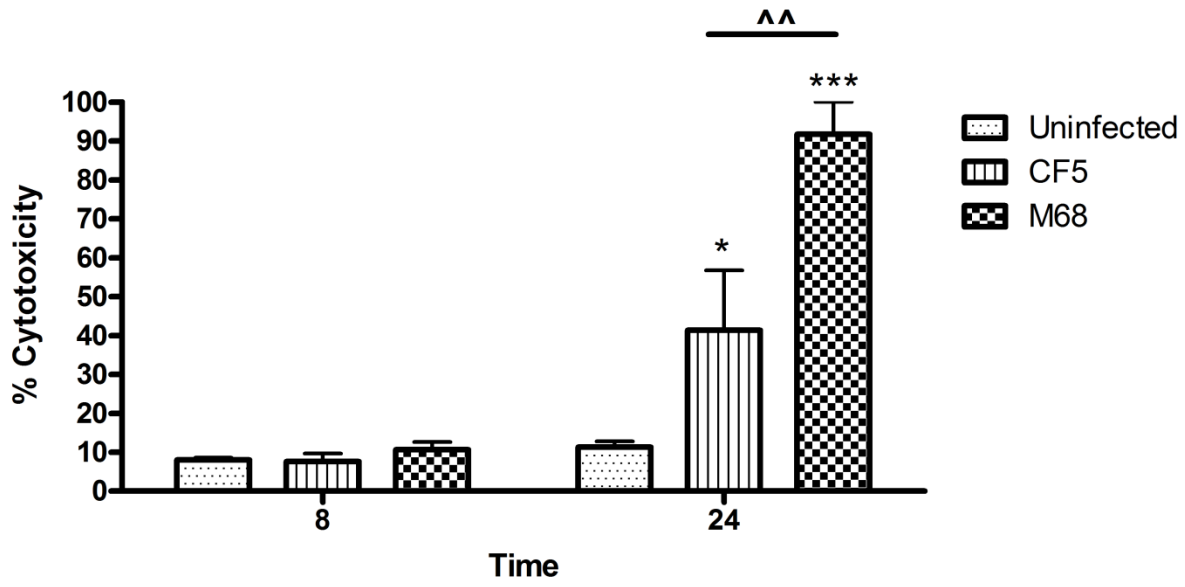


FIG S4. Lactate dehyrogenase (LDH) release by Caco-2 cell-lines exposed to CF5 and M68. Caco-2 monolayers were co-cultured with *C. difficile* strains at an MOI of 100 and LDH release was measured at 8 and 24h post-infection. Data represent mean ± SEM from five independent experiments. $^{*}p<0.05$, and $^{***}p<0.001$ represent significant difference from uninfected cells and $^{\wedge\wedge}p<0.01$ represents significant inter-strain difference. *P* values were obtained using ANOVA with Bonferroni post-test analysis

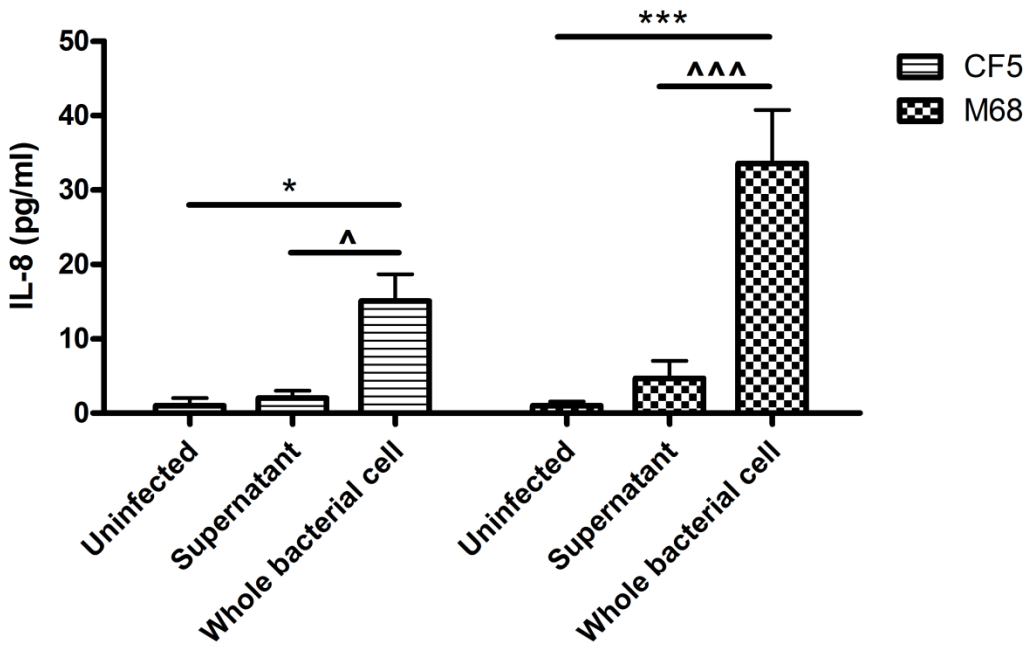
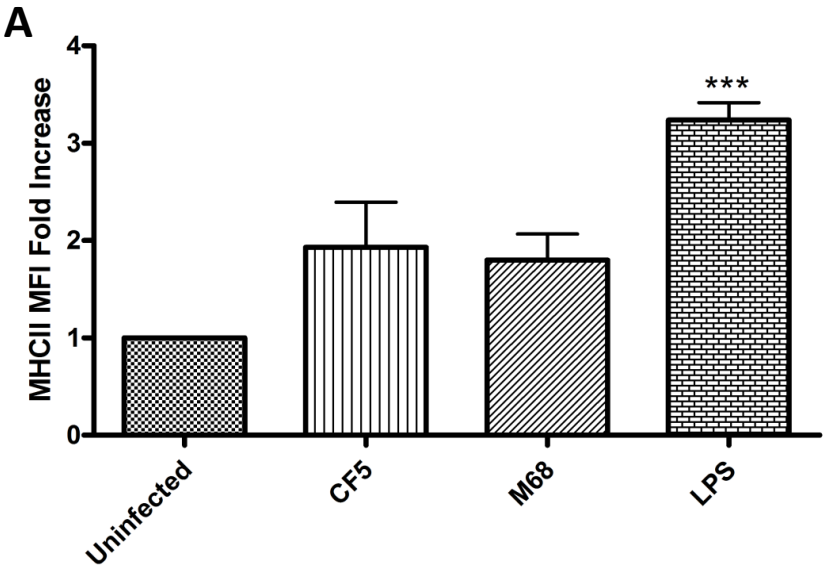
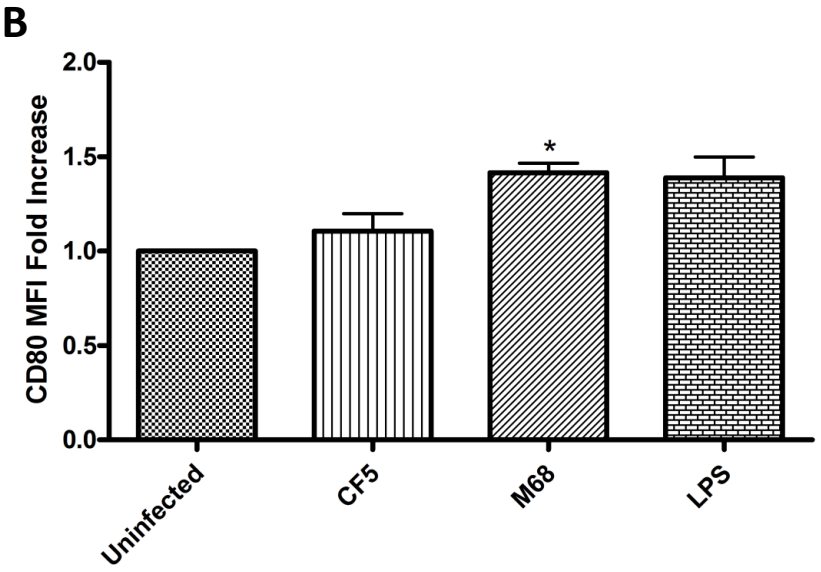
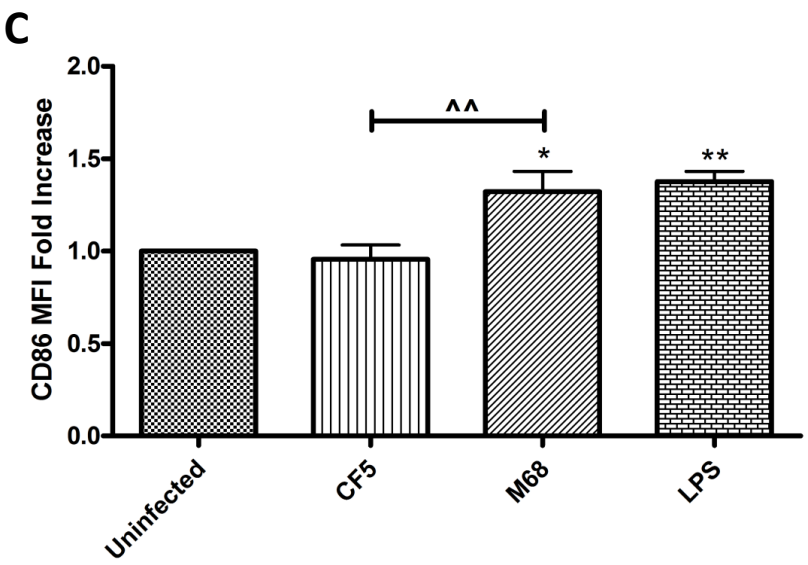


FIG S5. IL-8 protein expression in response to CF5 and M68.

Caco-2 monolayers were co-cultured with filter sterilised supernatant and whole bacterial cell of CF5 and M68 (MOI 500) and induction of IL-8 was measured 8h post-infection. Data represent mean ± SEM from three independent experiments. $^{*}p<0.05$ and $^{***}p<0.001$ represent significant difference from uninfected cells, and $^{\wedge}p<0.05$ and $^{\wedge\wedge\wedge}p<0.001$ represent significant inter-strain difference . *P* values were obtained using ANOVA with Bonferroni post-test analysis.


A
MHCII MFI Fold Increase
4
3
2
1
0
***
Uninfected
CF5
M68
LPS


B
CD80 MFI Fold Increase
2.0
1.5
1.0
0.5
0.0
*
Uninfected
CF5
M68
LPS


C
CD86 MFI Fold Increase
2.0
1.5
1.0
0.5
0.0
^^
*
**
Uninfected
CF5
M68
LPS

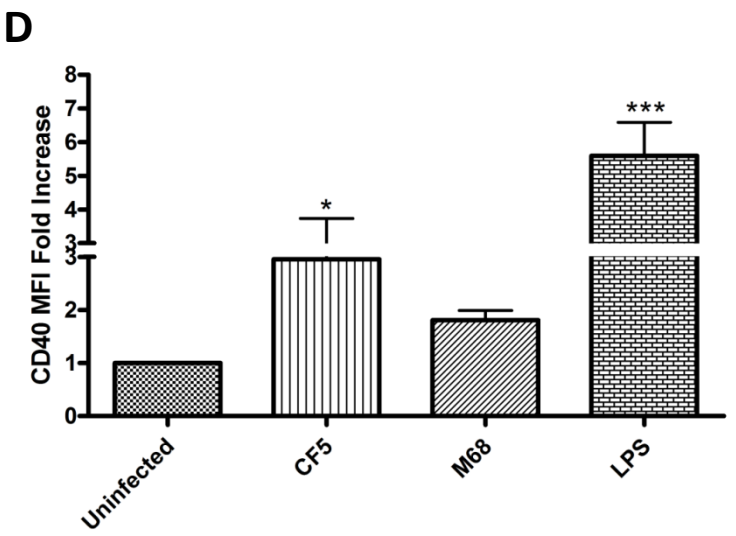

D
CD40 MFI Fold Increase
8
7
6
5
4
3
2
1
0
*
***
Uninfected
CF5
M68
LPS

FIG S6. *C. difficile* RT017 CF5 and M68 mediated BMDC activation.

BMDCs were stimulated with bacterial cultures (MOI 10). 8h post-stimulation, cells were labelled with anti-MHC II, CD80, CD86 and CD40 antibodies and analysed by flow cytometry. Surface expression was quantified and presented as mean fluorescence intensity (MFI) fold increase (A-D). 1 μg/ml LPS was included as a control. Data is represented mean ± SEM from three independent experiments performed in duplicate. $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ represent significant difference from uninfected cells and $^{\wedge\wedge}p<0.01$ represents significant inter-strain difference. *P* values were obtained using ANOVA with Bonferroni post-test analysis.

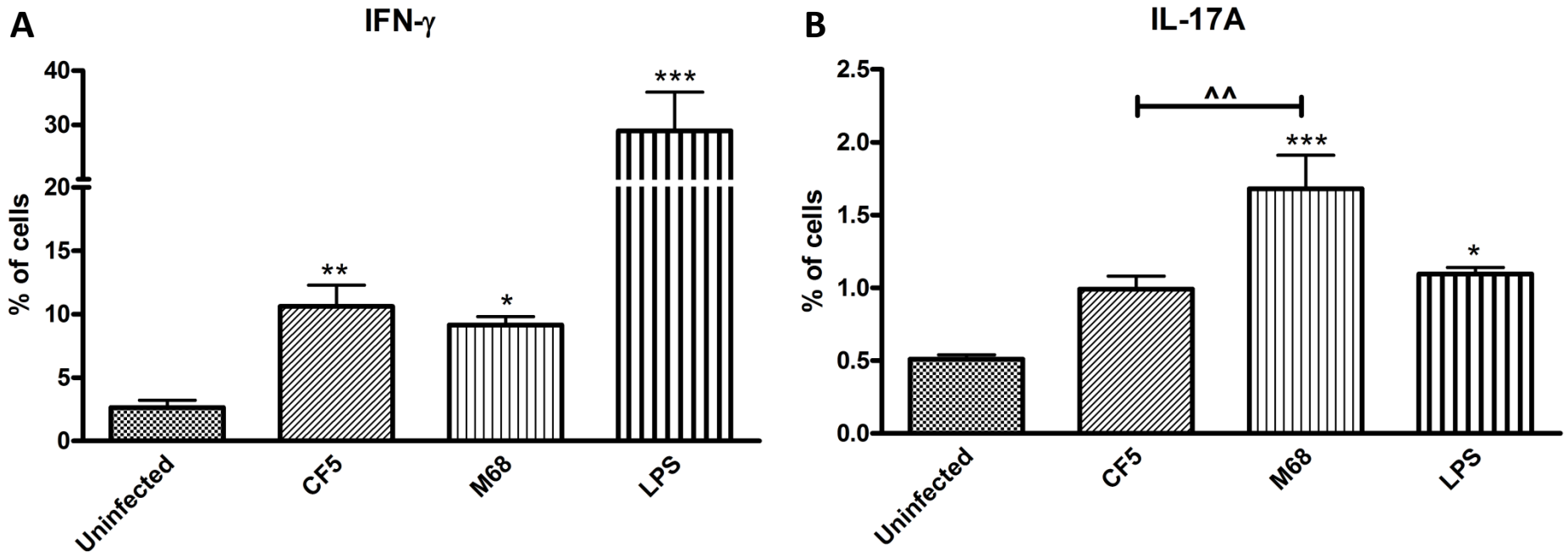


FIG S7. Increase in IFN-γ and IL-17 producing CD4⁺ T cells in response to *C. difficile* RT017 CF5 and M68.

Naive OT-II CD4⁺ T cells were co-cultured with CF5 and M68-stimulated BMDCs in the presence of $OVA_{323-339}$ for 96h. Intracellular expression of IFN-γ and IL-17 was quantified by flow cytometry gated on CD4⁺ cells. 1 µg/ml LPS served as a positive control. Quantified results were presented as percentage of IFN-γ⁺ (A) and IL-17⁺ (B) cells. Data represent mean ± SEM of three independent experiments. $^{*}p<0.5$, $^{**}p<0.01$ and $^{***}p<0.001$ represent significant difference from uninfected controls and ^^p<0.01 represents significant inter-strain difference. *P* values were obtained using ANOVA with Bonferroni post-test analysis.