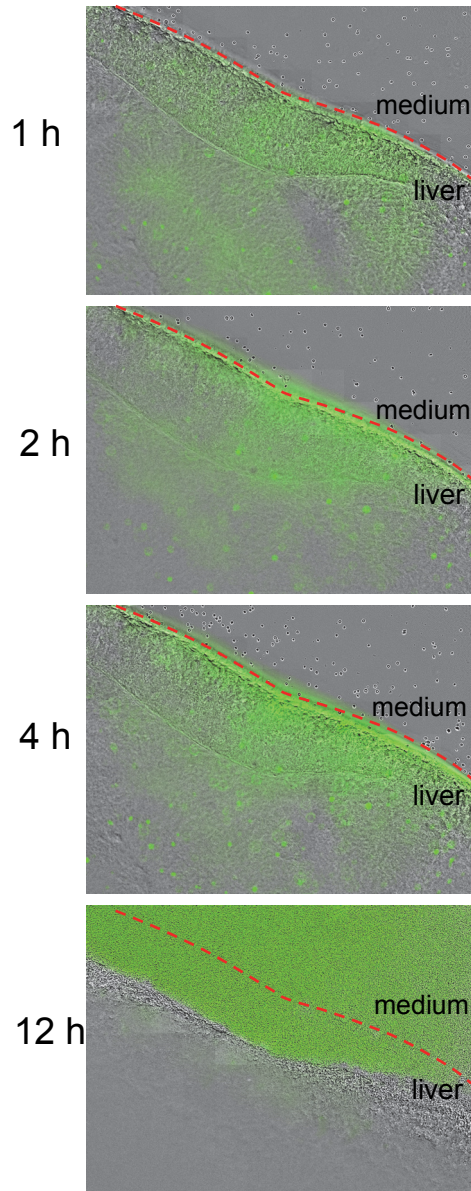


Peritoneal GATA6⁺ macrophages function as a portal for *Staphylococcus aureus* dissemination

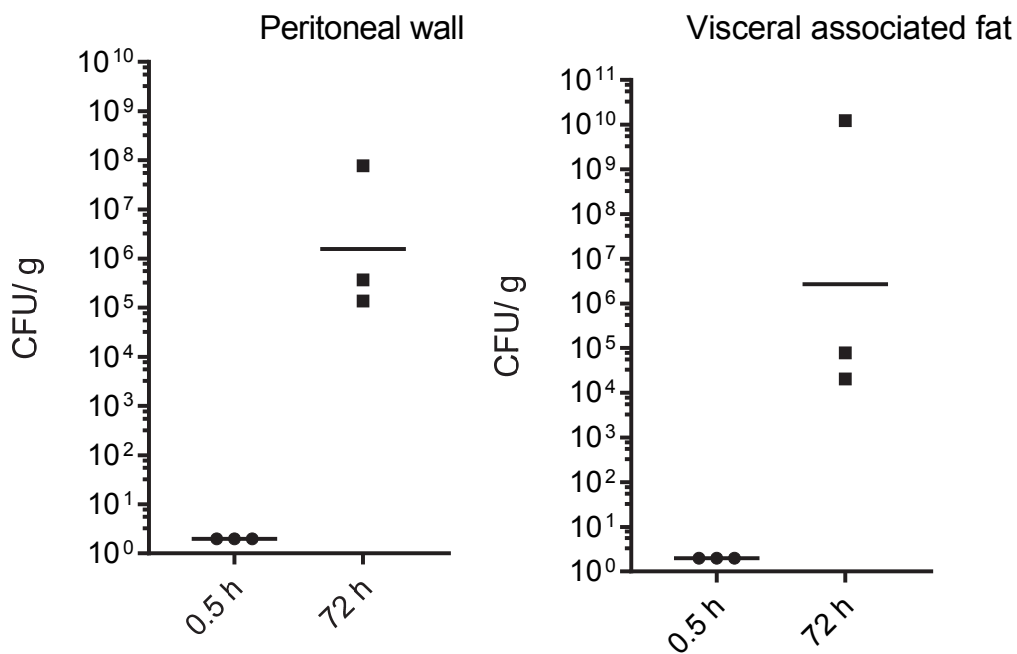
**Selina K. Jorch, Bas G. J. Surewaard, Mokarram Hossain, Moritz Peiseler, Carsten
Deppermann, Jennifer Deng, Ania Bogoslawski, Fardau van der Wal ,Abdelwahab Omri,
Michael J. Hickey and Paul Kubes**

Supplemental Figures



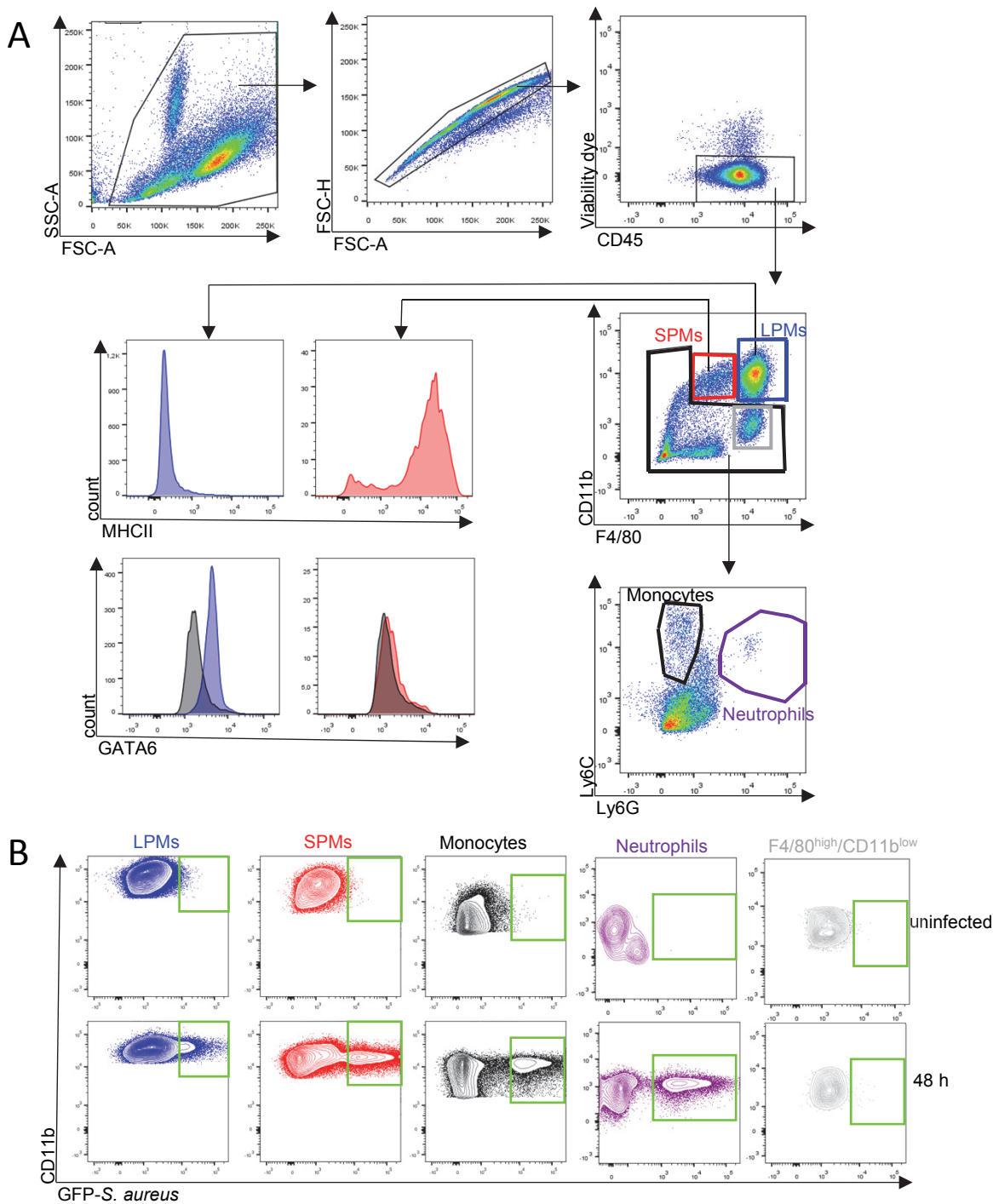
Supplemental Figure 1: *S. aureus* overgrows the liver

Liver was carefully removed 0.5 h post-infection with GFP-*S. aureus*. Vessels were cauterized for closure. Liver was incubated in a 6-well-plate with medium and imaged over time. Representative images of 3 independent experiments.



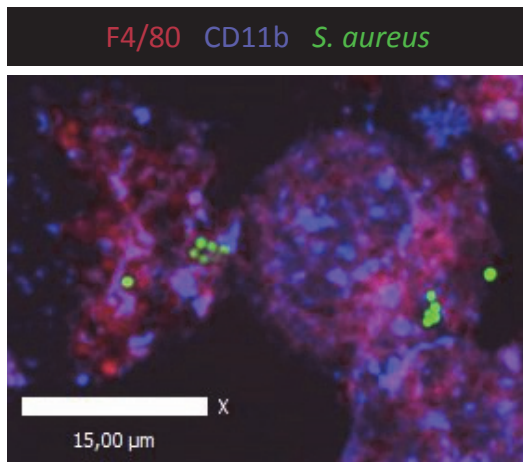
Supplemental Figure 2: *S. aureus* colonization of peritoneal wall and visceral associated fat after i.v. infection.

Mice were infected i.v. with 5×10^7 CFUs *S. aureus* and body parts were harvested at indicated time points prior CFUs were determined. Shown is the geometric mean, n = 3.



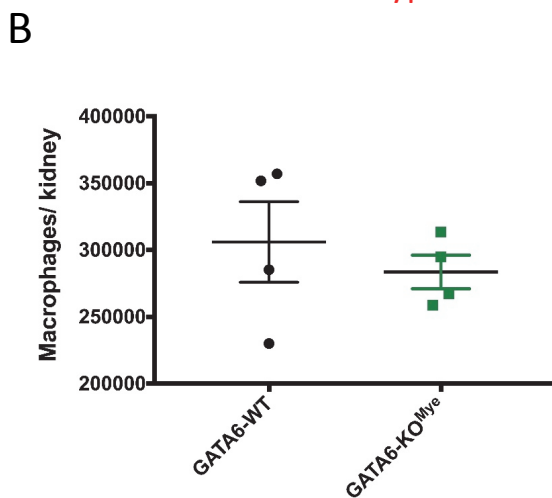
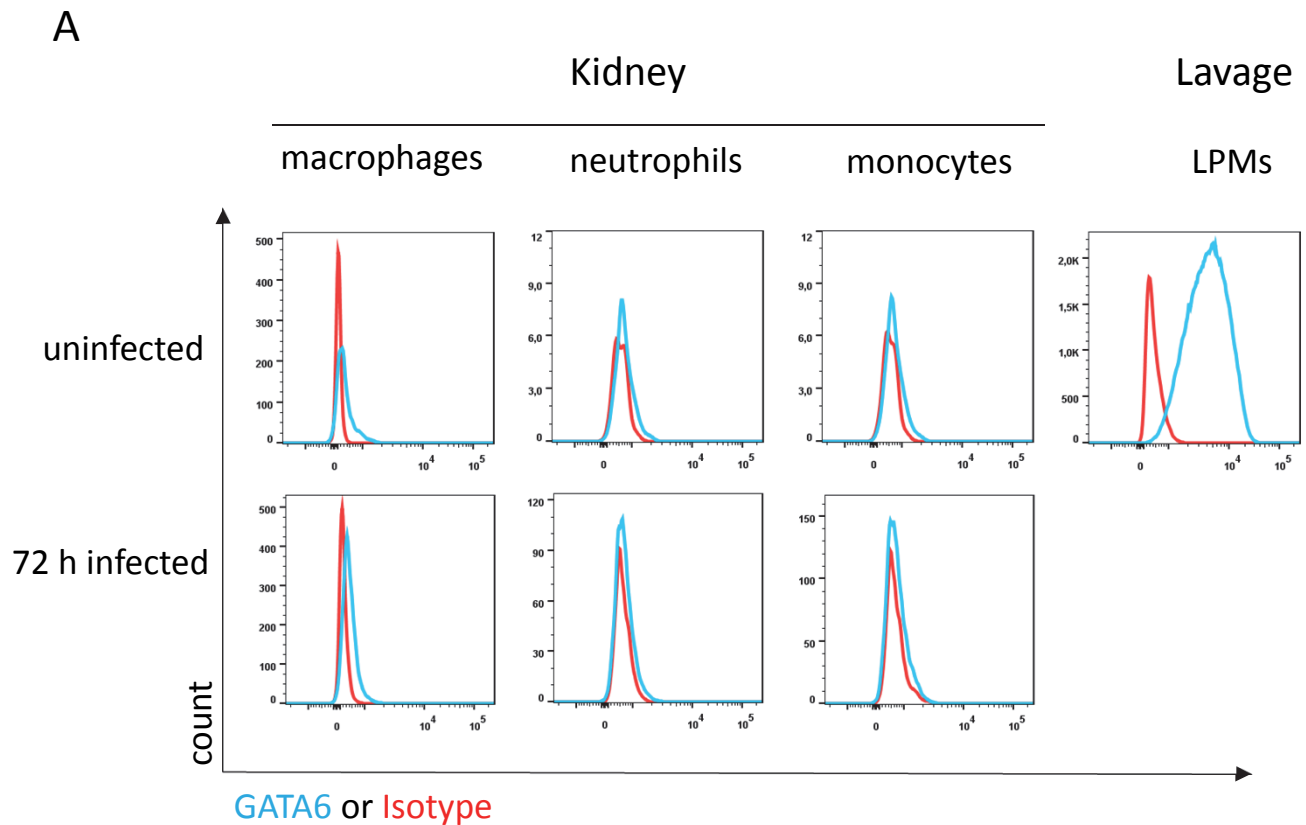
Supplemental Figure 3: Gating strategy for LPMs, SPMs, monocytes and neutrophils from peritoneal lavage.

(A) Cells were pre-gated on single, live and CD45⁺. Large peritoneal macrophages (LPMs) are F4/80 high, CD11b high and Gata6 positive (grey shows isotype control for Gata6 expression). Small peritoneal macrophages (SPMs) are F4/80 low, CD11b⁺ and MHCII high. Proinflammatory monocytes are defined as CD11b⁺ and Ly6C high, neutrophils were CD11b⁺ and Ly6G high. (B) Representative plots showing the *S. aureus*-GFP positive population in LPMs, SPMs, monocytes, neutrophils and F4/80^{high}/CD11b^{low} population.



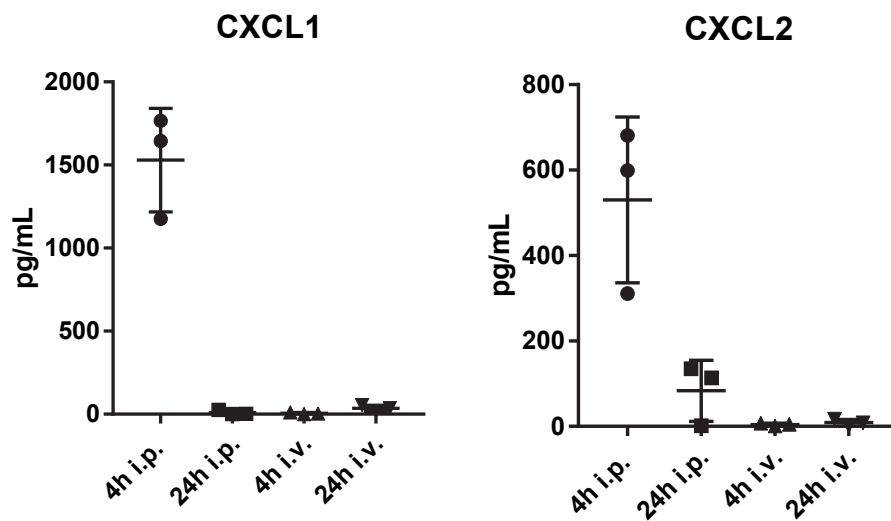
Supplemental Figure 4: GFP-*S. aureus* positive cell populations in the peritoneal cavity

Representative image (n=5 from two independent experiments) from confocal imaging of cytopsin slides of cells isolated from the peritoneal cavity 24 h post-infection. *S. aureus* appears in bright green inside F4/80+ (red) and CD11b+ (blue) cells.



Supplemental Figure 5: Renal mononuclear phagocytes do not express Gata6

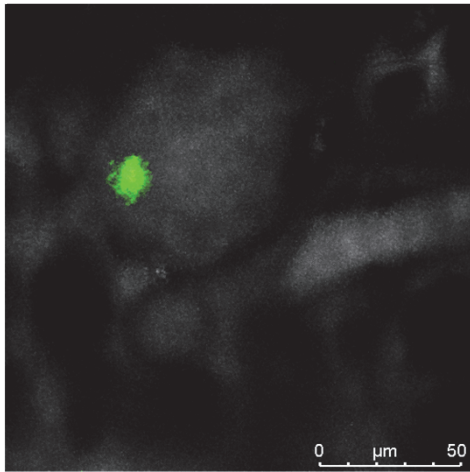
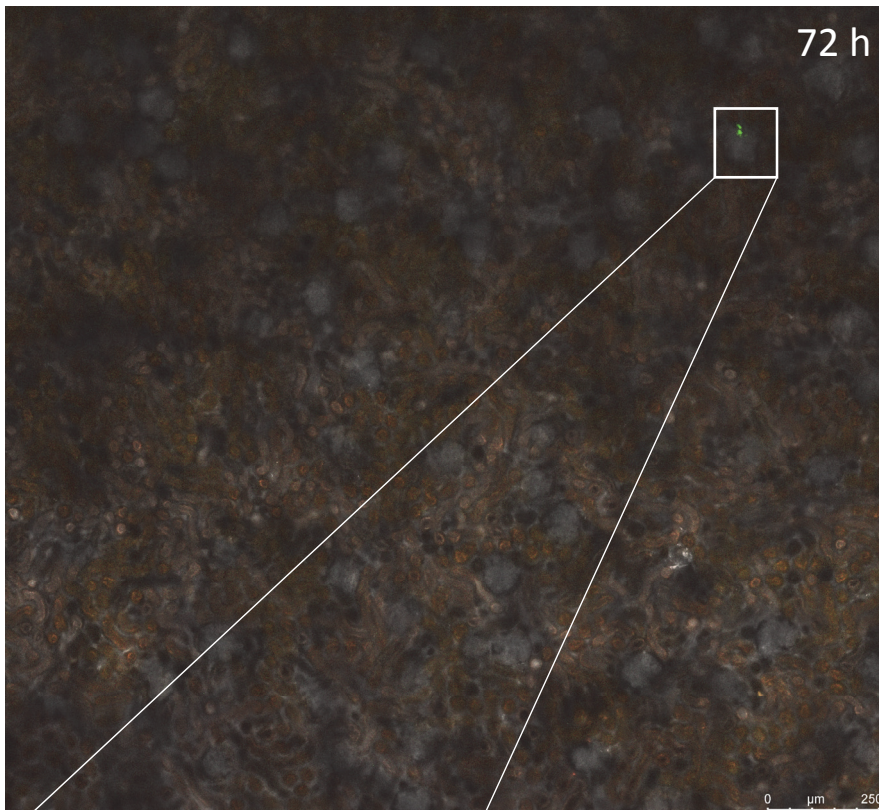
(A) Renal mononuclear phagocytes were isolated from WT mice at indicated time points post-infection and analyzed with flow cytometry. Representative histograms (n=3) are shown, gated on single, viable, CD45+, F4/80+, CX3CR1+ cells. Peritoneal lavage (LPMs) were used as positive control for Gata6 expression. (B) Number of renal mononuclear phagocytes (single, viable, CD45+, F4/80+, CX3CR1+) in Gata6 deficient mice and littermate controls analyzed by flow cytometry, n=4 from two independent experiments.



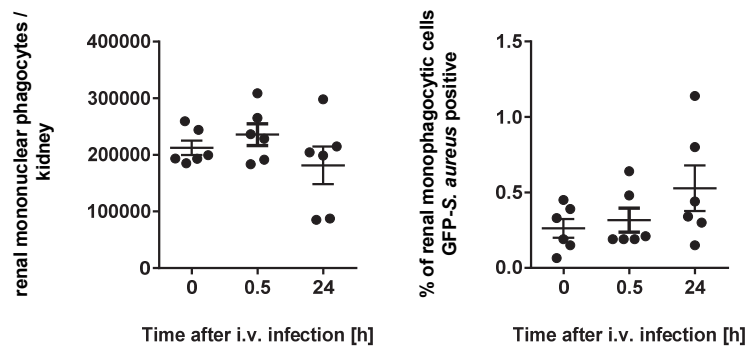
Supplemental Figure 6: Cytokine expression in the peritoneal cavity

Mice were infected for 4 h or 24 h i.v. or i.p. and peritoneal lavage was performed. Lavage supernatant was used to determine CXCL1 and CXCL2 concentration via ELISA, n = 3

A

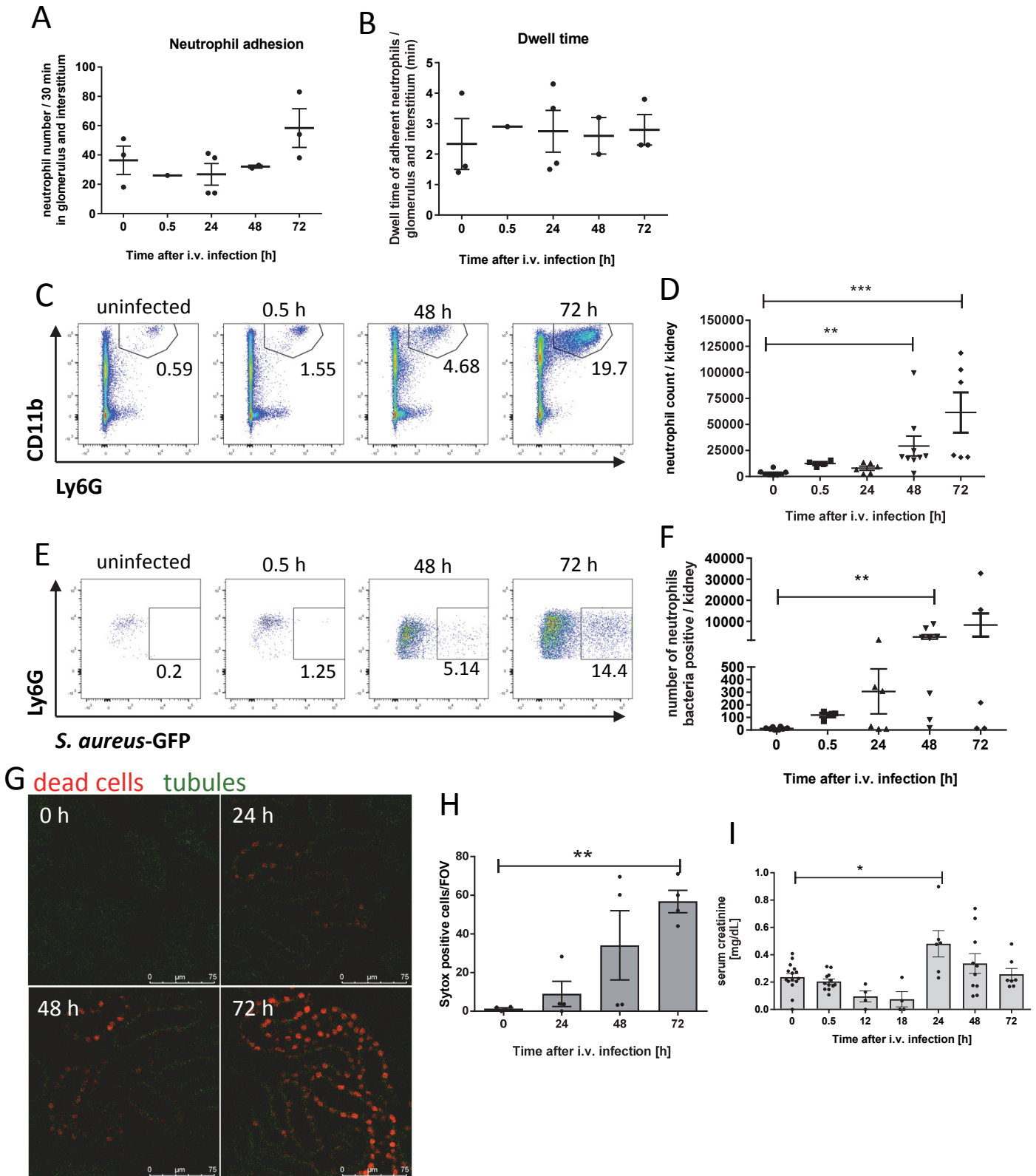


B



Supplemental Figure 7: *S. aureus* in the kidney

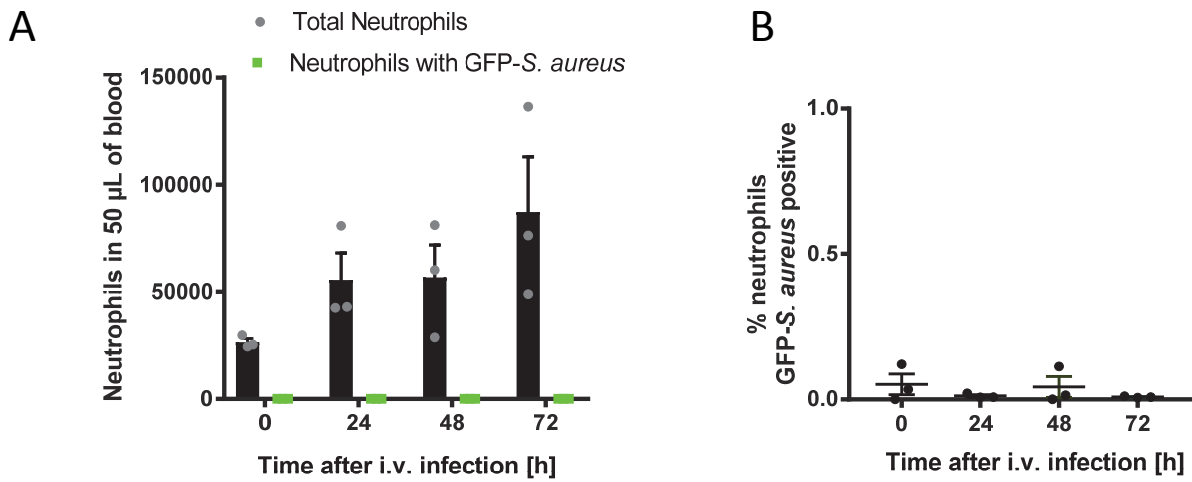
(A) 2-photon stitched IVM image from kidney of a 72 h infected mouse. Vasculature and therefore glomeruli appear in grey (AF680-albumin), tubules appear in dark yellow/red autofluorescence and GFP-*S. aureus* in bright green. From all mice that have been imaged (n=7), enlarged is the only glomerulus that ever had bacteria. (B) Total number of renal mononuclear phagocytic cells (single, viable, CD45+, F4/80+, CX3CR1+) of WT mice at different time points post-infection and their amount of GFP-*S. aureus* positive cells determined by flow cytometry, n = 6 from two independent experiments.



Supplemental Figure 8: Delayed neutrophil recruitment and neutrophil-bacterial interactions in the kidneys.

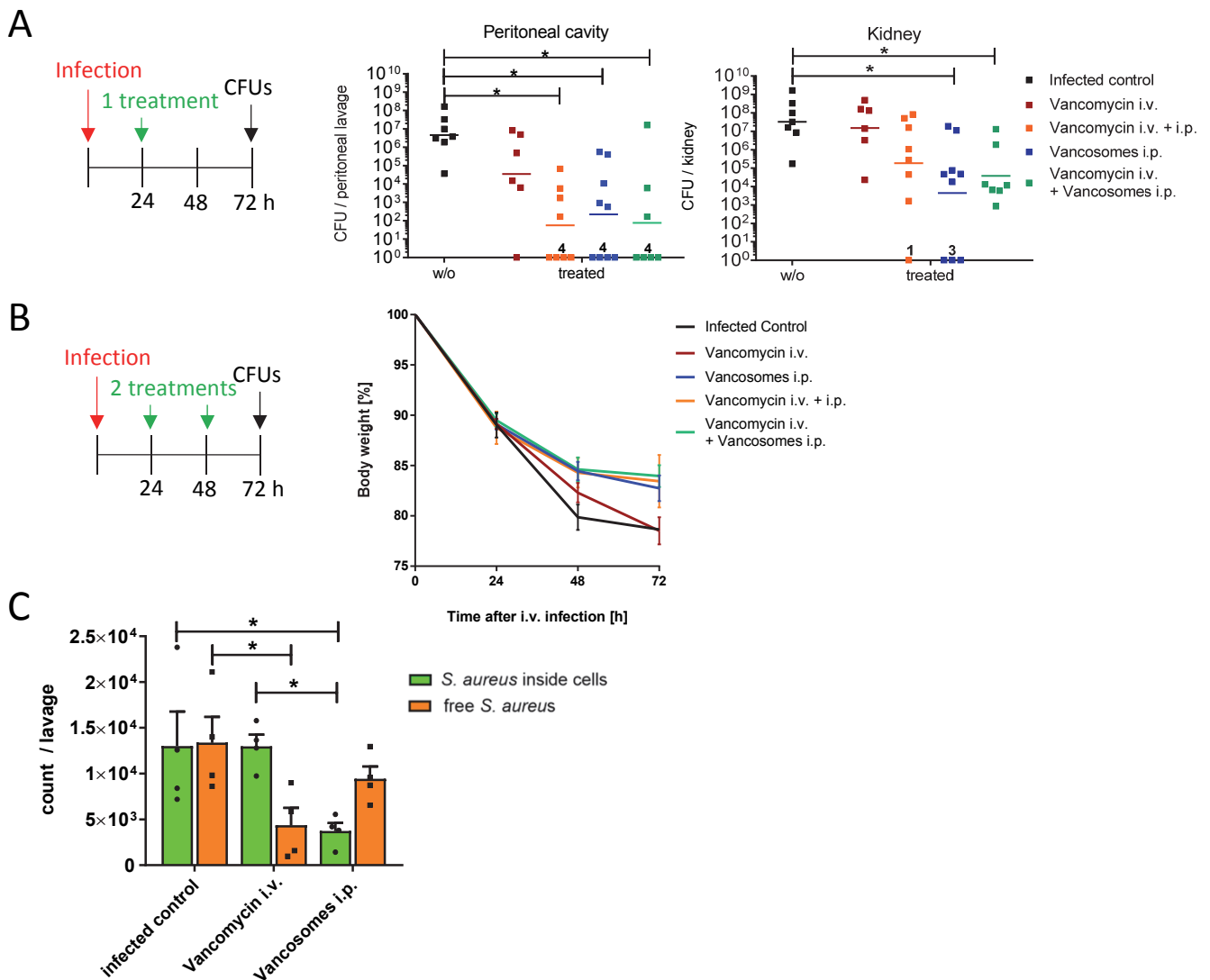
(A,B) Analysis of 2-photon IVM. Number (A) and dwell time (B) of adherent neutrophils from 30 min recordings. Neutrophils were defined as adherent when they were retained in

the kidney for longer than 30 sec. 3 FOV were analyzed per mouse, data represent mean \pm s.e.m. (error bars), n = 1-4. **(C-F)** Flow cytometry analyses of the whole kidney at indicated time points post infection with 5×10^7 *S. aureus* Newman. Gated on single, viable, CD45+, Ly6G+ and CD11b+ cells. **(C,E)** Representative flow cytometry plots showing Ly6G+, CD11b+ cells **(C)** and how many of these cells are positive for *S. aureus*-GFP **(E)**. **(D,F)** Quantification of total cell numbers from c **(D)** or e **(F)**. Data represent mean \pm s.e.m. (error bars), n = 6-8 from three independent experiments, one-way ANOVA followed by Bonferroni posthoc test, ** P < 0.01, *** P < 0.001. **(G)** Representative 2-photon IVM images of kidneys at indicated time points post-infection. Dead tubular cells are stained with Sytox Orange (red), tubules appear in dark green autofluorescence. **(H)** Analysis of g. Mean number of Sytox Orange-positive cells from 10 FOV per mouse, one-way ANOVA with Bonferroni's multiple comparisons test, n = 4 from two independent experiments, data represent mean \pm s.e.m. (error bars), ** P < 0.01 **(I)** Serum creatinine levels at indicated time points post-infection. One-way ANOVA with Bonferroni's multiple comparisons test, n = 5-9 from two independent experiments, data represent mean \pm s.e.m. (error bars), * P < 0.05.



Supplemental Figure 9: GFP-*S. aureus* in peripheral blood neutrophils.

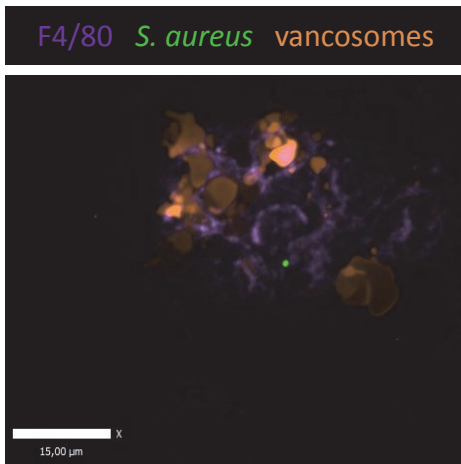
(A,B) Mice were infected with GFP-*S. aureus* i.v. and at indicated time points mice were euthanized and blood was collected. Neutrophil count (CD11b+, Ly6G+, **A**) and the percentage of neutrophils associated with *S. aureus* (**B**) was determined by flow cytometry analyses, n = 3.



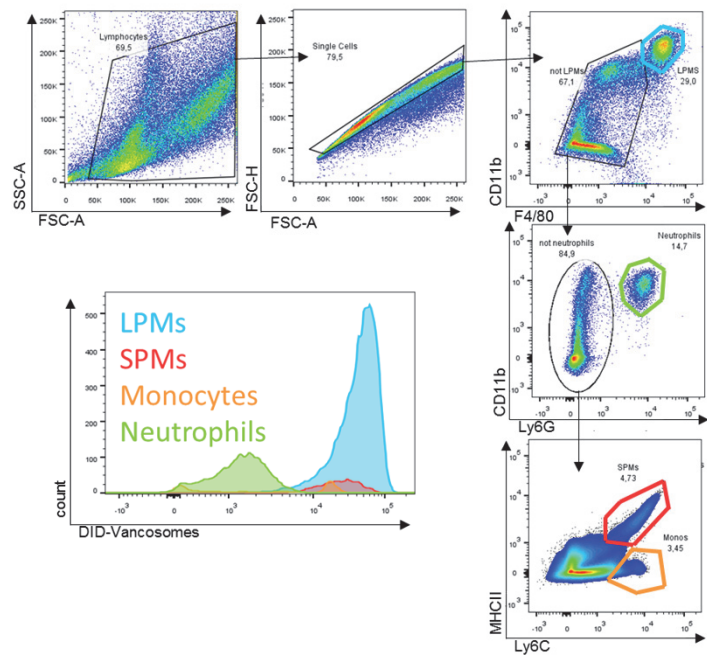
Supplemental Figure 10: Additional data for vancomycin and/or vancosomes treated mice.

(A) Mice were infected i.v. with 5×10^7 *S. aureus* Newman and treated with either vancomycin i.v. (red), vancosomes i.p. (blue), a combination of vancomycin i.v. + i.p. (orange) or vancomycin i.v. + vancosomes i.p. (green). 72 h post-infection organs were harvested, peritoneal lavage was performed and CFUs were determined. Data are presented as geometric mean, Kruskal-Wallis with Dunn's post-testing; $n = 6-9$ from three independent experiments * $P < 0.05$. **(B)** Body weight loss of mice that have been infected i.v. with 5×10^7 *S. aureus* and treated 24 h and 48 h post-infection as described in A., data are presented as mean \pm SEM, $n = 9$ from three independent experiments. **(C)** Flow cytometry analyses of peritoneal lavage 72 h post-infection of mice that were treated at 24 and 48 h with either vancomycin i.v. or vancosomes i.p. Total counts of bacteria inside single, viable, CD45+, cells in contrast to free bacteria, $n = 3$, two-way-ANOVA, followed by Bonferroni posthoc test, * $P < 0.05$.

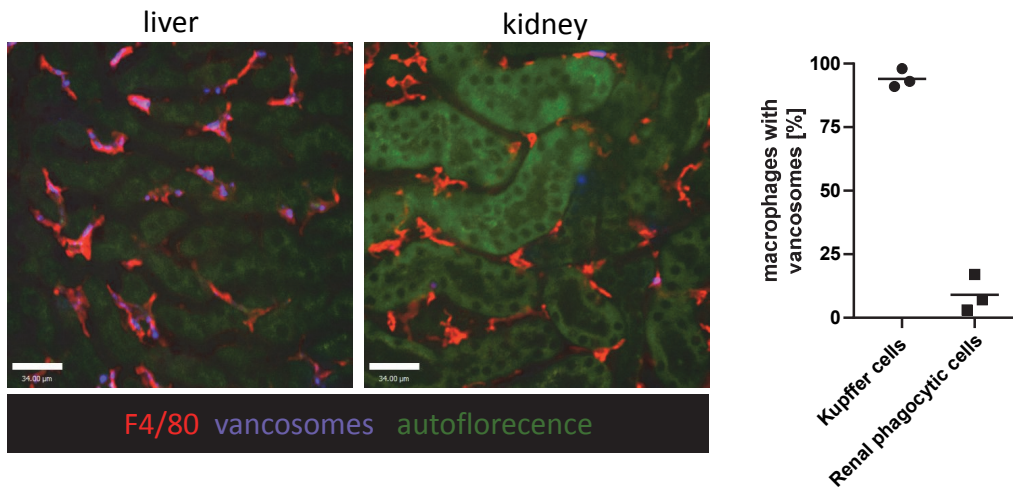
A



B



C



Supplemental Figure 11: Location of bacteria or vancosomes in F4/80+ cells of the peritoneal cavity, liver and kidney.

(A) Representative image (n=5 from two independent experiments) from confocal imaging of cytopsin slides of cells isolated from the peritoneal cavity 24 h post-infection that were treated with stained vancosomes. F4/80 positive cells appear in purple, vancosomes in orange. *S. aureus* appears in bright green and were rarely seen and only in macrophages that had not phagocytosed vancosomes. (B) Mice were injected i.p. with DID-stained vancosomes, peritoneal lavage was performed 2 h post injection and cells were analyzed by flow cytometry. Representative flow cytometry plots are shown, n = 2 (C) Representative images and analyzes of IVM from liver and kidney of animal that received stained vancosomes (blue) i.p. 30 min prior imaging, n = 3 (5 FOV were analyzed per mouse and used as n = 1).