

**Supplemental Figure 1: Increased granule release is not secondary to apoptosis, necrosis or NET formation.**

A. The pH of the media was measured under normoxic and hypoxic conditions, with no significant differences recorded throughout the incubation period. B&C. There is no increase in apoptosis under the degranulation assay conditions. Neutrophils were incubated under normoxia (N) or hypoxia (H) for 4 h prior to treatment with GM-CSF (10ng/ml, 30 min) and fMLP (100nM, 10 min) (GM) or media control and stained with May-Grünwald-Giemsa (B). C. Neutrophils were incubated under normoxia (N) or hypoxia (H) for 4 h prior to staining with FITC annexin-TO-PRO-3 (Invitrogen). Healthy cells, early apoptosis, late apoptosis and necrosis are represented by double negative, Annexin positive, double positive and TO-PRO-3 positive respectively. D. NET formation is not increased by hypoxia. Neutrophils were incubated under normoxia (N) or hypoxia (H) for 4 h prior to treatment with GM-CSF (10ng/ml, 30 min) and fMLP (100nM, 10 min) (GM), PMA (20mM) or media control. NETs were quantified with cell-impermeable nucleic acid dye Sytox Green (1:1000) in a fluorescence microplate reader at baseline, post 4 hour incubation and post treatment. NETs are expressed as extracellular DNA as % of total DNA from triton-X (0.5%) lysed neutrophils.

**Supplemental Figure 2: CSM does not affect degranulation**

A: Neutrophils were incubated under normoxia or hypoxia  $\pm$  CSM (25-50%) for 4 hours prior to priming with GM-CSF (10ng/ml, 5 min) and activation with fMLP (100 nM, 10 min). Degranulation was measured by elastase activity assay. Results represent mean  $\pm$  SEM (n=3) B: CSM induced intracellular stress. Neutrophils were loaded with DC-FDA (0.2 $\mu$ M) for 10 min prior to exposure to a range of CSM concentrations (5-100% CSM, 30 min) or PMA (200nM, 10 min). The production of intracellular ROS was analysed by flow cytometry (h). Results represent mean  $\pm$  SD (n=2, each performed in triplicate)

### **Supplemental Figure 3: Neutrophil supernatant-mediated damage to primary lung epithelial cells**

A. Primary ciliated HBECs were scraped from a Transwell® insert and exposed to normoxic and hypoxic neutrophil supernatants (1 in 2.5 dilution) for 2 h. Cells were fixed with Sorensen phosphate-buffered (pH 7.4) glutaraldehyde containing 0.1% (v/v) hydrogen peroxide and osmium tetroxide (2 h at 4<sup>o</sup> C), embedded in Spurr's resin and imaged using a Philips CM 100 TEM. Representative images of a single experiment, 6 images per treatment, magnification = x2500. B. ALI cultures of primary HBECs were exposed to neutrophil supernatants (1:2.5 dilution, 500 µl) applied to the basal surface. 10µl samples were aspirated from the basal well at 0, 4, 8, and 24 h and analyzed by commercial LDH assay (Sigma). Results represent a single experimental procedure with all conditions run in duplicate.

### **Supplemental Figure 4: AKT phosphorylation of serine 473 under hypoxic conditions**

Hypoxic AKT serine-473 phosphorylation is increased after 15 min of GM-CSF stimulation and this is PI3K-γ dependent. Neutrophils were incubated under normoxia or hypoxia for 4 hours prior to priming with GM-CSF (10ng/ml, 15 min or 30 min). C&D AKT phosphorylation is further increased by fMLP stimulation. Neutrophils were incubated under normoxia or hypoxia for 4 hours prior to priming with GM-CSF (10 ng/ml, 15 min or 30 min), prior to activation by fMLP (100nM, 10 min). p-AKT was quantified by western blotting. Results represent mean ± SD.

### **Supplemental Movies: granule products affect ciliary function**

Primary HBECs grown in ALI culture were scraped from the Transwell® inserts, transferred to a chamber slide and exposed to undiluted neutrophil supernatants for 2 h. Recordings

were made at 0 and 2 h with a high-speed camera (500 fps). Ciliary beat frequency determined by analysis of  $n=3$  movies with CiliaFA at  $t=2$  hours; fresh cells 0 hours.







