

Figure S1

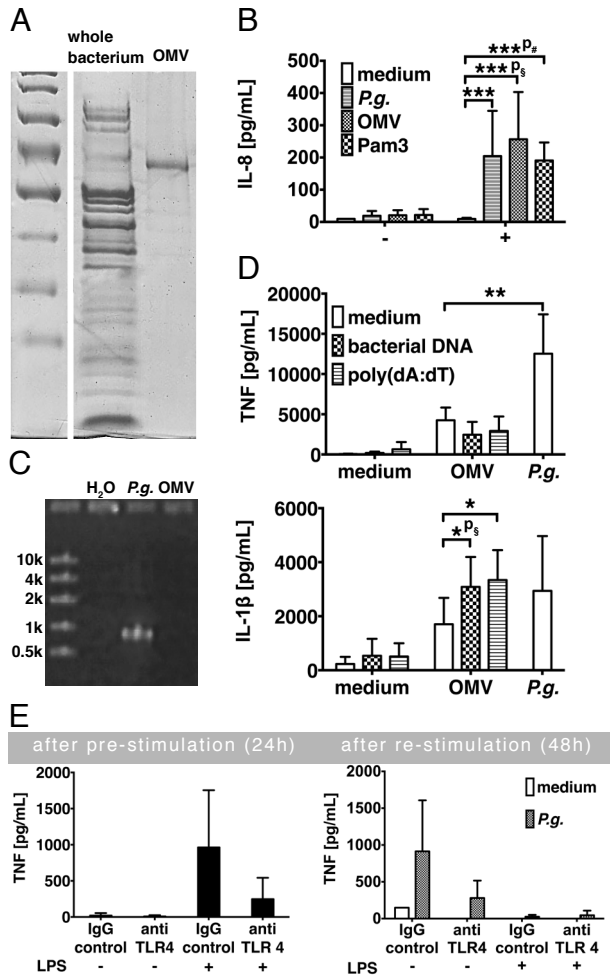


Figure S1: OMV protein content and TLR2-activity.

(A) The image shows a Coomassie stain to visualize proteins in *P.g.* (ATCC 33277) whole cell lysates and OMV. The blot is representative of $n=3$ independent experiments. (B) HEK 293 cells were transfected with and without TLR2 plasmid. Subsequent stimulation with *P.g.* (ATCC 33277), OMV and Pam3CSK4 (positive control) for 24 hours. IL-8 was quantified in the supernatants. Data are shown as mean \pm SD of $n=4$ donors ($n=3$ independent experiments). Statistical analysis: unpaired two-tailed Student's *t*-test; *** $p=0.00088$; *** $p_S=0.00016$; *** $p_Z\leq 0.0001$. (C) DNA was purified from OMV and *P.g.* (ATCC 33277) cell pellets. 16S rDNA PCR products were loaded on a 2% agarose gel stained with ethidium bromide. The results visualized on the representative gel were obtained $n=12$ experiments. (D) CD14⁺ monocytes isolated were stimulated with combinations of OMV alone, lipofetamine complexed bacterial DNA (purified from TLR2-deficient *S. aureus* mutant SA113DIgt), lipofetamine complexed poly(dA:dT) or live *P.g.* (ATCC 33277). TNF and IL-1 β concentrations were measured in the cellular supernatants harvested after 24 hours. The graphs summarize the data from $n=6$ donors ($n=3$ independent experiments) as mean values \pm SD. * $p=0.0218$; * $p_S=0.0447$. (E) Human monocytes were pre-stimulated with or without *Salmonella minnesota* LPS; live *P.g.* (ATCC 33277) was used for secondary challenge after washing. The experiments were performed in the presence of neutralizing anti-TLR4 antibody or the isotype control. TNF secretion was measured in the supernatants harvested after 24 (left panel) and 48 h (right panel). The diagrams summarize the data obtained as mean values \pm SD of $n=2$ donors ($n=2$ independent experiments).