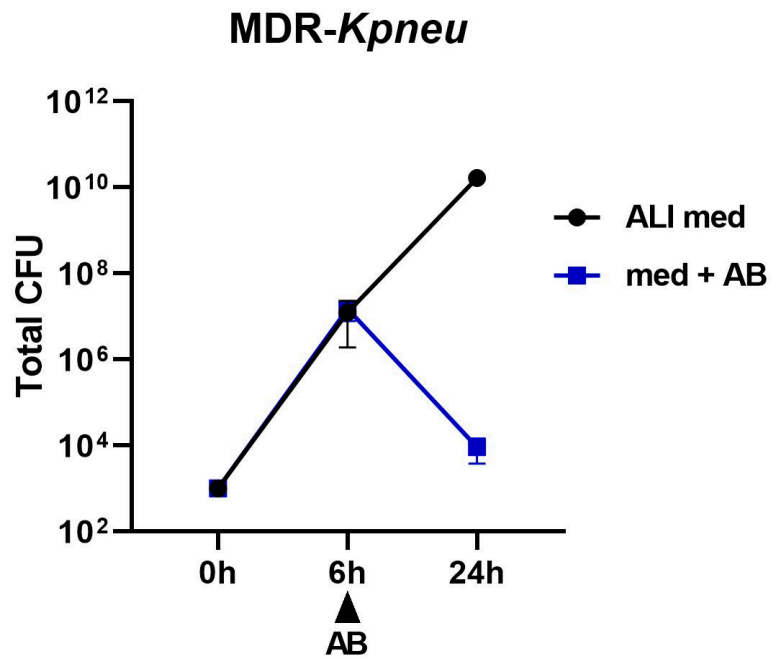


# **Immunostimulatory Effect of Flagellin on MDR-Klebsiella-Infected Human Airway Epithelial Cells**

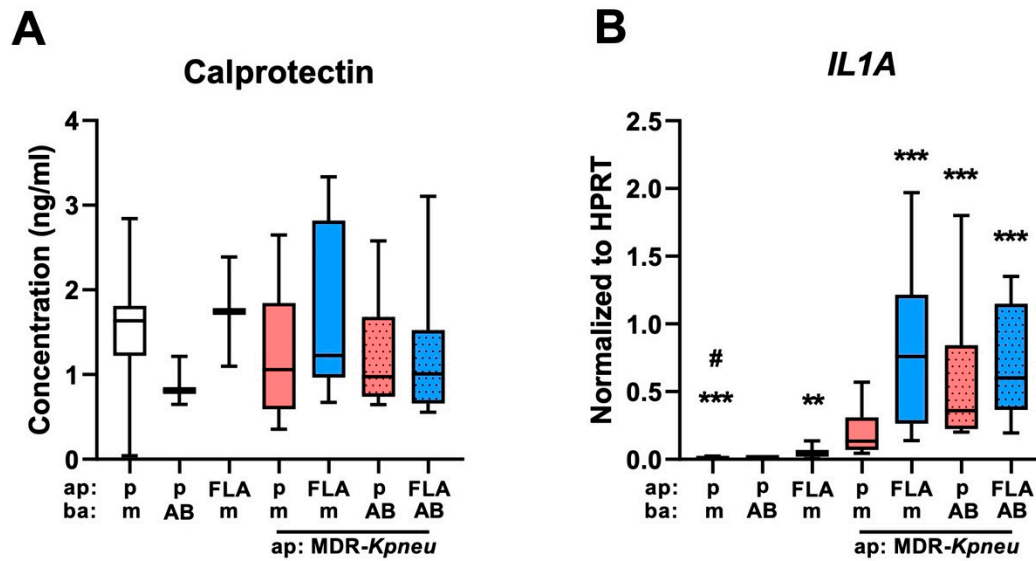
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## **Supplemental figures**

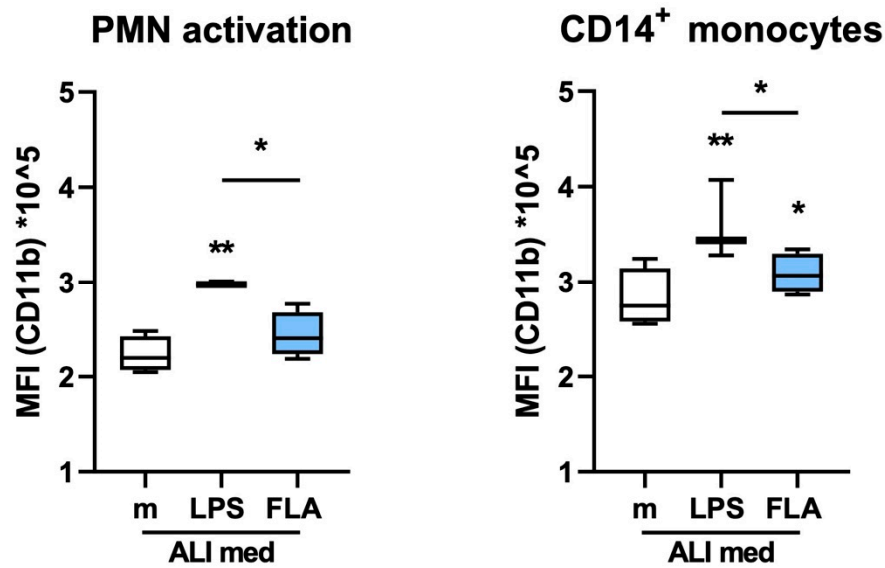
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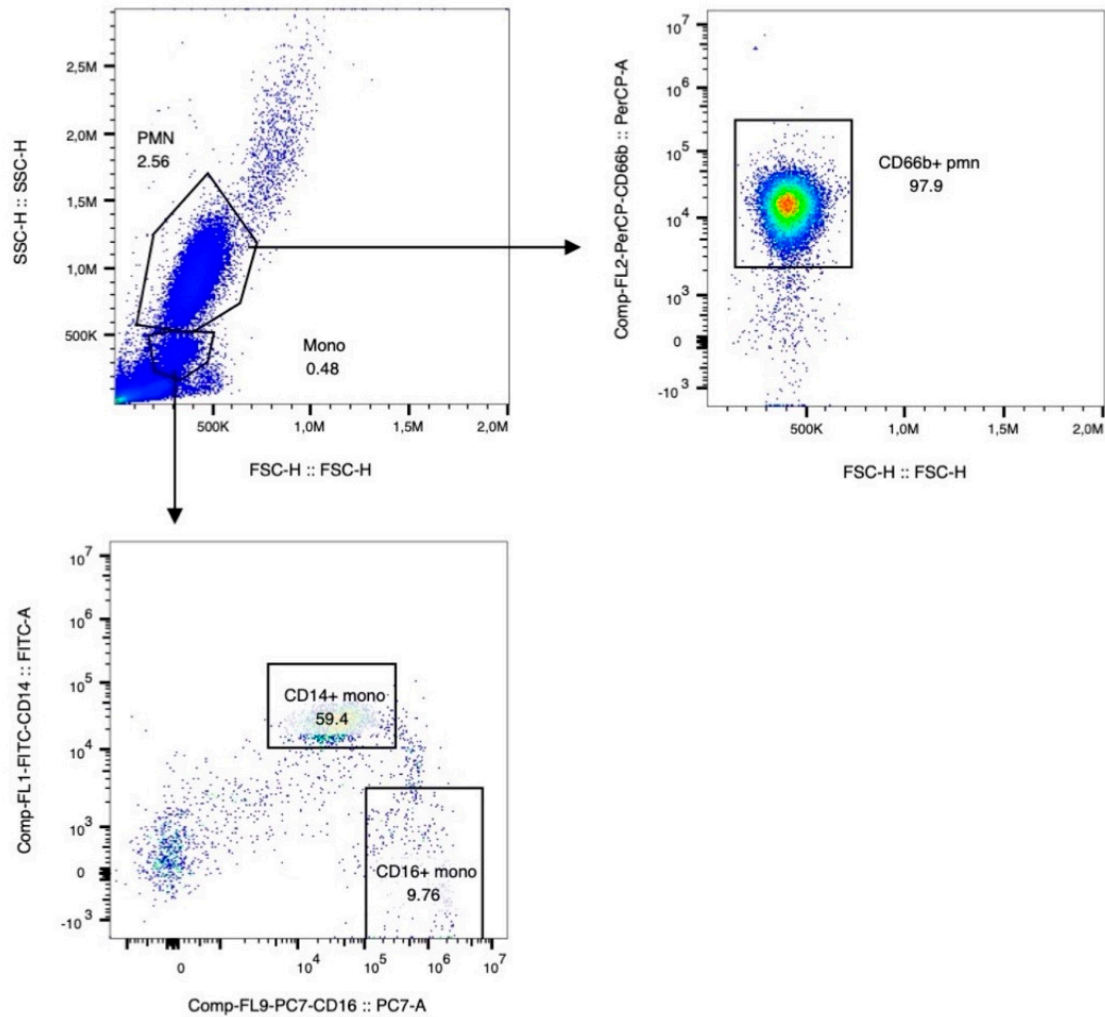
**Figure S1.** Effect of meropenem on outgrowth of MDR-*Kpneu* in ALI medium. ALI medium was inoculated with 1,000 CFU of log-phase MDR-*Kpneu* and incubated at 37°C and 5% CO<sub>2</sub>. After 6 hours, meropenem (AB) was added at a concentration of 50 µg/ml (> 6-fold of the MIC). Bacterial counts were determined at 6 and 24 hours after inoculation.



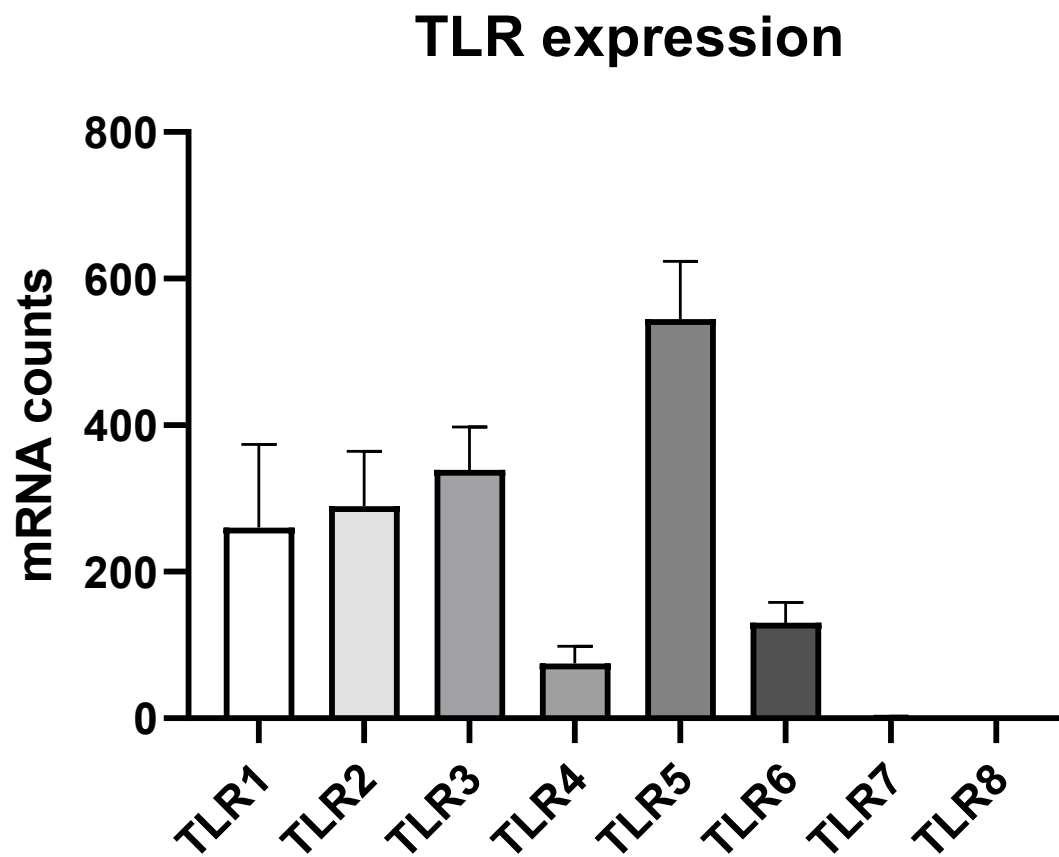
**Figure S2.** Effect of flagellin on Calprotectin secretion and IL1 $\alpha$  expression by MDR-*Kpneu* infected HBE cells. HBE cells were infected on the apical (ap) side with 1,000 CFU MDR-*Kpneu*, treated 6 hours later with 50  $\mu$ g/ml meropenem (AB) or medium (m) in the basolateral (ba) medium and 0.1  $\mu$ g flagellin (FLA) or PBS (P) on the apical side. Samples were collected 24 hours after infection. Protein levels of calprotectin (S100A8/A9 heterodimer) in basolateral medium (A). mRNA levels of IL-1 $\alpha$  (*IL1A*) relative to the housekeeping gene *HPRT* in HBE cells (B). Data are shown as box and whiskers representing 3 repeated experiments with n=4 per group. # indicate significant differences compared to FLA alone; \* indicate significant differences compared to MDR-*Kpneu* and medium treated cells. \*/#P<0.05, \*\*/#P<0.01, \*\*\*/###P<0.001.



**Figure S3.** Activation of PMNs and CD14<sup>+</sup> monocytes in whole blood. Plain ALI medium (m), supplemented with LPS (10 ng/ml) or flagellin (FLA; 1 µg/ml), was incubated with an equal volume of heparinized whole blood for 6 hours after which activation (CD11b mean fluorescence intensity (MFI)) was determined on PMN and CD14<sup>+</sup> monocytes by flow cytometry. Data are shown as box and whiskers representing data from 4 healthy blood donors with 4 technical replicates per group \*P<0.05, \*\*P<0.01.



**Figure S4.** Gating of PMNs and CD14<sup>+</sup> monocytes in whole blood. PMNs were defined as FSC<sup>high</sup>, SSC<sup>high</sup>, CD66b<sup>+</sup> cells and monocytes were defined either as FSC<sup>medium</sup>, SSC<sup>medium</sup>, CD14<sup>+</sup> cells or FSC<sup>medium</sup>, SSC<sup>medium</sup>, CD16<sup>+</sup> cells



**Figure S5.** Expression of different Toll-like-receptors (TLRs) in HBE cells. Total mRNA counts of TLRs as determined by RNA sequencing analysis described in reference 12 (accession number for the RNA sequencing data is GEO: GSE164704). Data represent duplicates of unstimulated HBE cells from two different donors.