

Supplementary Figures

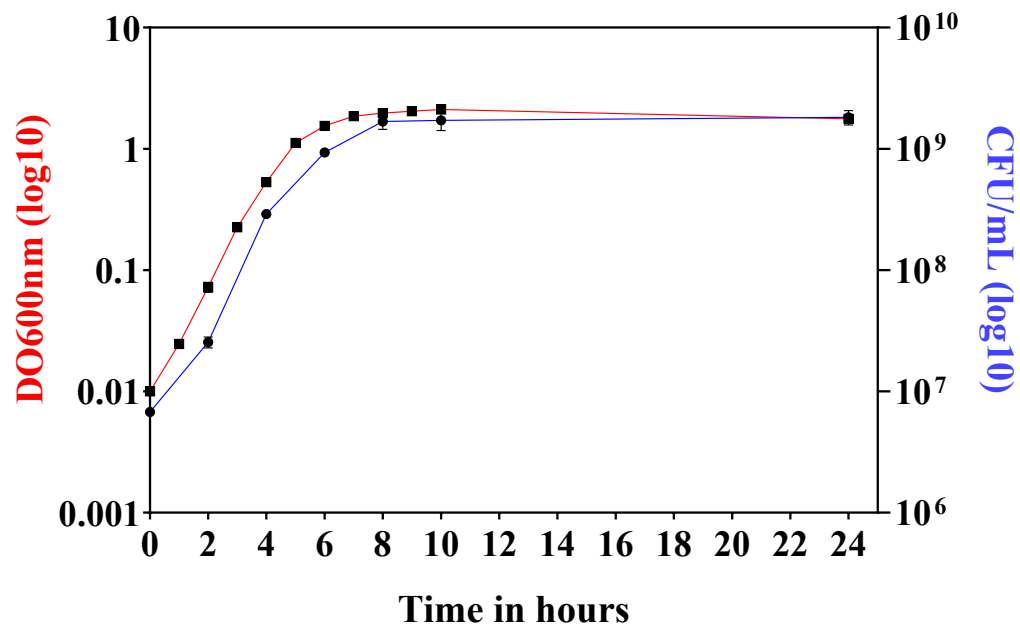


Figure S1. Growth curve of *S. aureus* N315 in RPMI+LB. Colony-forming units (CFU) were assessed every 2 hours. Data are presented as mean $\pm$ SD of biological triplicates.

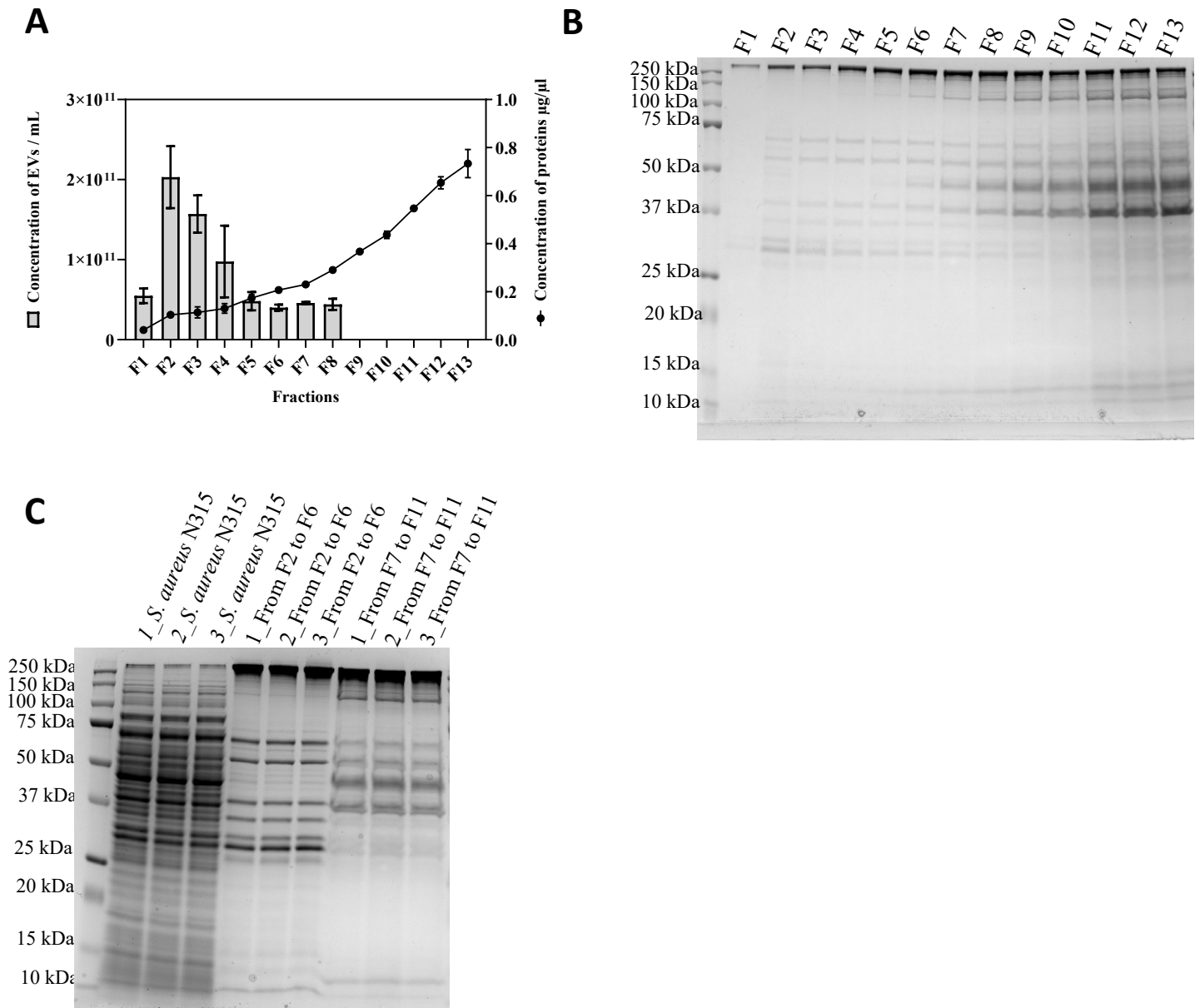


Figure S2. Selection of fractions to be sampled in size exclusion chromatography. The MRSA strain N315 was grown in eukaryotic cell culture medium RPMI+LB. After 16 h of culture, supernatant was filtered through 0.22 μm Nalgene top filters (Thermo Scientific) and concentrated with Amicon ultrafiltration systems (Millipore) 15 mL with a 100 kDa cut-off. SEC was performed according to the manufacturer's instructions by using qEV Cloumn Gen2 70nm. Each fraction of 0.4 mL was recovered and analyzed for proteins content with BCA (without EV lysis step) and NTA for particles concentrations (**A**) and analyzed in SDS-PAGE 12% gel (**B**). The same volume of each sample was deposited on each well to identify proteins contamination. Data are represented as mean $\pm$ SD of three biological replicates. (**C**) All EV extractions were performed in three biological replicates and fractions 2 to 6 and 7 to 11 were pooled together and concentrated with Amicon 0.5 mL 100 kDa. Seven μg of proteins in each sample were deposited on SDS-PAGE 12% gel with a N315 *S. aureus* lysate control.

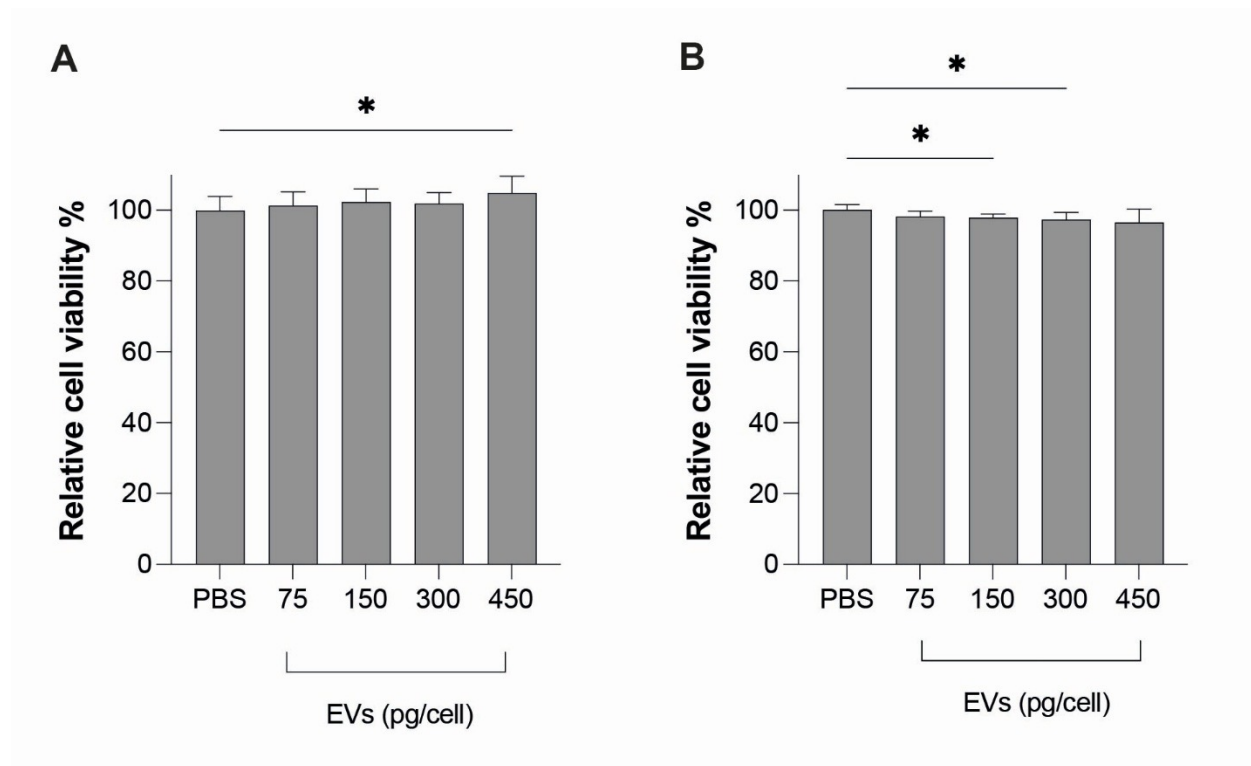


Figure S4. Effect of N315 derived EVs on MG-63 cells viability. After either 3 h (**A**) or 24 h (**B**) of interaction with quantity of EV ranging from 75 pg/cell to 450 pg/cell, cell viability was measured spectrophotometrically using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (G3580, Promega) and presented as percentage values relative to the control condition (PBS). Data are presented as mean $\pm$ SD of biological triplicates analyzed in technical triplicate (n = 9). Statistical analyses were performed using one-way ANOVA (panel A) or the Kruskal-Wallis test (panel B), depending on data normality : $p < 0.05$ (*).

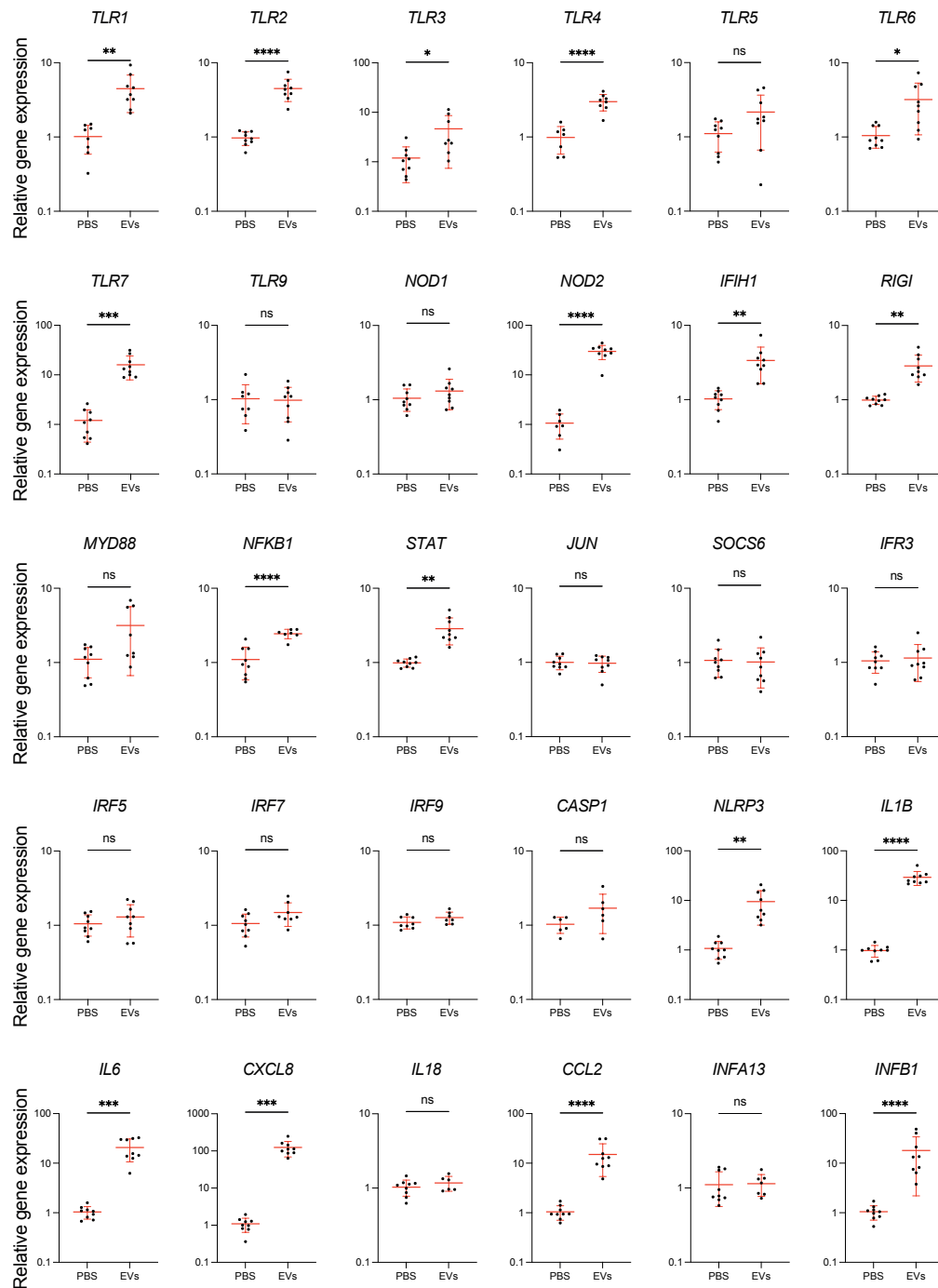


Figure S5. N315 EVs modulate the expression of immune-related genes in MG-63 cells. Relative fold change in mRNA expression of PPRs (*TLR1*, *TLR2*, *TLR3*, *TLR4*, *TLR5*, *TLR6*, *TLR7*, *TLR9*, *NOD1*, *NOD2*, *IFIH1*, *RIGI*), components of signaling pathways (*MYD88*, *NFKB1*, *STAT*, *JUN*, *SOCS6*, *IRF3*, *IRF5*, *IRF7*, *IRF9*, *CASP1*, *NLRP3*), and cytokines and chemokines (*IL1B*, *IL6*, *CXCL8*, *IL18*, *CCL2*, *INFA13*, *INFB1*) in MG-63 cells after 3 h stimulation with N315 EVs *versus* PBS-treated controls. Relative gene expression was calculated by the $2^{-\Delta\Delta C_t}$ method, using PBS-treated cells as control and *GAPDH*, *PGK1*, and *PPIA* genes for normalization. Data are presented as mean $\pm$ SD of biological triplicates analyzed in triplicate (n = 9). Statistical analyses were performed using unpaired t-test or Welch's t-test when required: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***); $p < 0.0001$ (****).

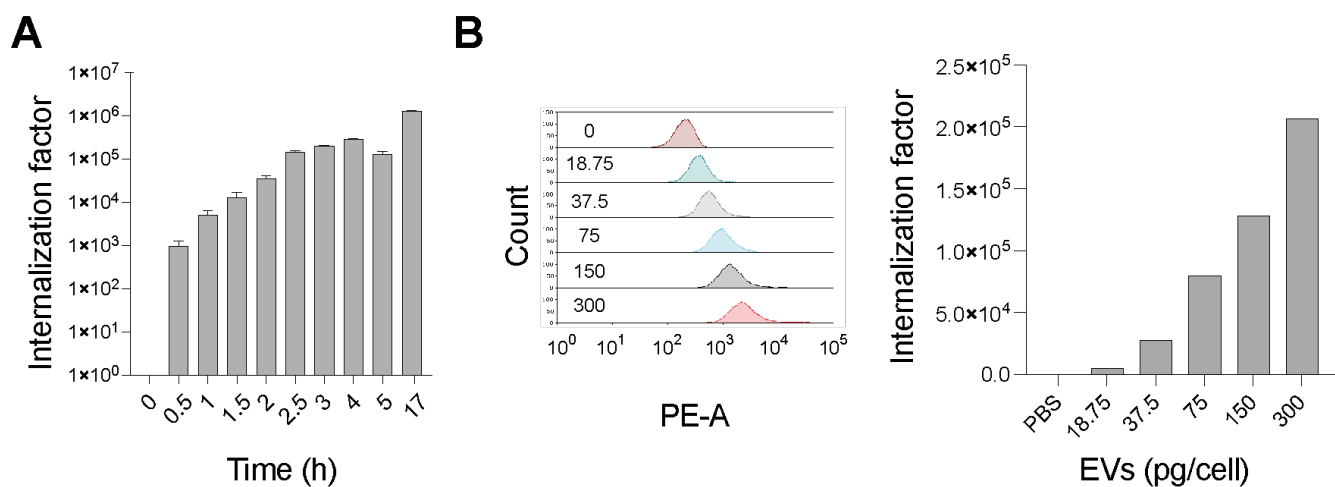


Figure S6. Interaction of N315 EVs with MG-63 cells. **(A)** Flow cytometry analysis of MG-63 cells incubated with DiI-labelled EVs at different incubation time. The graph shows a representative biological replicate, performed in technical triplicates, from three independent biological replicates. **(B)** Flow cytometry analysis of MG-63 cells after 3 h of incubation with increasing quantities of DiI-labelled EVs. Graphs correspond to one experiment with two technical replicates. Data were expressed as an internalization factor, calculated as the median fluorescence multiplied by the percentage of marker-positive events. Data were expressed as an internalization factor, calculated as the median fluorescence multiplied by the percentage of marker-positive events (Further details are provided in the Methods section).