

## **Supplementary Materials and Methods**

### **Chemicals.**

All chemicals and reagents were of the highest purity available and were purchased from Sigma Aldrich Chemical Co. Ltd., unless indicated otherwise.

### **LC-MS/MS analysis**

For neutrophil cell membrane phox protein analysis, membrane samples underwent in-solution digestion using sequencing grade modified trypsin (Promega) at a 1:20 enzyme:protein ratio. A Dionex Ultimate 3000 nanoLC system (Thermo Fisher Scientific) coupled to an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific) was used for the nano LC-MS/MS analysis as previously described [1]. Quantitative label-free data analysis was carried out using Progenesis QI for Proteomics (software version 3.1; Non-linear dynamics, a Waters company) as described by the manufacturer ([www.nonlinear.com](http://www.nonlinear.com)). Peptide and protein identification was achieved using Proteome Discoverer 2.1 (Thermo Scientific) with Sequest HT, Mascot and Percolator. The data was searched against the NCBI Uniprot Swissprot 2017 Homo sapiens database containing 20,148 sequences.

### **AAT/fMLP binding protocol.**

For experiments investigating binding of fMLP to AAT, 10µm polystyrene beads (Polysciences Europe, Eppelheim, Germany) were coated with AAT in saline solution (0.9% NaCl [w/v]) as previously described [2]. In brief, the beads were suspended in saline with added AAT (100µg in 100µl) for 24 h in the dark at 4°C with gentle rotation. The beads were centrifuged ( $15,000 \times g$  for 4 min at 4°C) and the amount of AAT binding to the beads was indirectly determined by measuring the unbound levels by UV spectrometry. After washing with saline, the beads were blocked with 2% (w/v) BSA. AAT-loaded beads were washed and resuspended in solutions of a FITC-labelled fMLP for 30 min at room temperature. Uncoated beads (no added AAT) were also

suspended in solutions with the FITC-labelled fMLP. Fluorescence was counted using a BD FACSCalibur flow cytometer (BD Biosciences, Heidelberg, Germany) with a total of 10,000 events acquired. The data were analysed and the mean fluorescence units (MFU) for each experiment were determined using FlowJo software.

### **Supplementary references**

1. Murphy MP, McEnery T, McQuillan K, McElvaney OF, McElvaney OJ, Landers S, Coleman O, Bussayajirapong A, Hawkins P, Henry M, Meleady P, Reeves EP, McElvaney NG. alpha1 Antitrypsin therapy modulates the neutrophil membrane proteome and secretome. *Eur Respir J* 2020: 55(4).
2. Reeves EP, Ali T, Leonard P, Hearty S, O'Kennedy R, May FE, Westley BR, Josenhans C, Rust M, Suerbaum S, Smith A, Drumm B, Clyne M. Helicobacter pylori lipopolysaccharide interacts with TFF1 in a pH-dependent manner. *Gastroenterology* 2008; 135(6): 2043-2054, 2054 e2041-2042.