

Supplemental Material

Differential recognition of *Vibrio parahaemolyticus* OmpU by TLRs in monocytes and macrophages for the induction of pro-inflammatory responses

Running title: Differential TLR recognition by VpOmpU

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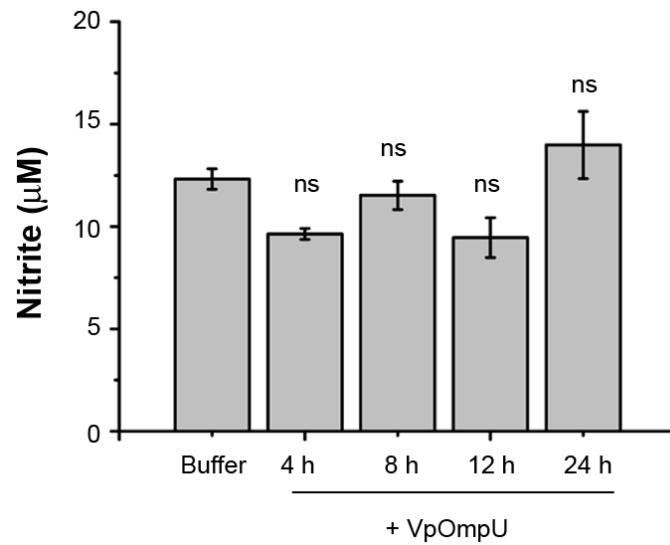


Figure S1. THP-1 cells do not produce nitric oxide in response to VpOmpU. THP-1 cells were treated with 10 µg/mL of VpOmpU, and were incubated for different time points. Supernatants were collected following each incubation, and were analysed for nitric oxide production (in terms of nitrite) using Griess' reagent. Bar graphs are expressed as mean ± SEM from three independent experiments (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns $p > 0.05$ versus buffer-treated cells).

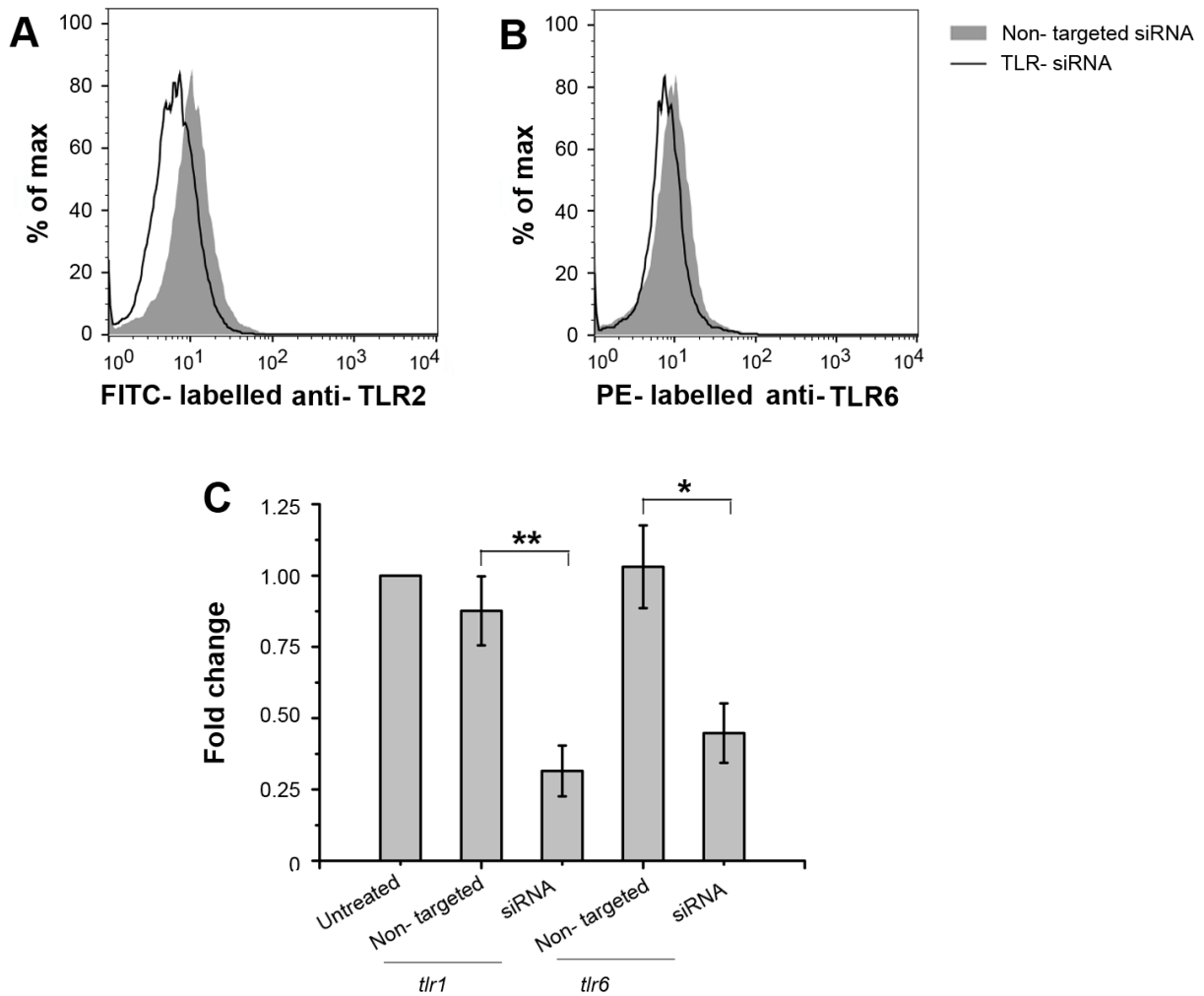


Figure S2 (A) Decrease in the surface expression of TLR2 upon siRNA-mediated knockdown in THP-1 cells. (B) Decrease in the surface expression of TLR6 upon siRNA-mediated knockdown in THP-1 cells. (A-B) For knock down, siRNA (TLR2, TLR6 and non-targeted) transfection was done for 48 h and cells were analysed by flow cytometry using FITC-conjugated anti-TLR2 antibody and PE-conjugated anti-TLR6 antibody. Data are represented as overlay histogram of targeted siRNA-transfected cells versus non-targeted siRNA-transfected cells. (C) Decrease in gene- expression of TLR1 and TLR6 upon siRNA-mediated knockdown in RAW 264.7 cells. Following siRNA knock-down for 24 h, cells were analysed by semi-quantitative PCR using primers for *tlr1* and *tlr6*. Fold change is calculated above untreated cells and bar graphs are expressed as mean \pm SD from three independent experiments (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns $p > 0.05$ versus VpOmpU-treated cells transfected with non-targeted siRNA).