Supplemental Information

Supplemental Methods

Lung bacterial load

To evaluate the pulmonary bacterial load, the right lung of the euthanized mice from all three experimental groups were homogenized in PBS supplemented with 10% FCS and EDTA using a 70 µm cell strainer. Half of the homogenate was used to evaluate the pulmonary bacterial load. Serial dilutions of the homogenate were plated on LB-agar plates, and bacterial CFU were counted after 16 hours of incubation at 37 °C.

Flow cytometry

For flow cytometric analysis, cells were resuspended in PBS supplemented with 10% FCS and EDTA to prevent unspecific antibody binding, stained with 1 µl of the desired antibody and analyzed at a CytoFLEX S cytometer (Beckman Coulter). The generated data were analyzed with FlowJo software (TreeStar, Ashland, OR).

Half of the lung homogenate generated from the right lung lobe was used for the flow cytometry analysis to assess infiltrating granulocytes. The cells were fixed in 4% PFA for 15 min on ice, washed with PBS, incubated with 1 µl fc receptor blocking antibody for 20 minutes and finally stained with 1 µl of the respective antibody for 1 h at 4 °C in the dark. Cells were analyzed at a BD LSR II Flow Cytometer (Beckton & Dickinson) and analyzed with FlowJo software (TreeStar).

Antibodies

Antibodies were purchased from eBioscience: hCD45- PE-Cy7 (Cat-No: 25-0459), hCD14-FITC (Cat-No: 11-0149-41), hCD86-PE (Cat-No: 12-0869-41) , hCD64-FITC (Cat-No: 11-0649-42), mGR1-eFlour450 (Cat-No: 48-5931-82), and mCD16/CD32 (Cat-No: 130-092-574) was purchased from Miltenyi Biotec.

Lung histology

The left lung of euthanized mice was filled with PBS, ligated, resected and fixed in neutral buffered 4% PFA for at least 4 hours at 4 °C. Afterward, the lung tissues were dehydrated (Shandon Hypercenter, XP), formalin-fixed, embedded in paraffin (TES, Medite), and sectioned at 2 - 3 µm thickness (microtome Reichert-Jung 2030). The sections were deparaffinized and stained with hematoxylin and eosin. The histological scoring of the sections was done as described before (Dutow et al., 2013) by a trained pathologist. Representative microphotographs were taken (AxioCam MRc, Zeiss).

Microarray analysis

For microarray analysis, macrophages were infected with *S. aureus* strain Newman with an MOI of 5, and non-infected cells were served as control. Two hours post-infection, non-infected (Ctrl) and infected samples (2h) were collected for RNA isolation. The extracellular bacteria of 24h infected samples were cleared by adding 1mM of penicillin-streptomycin 2-hours after the infection. According to the manufacturer's instructions, RNA isolation was performed using RNAeasy Micro Kit (#74004, Qiagen). 150-200ng of total RNA per sample was used as input to analyze transcriptome changes of cells upon the infection.

The Microarray used in this study is a refined version of the Whole Human Genome Oligo Microarray 4x44K v2 (Design ID 026652, Agilent Technologies), called '026652QM_RCUG_HomoSapiens' (Design ID 084555) established by the Research Core Unit Genomics (RCUG) of Hannover Medical School. The Microarray design was performed at Agilent's eArray portal using a 1x1M format for mRNA expression as a template. All non-control probes have been printed five times within a region of 181560 Features (170 columns x 1068 rows). Four regions were located within one 1M region bringing about four microarray fields per slide, which result in individual hybridizations (Customer Specified Feature Layout). Control probes needed for proper Feature Extraction software operation were automatically determined and located by eArray using default settings.

Cy3-labeled cRNA was synthesized in ¾ reaction volumes using the 'Low Input Quick Amp Labeling Kit One-Color' (#5190-2305, Agilent Technologies) according to the manufacturer's instruction. Fragmentation, hybridization, and washing procedures of cRNA were performed as instruction in the 'One-Color Microarray-Based Gene Expression Analysis Low Input Quick Amp Labeling Protocol V6.7'. Except for hybridization, 2500 ng of labeled cRNA was applied.

Agilent Micro Array Scanner G2565CA (pixel resolution 3 µm, bit depth 20) was used to scan the slides, and the data were extracted using the 'Feature Extraction Software V10.7.3.1' applying the default extraction protocol file 'GE1_107_Sep09.xml'. The average of on-chip replicates (quintuplicates) was measured using the geometric mean of processed intensity values of the green channel, 'gProcessedSignal' (gPS) to gain one value per unique non-control probe. Single Features were excluded if they were 1) identified as Outliers by the Feature Extraction Software, 2) manually flagged, 3) outside the interval of '1.42 x interquartile range 'based on the normalized gPS distribution or if 4) showing a coefficient of variation of pixel intensities that exceeded 0.5. Subsequently, the average of gPS values were normalized by global linear scaling: the gPS values of each sample were multiplied by an array-specific scaling factor. To calculate this factor, a 'reference 75th Percentile value' (set as 1500 for the whole series) was divided by the 75th Percentile value of the particular Microarray to be normalized ('Array I' in the formula shown below).

Therefore, the following formula was used to calculate the normalized gPS values for all samples:

$$normalized\ gPS_{Array\ i} = gPS_{Array\ i}\ x\ (1500 / 75^{th}\ Percentile_{Array\ i})$$

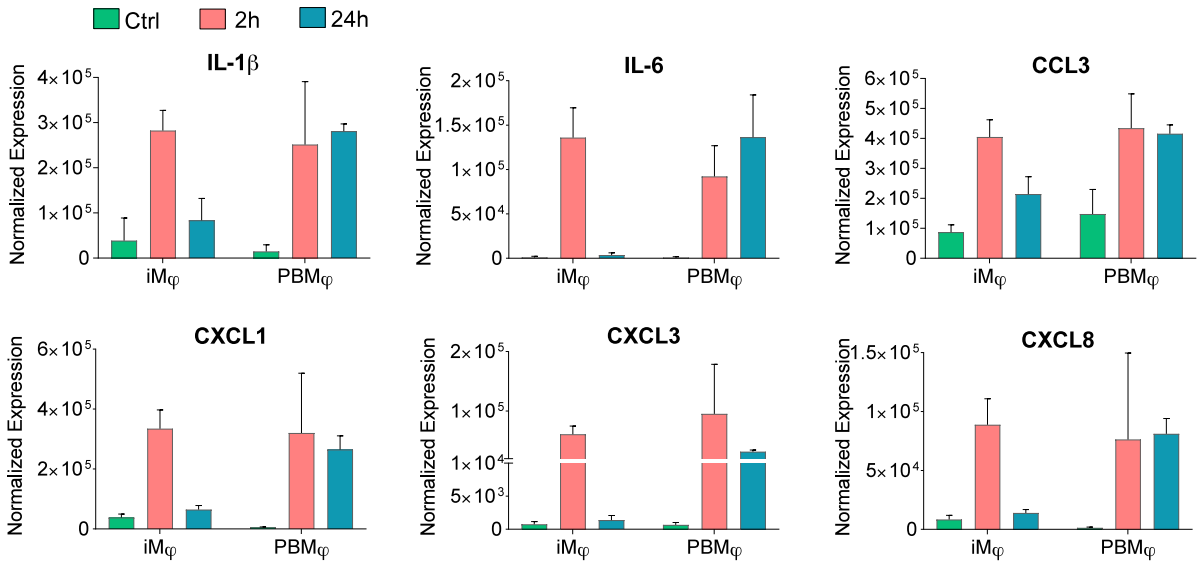
The intensity distribution of negative control was used to define the lower intensity threshold, and this value was fixed at 10 normalized gPS units. All measurements below this intensity cutoff were substituted by the respective value of 10. Finally, normalized processed data were imported into Omics Explorer software v3.5 (Qlucore) under default import settings for Agilent One Color mRNA Microarrays. PCA, heatmap clustering, and GO-based heatmaps visualization were performed in Omics Explorer. Venn diagram was illustrated using Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/). Gene ontology analysis of biological processes and molecular function on the human gene atlas were conducted using Enrichr (https://amp.pharm.mssm.edu/Enrichr).
PCA, heatmap clustering, and GO-based heatmaps visualization were performed in Omics Explorer software 3.5 (Qlucore). Venn diagram was illustrated using Venny 2.1 (http://bioinfogp.cnb.csic.es/tools/venny). Gene ontology analysis of biological processes and molecular function on the human gene atlas were conducted using Enrichr (https://amp.pharm.mssm.edu/Enrichr). Microarray data are deposited under accession number GSE179815 in Gene Expression Omnibus (GEO) database repository (https://www.ncbi.nlm.nih.gov/geo/).

qRT-PCR

The extracted RNA for microarray analysis were used for cDNA synthesis using RevertAid reverse transcriptase (Fermentas) and oligo (dT)18 primer (Thermo scientific). For qRT-PCR analysis TaqMan Universal Master Mix II (Applied Biosystems) was used according to the manufacturer's instructions. The following primers were used: GAPDH (#Hs99999905_m1 Thermo Fisher), IL-6 (#Hs00174131_m1 Thermo Fisher), IL-1β (#Hs00174097_m1 Thermo Fisher), and CXCL8 (#Hs00174103_m1 Thermo Fisher).

Supplementary Figure 1

A



Supplementary Figure 1: Expression of pro-inflammatory genes in iMφ and PBMφ. (A) The expression value of each probe in the microarray chip was measured using the geometric mean of processed intensity values of the green fluorescent channel and it was normalized by global linear scaling. Bar graphs are representing the expression value of each gene in noninfected macrophages non-infected macrophages (Ctrl), and S. aureus infected macrophages 2 and 24 hours post-infection (2h, and 24h; n = 2 biological replicates, mean ± SD).