Supporting Information

Salmonella Typhimurium adhesin OmpV activates host-immunity to confer protection against systemic and gastro-intestinal infection in mice

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Fig. S1: Knock-down profile of TLR6 (A) and TLR1(B) with siRNA in RAW 264.7 macrophages.
Fig. S2: Knock-down profile of TLR1 (A), TLR2 (B) and TLR6 (C) with shRNA in THP-1 monocytes.
Fig. S3: Knock-down profile of TLR, TLR2 and TLR6 with shRNA in HT29 cells.
Fig. S4: OmpV/ OmpV proteoliposome (PL) induces significant production of pro-inflammatory mediators by macrophages, monocytes and intestinal epithelial cells without affecting cell health adversely.
Fig. S5: Quantification of flow cytometry plots (histograms) showing surface expression of TLRs in response to OmpV-treatment.
Fig. S6: Densitometric analysis of western blots showing translocation of p65 and cRel from cytoplasm to nucleus in RAW 264.7 and THP-1 cells.
Fig. S7: Densitometric analysis of western blots showing translocation of AP-1 subunits in RAW 264.7 and THP-1 cells.
Fig. S8: Densitometric analysis of western blots showing phosphorylation of JNK and p38 in RAW 264.7 and THP-1 cells.
Fig. S9: No significant decrease in IL-6 production in OmpV-treated RAW 264.7 macrophages upon pretreatment with NOD signalling inhibitor indicating absence of peptidoglycan contamination in OmpV protein.

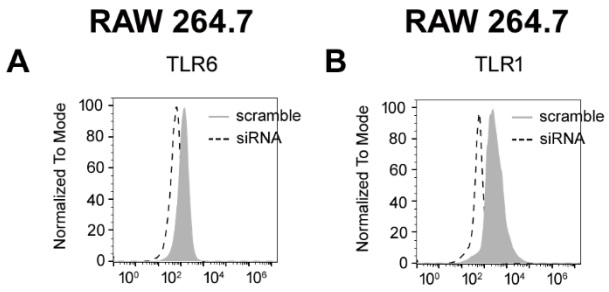

Fig. S1: Knock-down profile of TLR6 (A) and TLR1 (B) in RAW 264.7 macrophages upon transfection with siRNA. RAW 264.7 cells were transfected with siRNA. Following incubation, cells were harvested and surface expression of TLR6 and TLR1 was checked using flow cytometry. Scrambled siRNA transfected cells were used as control.

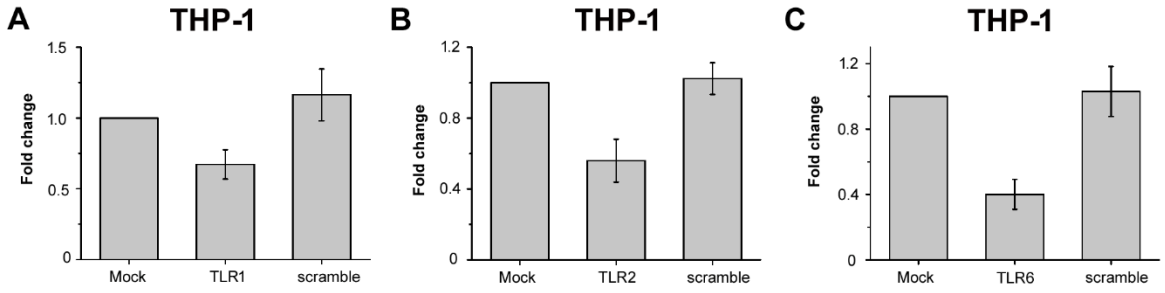

Fig. S2: Knock-down profile of TLR1 (A), TLR2 (B) and TLR6 (C) in THP-1 monocytes upon transfection with shRNA. THP-1 cells were transfected with shRNA. Following incubation, cells were harvested and whole-cell lysates were prepared. The levels of TLRs were checked using western blots and quantified using densitometry studies. Scrambled shRNA and mock transfected cells were used as control.

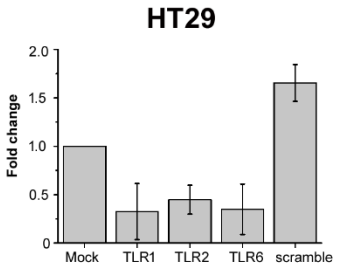

Fig. S3: Knock-down profile of TLR1, TLR2 and TLR6 in HT29 cells upon transfection with shRNA. HT29 cells were transfected with shRNA. Following incubation, cells were harvested and RNA was isolated. The levels of TLRs were checked using RT-PCR studies. Scrambled shRNA and mock transfected cells were used as control.

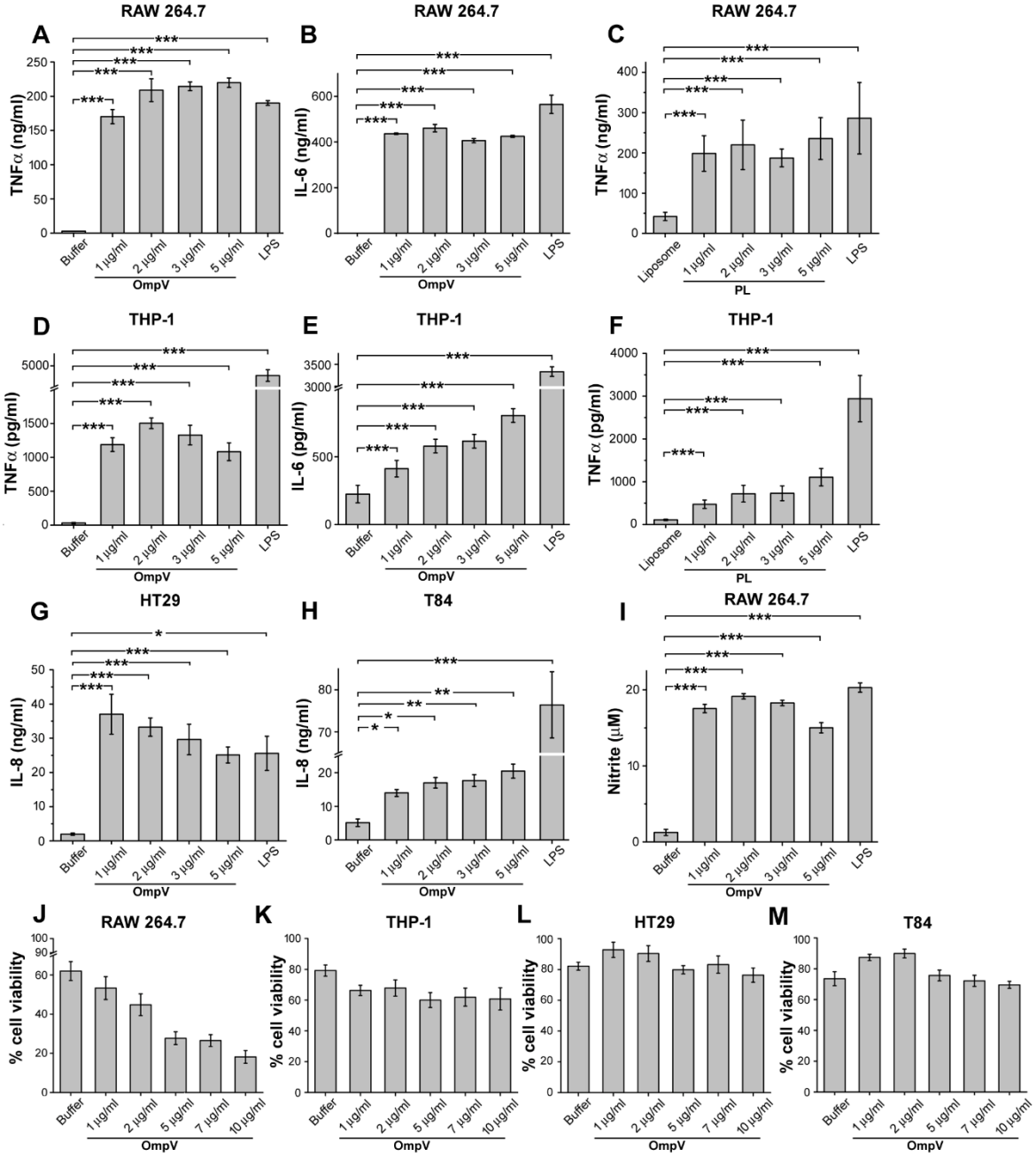

Fig. S4: (A-F) Significant production of TNFα and IL-6 in response to different doses of OmpV or OmpV-proteoliposome (PL) in RAW 264.7 macrophages (A-C) and THP-1 monocytes (D-F). (G-H) Significant production of IL-8 was observed in HT29 (G) and T84 (H) in response to different doses of OmpV. (I) Significant production of nitric oxide (NO) was observed in RAW 264.7 macrophages upon treatment with different doses of OmpV. (A-I) Cells were treated with PmB followed by different concentrations of OmpV or OmpV-proteoliposome (PL) as indicated and incubated for 24 h (A-C, E, G-I) or 4 h (D, F). Following respective incubations, supernatants were collected and estimated for the presence of TNFα and IL-6 (A-F) or IL-8 (G-H) or nitric oxide (I). LPS was used as a positive control. 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 the buffer-treated cells). (J-M) Cell viability assay indicated that OmpV in low concentration (1-2 μg/ml) minimally affects the cell health in case of macrophages, whereas, in cases of monocytes and IECs even higher concentrations of OmpV do not decrease cell viability. Cells were treated with PmB followed by different concentrations of OmpV as indicated and incubated for 24 h. Cell viability was calculated using MTT assay.

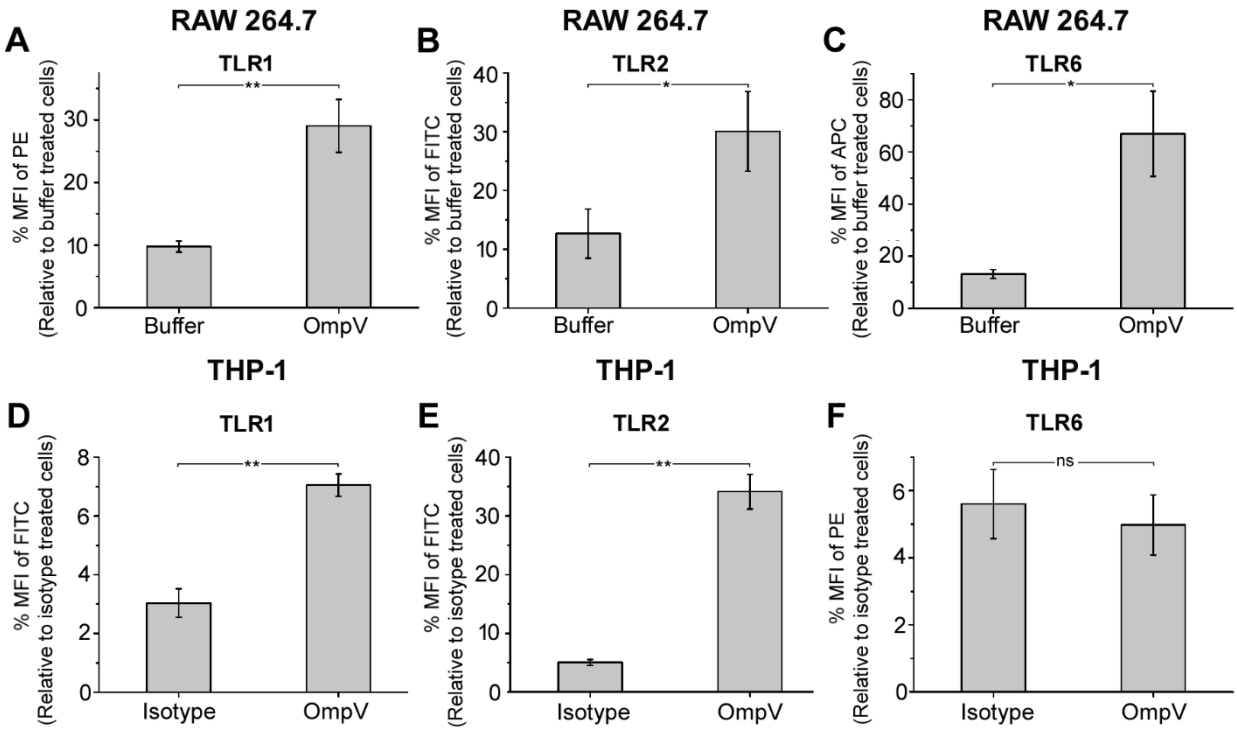

Fig. S5: OmpV induces increased surface expression of TLR1, TLR2 and TLR6 on RAW 264.7 macrophages (A-C) while TLR1 and TLR2 on THP-1 monocytes (D-F). Cells were treated with PmB followed by OmpV and incubated for 24 h. Following incubations, the surface expression of TLRs was analysed using flow cytometry. Bar graphs represent the percentage of MFI and are expressed as mean ±SEM from three independent experiments (*$p<0.05$, **$p<0.01$, ***$p<0.001$, ns $p>0.05$ versus the buffer-treated or isotype-treated cells).

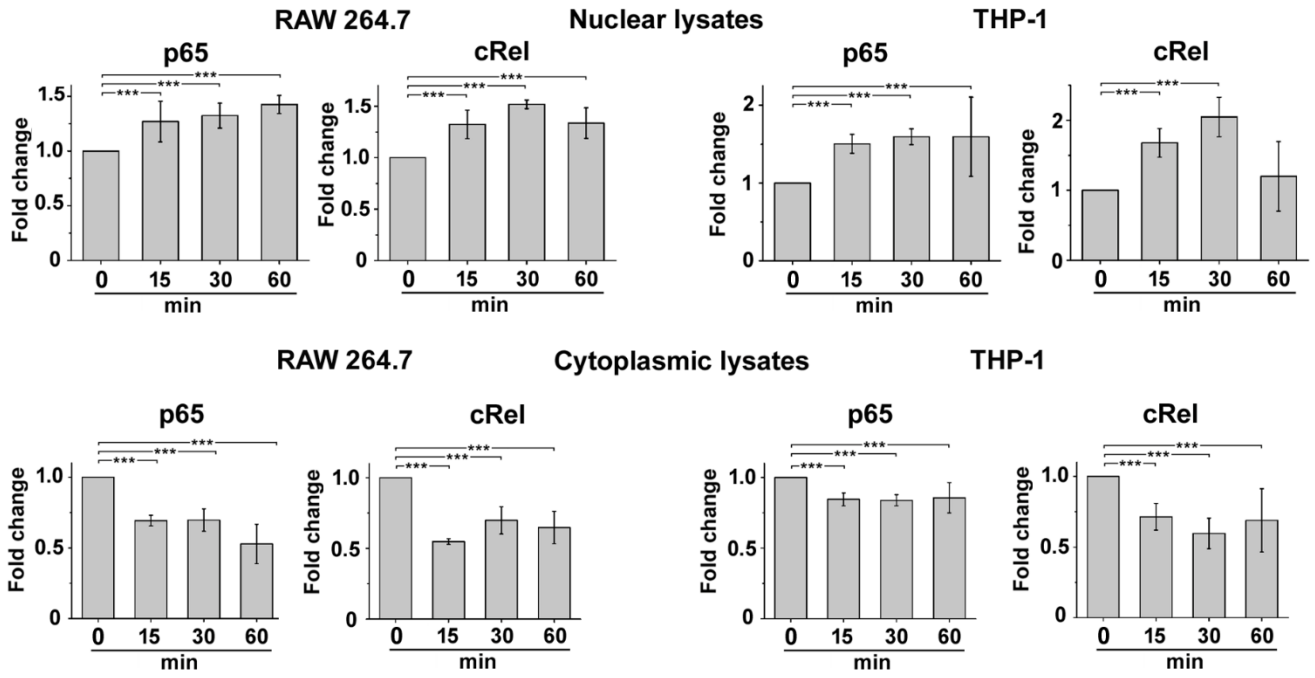

Fig. S6: Densitometric analysis of western blots indicating translocation of NFκB subunits p65 and cRel from the cytoplasm to the nucleus in RAW 264.7 macrophages and THP-1 monocytes. Bar graphs represent the fold change of proteins in nuclear and cytoplasmic lysate and are expressed as mean ±SEM from three independent experiments (*p<0.05, **p<0.01, ***p<0.001, ns p>0.05 versus 0 min).

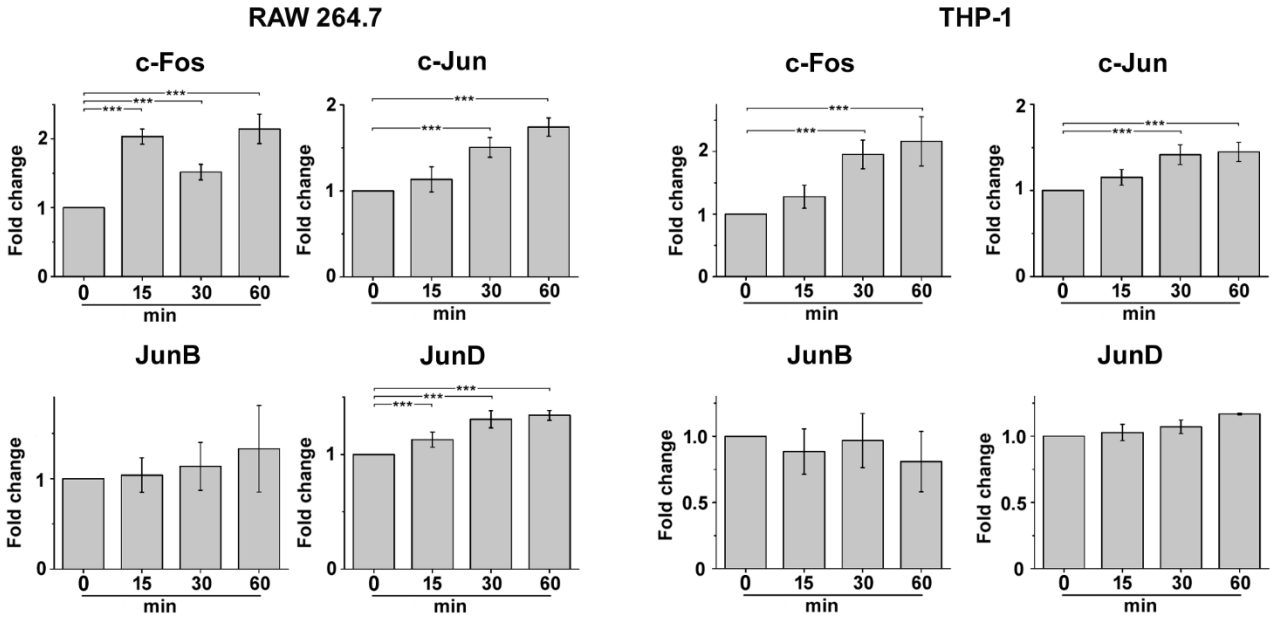

Fig. S7: Densitometric analysis of western blots indicating translocation of AP-1 subunits to the nucleus in RAW 264.7 macrophages and THP-1 monocytes. Bar graphs represent the fold change of proteins in nuclear and cytoplasmic lysate and are expressed as mean ±SEM from three independent experiments (*p<0.05, **p<0.01, ***p<0.001, ns p>0.05 versus 0 min).

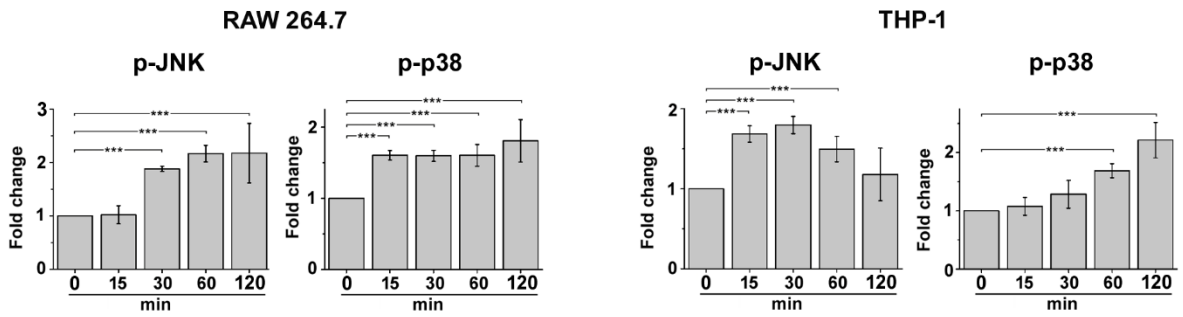

Fig. S8: Densitometric analysis of western blots indicating phosphorylation of p38 and JNK in RAW 264.7 macrophages and THP-1 monocytes. Bar graphs represent the fold change of proteins in nuclear and cytoplasmic lysate and are expressed as mean ±SEM from three independent experiments (*$p<0.05$, **$p<0.01$, ***$p<0.001$, ns $p>0.05$ versus 0 min).

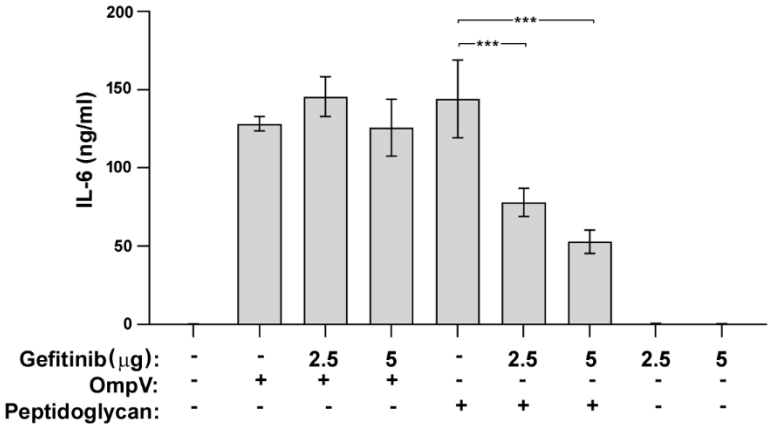

Fig. S9: No significant decrease in IL-6 production in OmpV-treated RAW 264.7 macrophages upon blocking NOD signalling indicating absence of peptidoglycan contamination in OmpV protein. RAW 264.7 cells were pre-treated with Gefitinib, a NOD signalling inhibitor for 30 min and then activated with OmpV. Peptidoglycan-activated macrophages were taken as positive control to check the efficacy of the inhibitor. Only inhibitor was taken as negative control. 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 peptidoglycan treated cells).