

## Supplemental Material and Methods

### *Elastase and LDH assays*

According to the literature, elastase-release was used as marker for neutrophil activity and degranulation [i] and LDH-release was used as marker for cytotoxicity [iii]. Human neutrophils were resuspended in RPMI without phenol red containing 2% nuclease-free FCS (70°C heat-inactivated) and plated in non-treated tissue culture plates (Greiner Bio-One, CELLSTAR®) at a concentration of  $2 \times 10^6$  cells/ml. Neutrophils were stimulated with 25nM PMA for 20 min at 37°C in 5% CO<sub>2</sub>. Then, neutrophils were infected with bacteria at MOI of 2, the plates were centrifuged at 1600 rpm for 5 min and incubated for 90 min at 37°C in 5% CO<sub>2</sub>. After incubation, micrococcal nuclease (Worthington) was added at a concentration of 500 mU/ml to degrade NETs and to release elastase from NETs. The reaction was stopped with 5 mM EDTA and the plate was centrifuged at 1000 rpm for 8 min. For elastase measurement, 50 µl of the supernatant was incubated with 50 µl of 200 µM elastase substrate (N-(Methoxysuccinyl)-Ala-Ala-Pro-Val 4-nitroanilide, Sigma) for 30 min at room temperature. Optical density was measured at 405nm (VersaMax Tunable Microplate Reader, Molecular Devices). For LDH measurement, the CytoTox 96® Non-Radioactive Cytotoxicity Assay (Promega) was used according to the manufacturer's recommendations. The percentage of LDH or elastase release was calculated compared to 100% cell lysis control (cells lysed with 0.25 % Triton X-100 for 10 min).

### *Neutrophil killing assays*

Human neutrophils were resuspended in RPMI containing 2% nuclease-free FCS and plated in non-treated tissue culture plates at a concentration of  $2 \times 10^6$  cells/ml. Neutrophils were stimulated with 25 nM PMA for 20 min at 37°C in 5% CO<sub>2</sub>. Then, neutrophils were infected with bacteria at MOI of 2, the plates were centrifuged at 1600 rpm for 5 min and incubated for 30 and 90 min at 37°C in 5% CO<sub>2</sub>. After incubation, cells were lysed with 0.25 % Triton X-100 by pipetting up and down. Serial dilutions in sterile PBS were plated on THA plates for enumeration of surviving cfu. The percentage of surviving bacteria was calculated in comparison to bacterial growth control grown under the same conditions in the absence of cells.

### **References**

- i Pham CT: Neutrophil serine proteases: specific regulators of inflammation. *Nat Rev Immunol* 2006;6:541-550.
- ii Fotakis G, Timbrell JA: In vitro cytotoxicity assays: comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. *Toxicol Lett* 2006;160:171-177.