**Online data supplement belonging to:**

**Myeloid-related protein-8/14 facilitates bacterial growth during pneumococcal pneumonia by Ahmed Achouiti *et al*.**

**Cytokine measurements:** MRP8/14 was measured by ELISA(1). Lung tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-10, Keratinocyte-derived chemokine (KC) and macrophage inflammatory protein 2 (MIP-2)(all R&D systems, Minneapolis, MN) and myeloperoxidase (MPO; Hycult Biotechnology BV, Uden, the Netherlands) were measured using ELISAs.

**Histology:** Lung pathology scores were determined as described(2-4). In brief, lungs were harvested at the indicated time points, fixed in 4% buffered formalin, and embedded in paraffin. 4 μm sections were stained with haematoxylin and eosin, and analyzed by a pathologist blinded for groups as described earlier. To score lung inflammation and damage, the entire lung surface was analyzed with respect to the following parameters: bronchitis, edema, interstitial inflammation, intra-alveolar inflammation, pleuritis, endothelialitis and percentage of the lung surface demonstrating confluent inflammatory infiltrate. Each parameter was graded 0–4, with 0 being 'absent' and 4 being 'severe'. The total pathology score was expressed as the sum of the score for all parameters. Granulocyte staining was done using FITC-labeled anti-mouse Ly-6G monoclonal antibody (BD PharMingen, San Diego, Calif., USA) as described earlier (5). MRP8 and MRP14 staining of lung tissue were performed as described previously(6).

**Whole blood stimulation:** Growth-arrested bacteria were prepared as described(7) In brief, *S. pneumoniae* were cultured and washed with pyrogen-free sterile saline and resuspended in sterile PBS to a concentration of $2\times10^9$ bacteria/ml. The concentrated *S. pneumoniae* preparation was treated for 1 hour at 37°C with 50 μg/ml

Mitomycin C (Sigma-Aldrich; Zwijndrecht, the Netherlands) to prepare alive but growth-arrested bacteria. Subsequently, the growth-arrested *S. pneumoniae* preparation was washed twice in ice-cold sterile PBS by centrifugation at 4°C, and the final pellet was dispersed in ice-cold PBS in the initial volume and transferred to sterile tubes. Undiluted samples of these preparations failed to generate any bacterial colonies when plated on BA plates, indicating successful growth arrest. Bacteria were washed and resuspended in RPMI and diluted to ten-fold lower bacterial concentrations ($2\times10^{3-6}$ cfu/ml). 100 µl of heparinized whole blood obtained from 4 individual Wt and *mrp14*$^{-/-}$ mice were then incubated with 100 µl of the different bacterial concentrations in a 96 wells plate and incubated for 5 hours at 37°C, 5% CO2. After incubation, plates were centrifuged at 4°C and supernatant was harvested for determination of TNF-α.

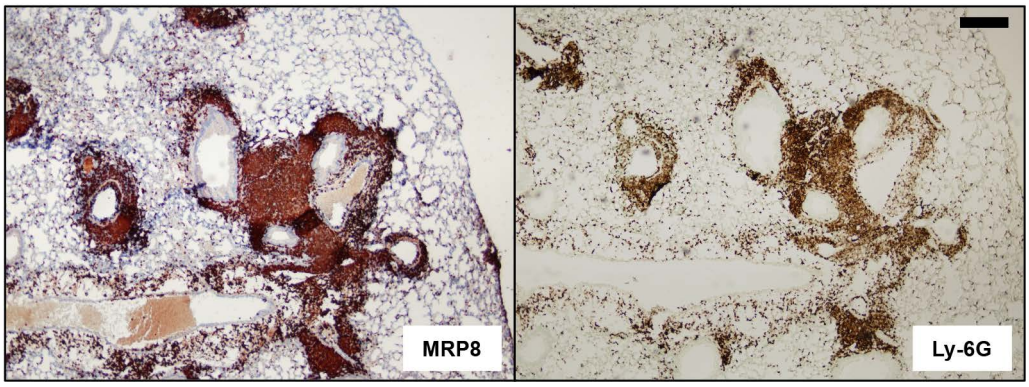
***In vitro* growth measurements:** *S. pneumoniae* and *K. pneumoniae* were grown to log phase and diluted to approximately 2000 cfu/ml in HEPES buffered IMDM. 50 µl of this bacterial suspension was added to 50 µl of HBSS without calcium and magnesium, (Gibco) supplemented with zinc (end concentration 100 µM, unless indicated otherwise), manganese (end concentration 0.1 µM) and heat inactivated fetal calf serum (HI-FCS; end concentration 10%) and incubated for 6 and 20 hours at 37°C, 5% $CO_2$ (n=4–6). Recombinant mouse MRP8 and MRP14 homodimers as well as MRP8/14 heterodimers were generated as previously described(8). Approximately 2000 cfu *S. pneumoniae* in 50 µl HEPES buffered IMDM, were added to a 50 µl HBSS solution with zinc (end concentration 100 µM), manganese (end concentration 0.1 µM) and HI-FCS (end concentration 10%) and increasing concentrations of MRPs. Bacteria and MRPs were incubated for 6 hours at 37°C, 5% $CO_2$. To test the growth inhibitory effect of MRP8/14 on *S. pneumoniae*, without supplementation of zinc and manganese, bacteria were grown to log phase and diluted to approximately 2000 cfu/ml in HEPES buffered

IMDM. 50 µl of this bacterial suspension was added to 50 µl of recombinant murine MRP8/14 in HBSS without calcium and magnesium (end concentration 0, 10, 50 and 100 µg/ml) and HI-FCS (end concentration 10%; n = 3) and incubated for a maximum of 24 hours. Growth was assessed by plating out ten-fold dilutions of bacterial concentrations on BA plates and incubation at 37°C for a maximum of 16 hours.

**Figure legends**

**Figure S1. MRP8 and Ly-6G staining in Wt mice**. Representative MRP8 (A) and neutrophil staining (B) of Wt mice 24 hours after *S. pneumoniae* infection ($5x10^4$ cfu). Scalebar indicates 200 µm.

Fig.S1



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