Table II. Change in TLR mRNA levels following TNFα or FliC stimulation.

| Gene | Fold change TNFα stimulated | Fold change FliC stimulated |
|---|---|---|
| TLR1 | ND | ND |
| TLR2 | 0.6 | 1.3 |
| TLR3 | 1.6 | 0.6 |
| TLR4 | 3.5 | 1.1 |
| TLR5 | 0.9 | 0.5 |
| TLR6 | 0.9 | 0.6 |
| TLR7 | M | M |
| TLR8 | ND | ND |
| TLR9 | 0.2 | 0.5 |
| TLR10 | 0.3 | 0.5 |
| GAPDH | 1.0 | 1.0 |

ND None detected by $RT^2PCR$
M None detected above level of minus RT control.

Reverse Transcription and Real Time PCR ($RT^2PCR$)- RNA was prepared from cells left untreated, stimulated with TNFα (10ng/ml) or flagellin (1μg/ml) for 3h. $RT^2PCR$ was performed with an iCycler (Bio-Rad) to quantify SYBR-green labeled products generated from PCR products of 1st strand cDNA prepared from TLR1 through TLR10 mRNA, 18S rRNA, and GAPDH mRNA. $RT^2PCR$ (25ul reaction volume) was performed with the appropriate primers (SuperArray) in triplicate with HotStart Taq DNA polymerase (SuperArray) at 95°C for 5 min to activate Taq and amplified for 40 cycles (95°C, 30 sec, 55°C, 30 sec, 72°C, 30 sec). $RT^2PCR$ was performed on the minus RT controls with TLR5 primers to detect DNA contamination. Fold change in mRNA expression was expressed as $2^{\Delta\Delta Ct}$. ΔCt is the difference in threshold cycles for the TLR mRNAs and 18S rRNA. ΔΔCt is the difference between ΔCt non-simulated control and ΔCt stimulated sample.