SUPPLEMENTARY MATERIAL

Poly-N-acetyl glucosamine production by *Staphylococcus epidermidis* cells increases their *in vivo* pro-inflammatory effect

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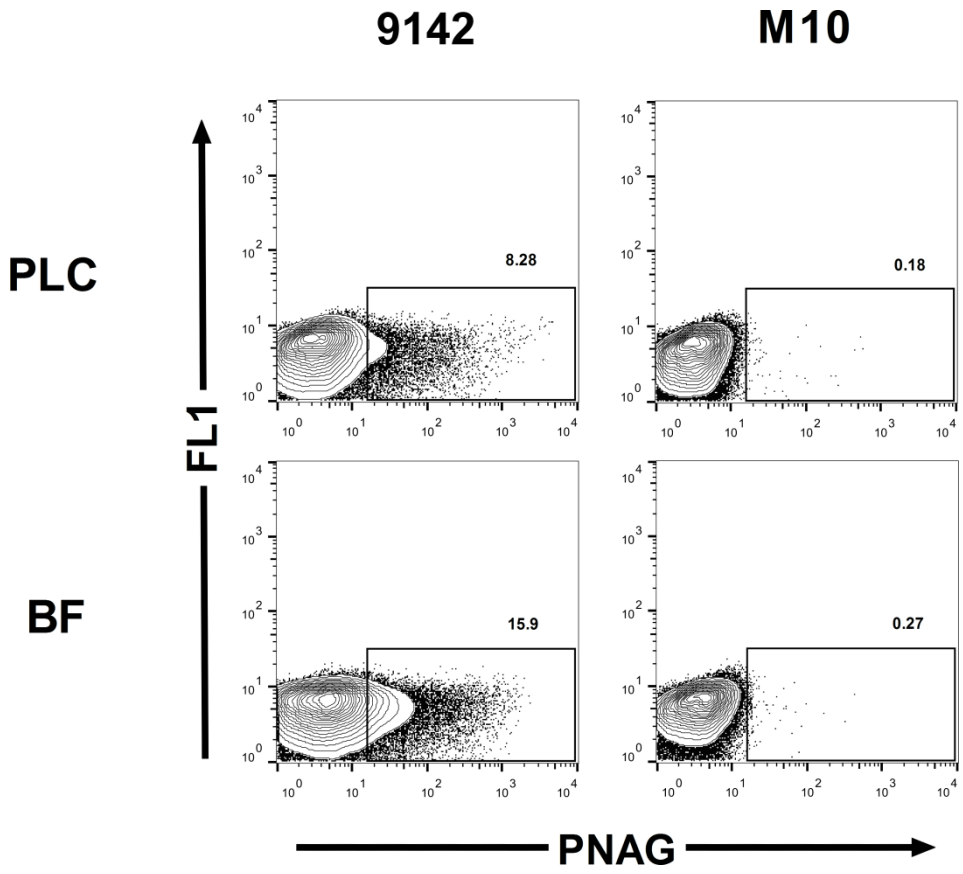
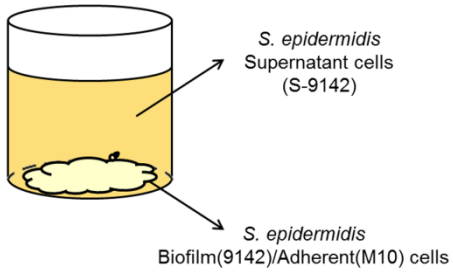


FIG S1. Flow cytometric analysis of poly-N-acetyl glucosamine (PNAG) on the surface of *S. epidermidis* 9142 or M10 planktonic (PLC) or biofilm (BF) cells. Planktonic and biofilm cell cultures were grwon as previously described (S1) and used to prepare biofilm (BF) or planktonic (PLC) cell suspensions that were washed with PBS and stained with a PNAG-specific human monoclonal antibody (mAb) (clone F598) (S2). A polyclonal biotinylated anti-human IgG was used as secondary antibody and revealed using PE-Cy7-conjugated streptavidin (eBioscience, San Diego, CA).

A



B

Intraperitoneal injection		**Group nomenclature**
PBS	→	PBS
2×10^8 S. epidermidis 9142	→	9142
2×10^8 S. epidermidis M10	→	M10

C

Intraperitoneal injection twice one week interval	**Intravenous injection**	**Group nomenclature**
PBS	→	PBS/-
PBS	→ 1×10^8 S. epidermidis 9142 →	PBS/S-9142
1×10^6 S. epidermidis 9142	→	9142/-
1×10^6 S. epidermidis 9142	→ 1×10^8 S. epidermidis 9142 →	9142/S-9142
1×10^6 S. epidermidis M10	→	M10/-
1×10^6 S. epidermidis M10	→ 1×10^8 S. epidermidis 9142 →	M10/S-9142

FIG S2. (A) Schematic representation of *S. epidermidis* growth. *S. epidermidis* 9142 and M10 cells were plated in 24-well plates (Nunc, Denmark) in tryptic soy broth (TSB, Merck KGaA, Darmstadt, Germany) supplemented with 0.3% glucose and incubated at 37 ºC with 80 RPM in a GML Orbital Shaker-3031 (Gesellschaft für Labortechnik mbH, Burgwedel, Germany). After 24 h incubation, the medium was renewed with the addition of fresh TSB supplemented with 1% glucose and cells were similarly incubated for another 24 h. Biofilm grown *S. epidermidis* 9142 or M10 cells, loosely adherent to the culture plate, were collected separately following removal of medium supernatant and washed twice with sterile PBS. Bacteria was then gently scraped and sonicated for 10 seconds at 18W (W185D model, MSE) to dissociate cell clusters to create a homogeneous cell suspension for quantification. To obtain biofilm released/detached *S. epidermidis* cells, the supernatant of 48 h 9142 biofilm cultures set as described was gently collected and the recovered cells (S-9142) were mildly sonicated to dissociate cell clusters and washed twice in sterile PBS for quantification. Bacterial quantification following SYBR/PI staining and flow cytometry analysis was carried out as previously described (3). (B) Mouse groups used to study the early inflammatory response following *S. epidermidis* intraperitoneal infection with either biofilm-grown 9142 or M10 cells (9142 and M10, respectively) or sham-infected with PBS alone (PBS). (C) Mouse groups used to study the immune response elicited following i.p. priming with 9142 or M10 cells and i.v. infection with S-9142 cells. WT or *IL-10*$^{-/-}$ mice were primed i.p. with either *S. epidermidis* biofilm cells (9142/S-9142) or M10 cells (M10/S-9142) or sham-primed with PBS alone (PBS/S-9142), twice with one-week interval and i.v. infected with S-9142 cells. Control mice receiving the i.p. priming alone were also performed (9142/-, M10/- and PBS/-).

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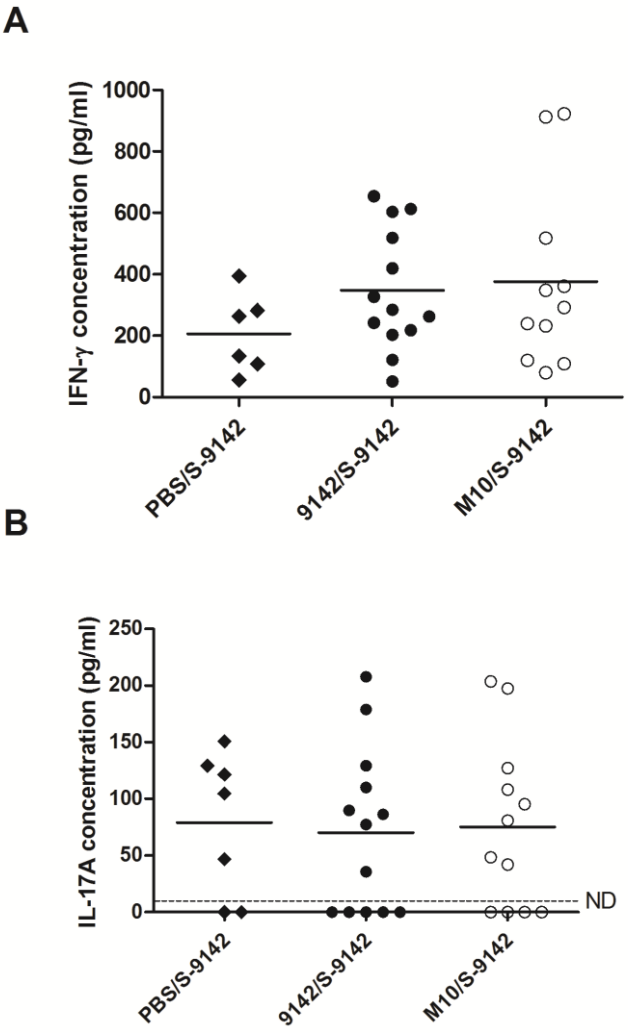

FIG S3. Serum levels of (A) IFN-γ and (B) IL-17A detected in 9142/S-9142, M10/S-9142 and PBS/S-9142 mouse groups. Cytokine levels were determined in serum samples collected 4 days upon the i.v. infection. Results correspond to pooled data of two independent experiments. Each dot represents an individual mouse. Horizontal lines in each group represent the mean value.

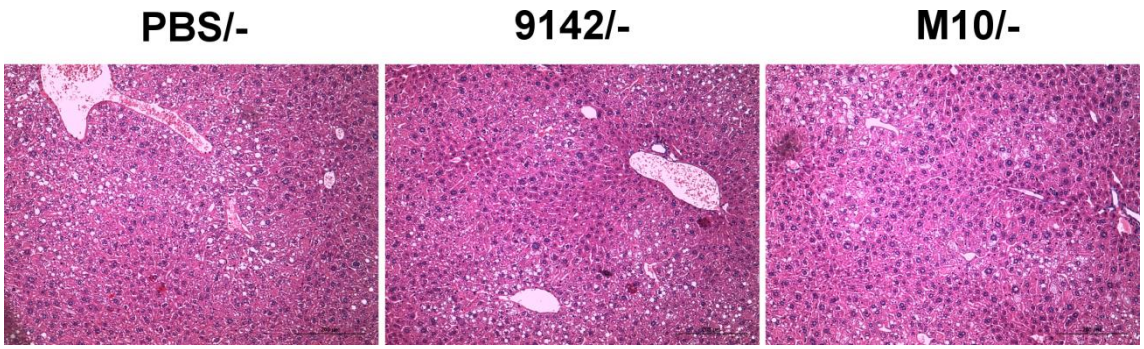

FIG S4. Representative micrographs taken at 100× magnification depicting the histopathology profile observed in the liver of mice primed i.p., twice at one-week interval with either 1×10^6 *S. epidermidis* 9142 (9142/-) or M10 (M10/-) cells or with PBS alone (PBS/-). Bar = 200µm

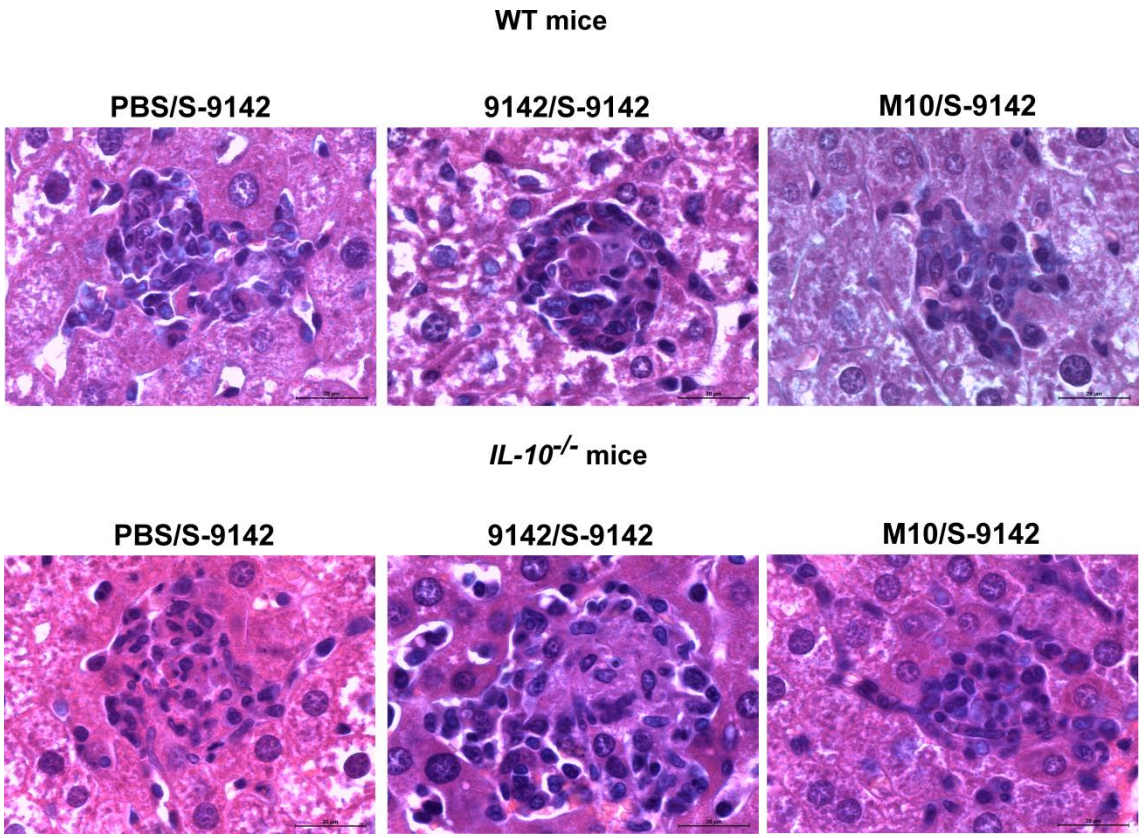

FIG S5. Representative micrographs taken at 1000× magnification depicting the histopathology profile of the cellular infiltrates observed in the liver WT ans *IL10*$^{-/-}$ of 9142/S-9142, M10/S-9142 and PBS/S-9142 mouse groups, 4 days following i.v. infection. Bar = 20µm

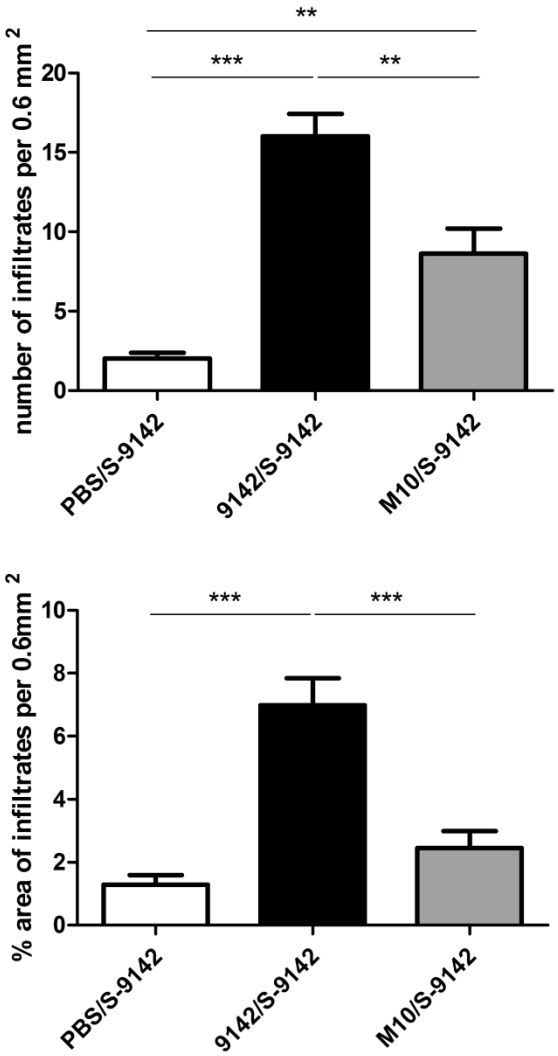

FIG S6. Quantitative analysis of the number of cellular infiltrates and percentage of area containing cellular infiltrates per 0.6 mm^2 in the livers of immunized and *S. epidermidis* infected 9142/S-9142, M10/S-9142 and PBS/S-9142 mouse groups, 9 days following i.v. infection. Quantification was performed by analyzing 6 liver samples from each group were from where 5 micrographs at 100× magnification, from 5 different liver lobes, were randomly taken. (one-way ANOVA, $p<0.05$; Tukey's post-hoc test: ** $p<0.01$; *** $p<0.001$)

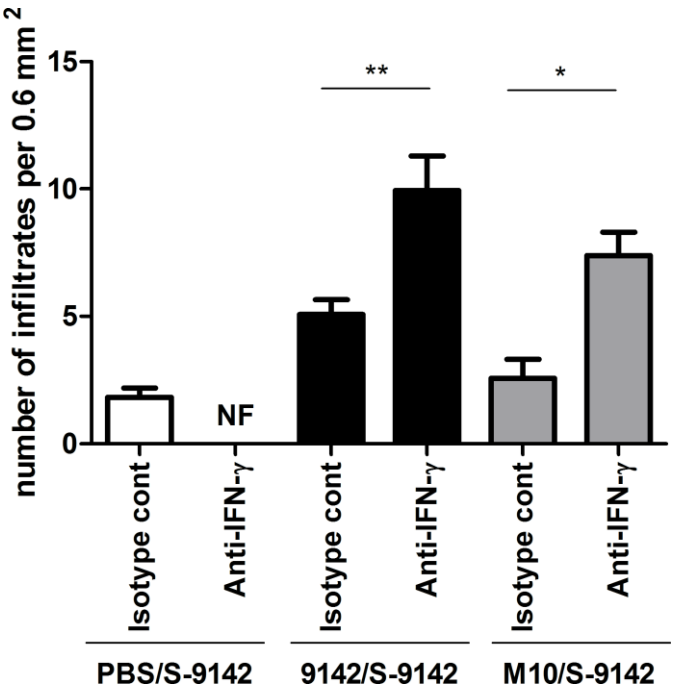

Fig. S7 - Quantitative analysis of the number of cellular infiltrates per 0.6 mm^2 in the livers of immunized, or sham-immunized, and infected 9142/S-9142, M10/S-9142 or PBS/S-9142 mouse groups, 4 days following *S. epidermidis* i.v. infection. Mouse groups were either treated with 500 µg of anti-IFN-γ mAb or its respective isotype control administered 1h prior to the i.v. infection. Quantification was performed by analyzing 5 liver samples from each group were from where 4 micrographs at 100× magnification, from 4 different liver lobes, were randomly taken; NF, not found (one-way ANOVA, $p<0.05$; Tukey's post-hoc test: * $p<0.05$; ** $p<0.01$)

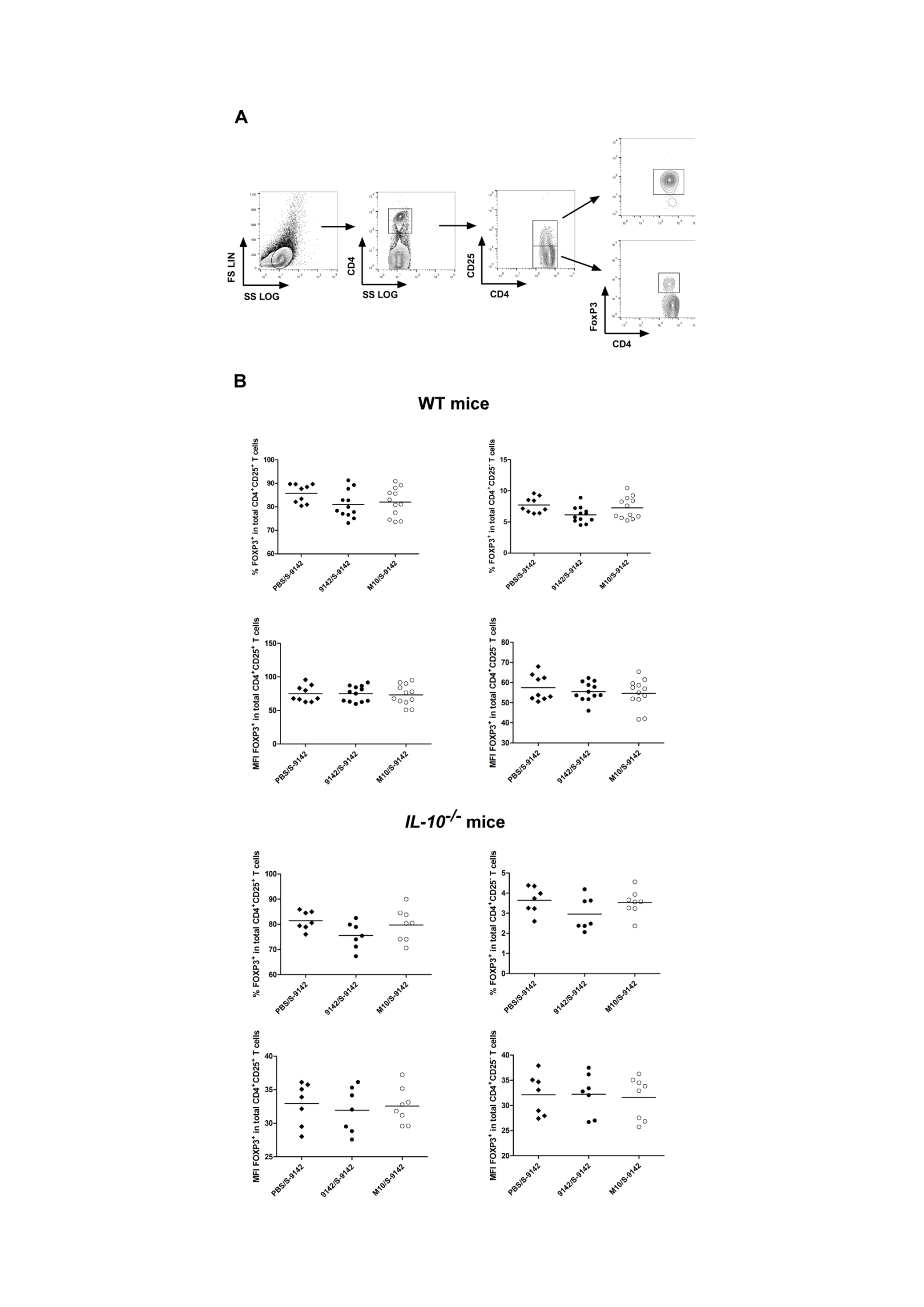
A
FS LIN
SS LOG
CD4
SS LOG
CD25
CD4
FoxP3
CD4
B
WT mice
% FOXP3+ in total CD4+CD25+ T cells
% FOXP3+ in total CD4+CD25- T cells
MFI FOXP3+ in total CD4+CD25+ T cells
MFI FOXP3+ in total CD4+CD25- T cells
PBS/S-9142
9142/S-9142
M10/S-9142
IL-10-/- mice

FIG S8. (A) Contour plots representative of the gating strategy used to define $Foxp3^{+}CD25^{+}CD4^{+}$ and $Foxp3^{+}CD25^{-}CD4^{+}$ T cells within gated $CD4^{+}$ T cells. (B) Frequency and mean fluorescence intensity (MFI) due to Foxp3 staining in $Foxp3^{+}CD25^{+}CD4^{+}$ and $Foxp3^{+}CD25^{-}CD4^{+}$ T cells in the spleen of WT and *Il10*$^{-/-}$ 9142/S-9142, M10/S-9142 and PBS/S-9142 mouse groups, 4 days following i.v. infection. Results are from pooled data of 2 independent experiments. Each dot represents an individual mouse. Horizontal lines in each group represent the mean value.

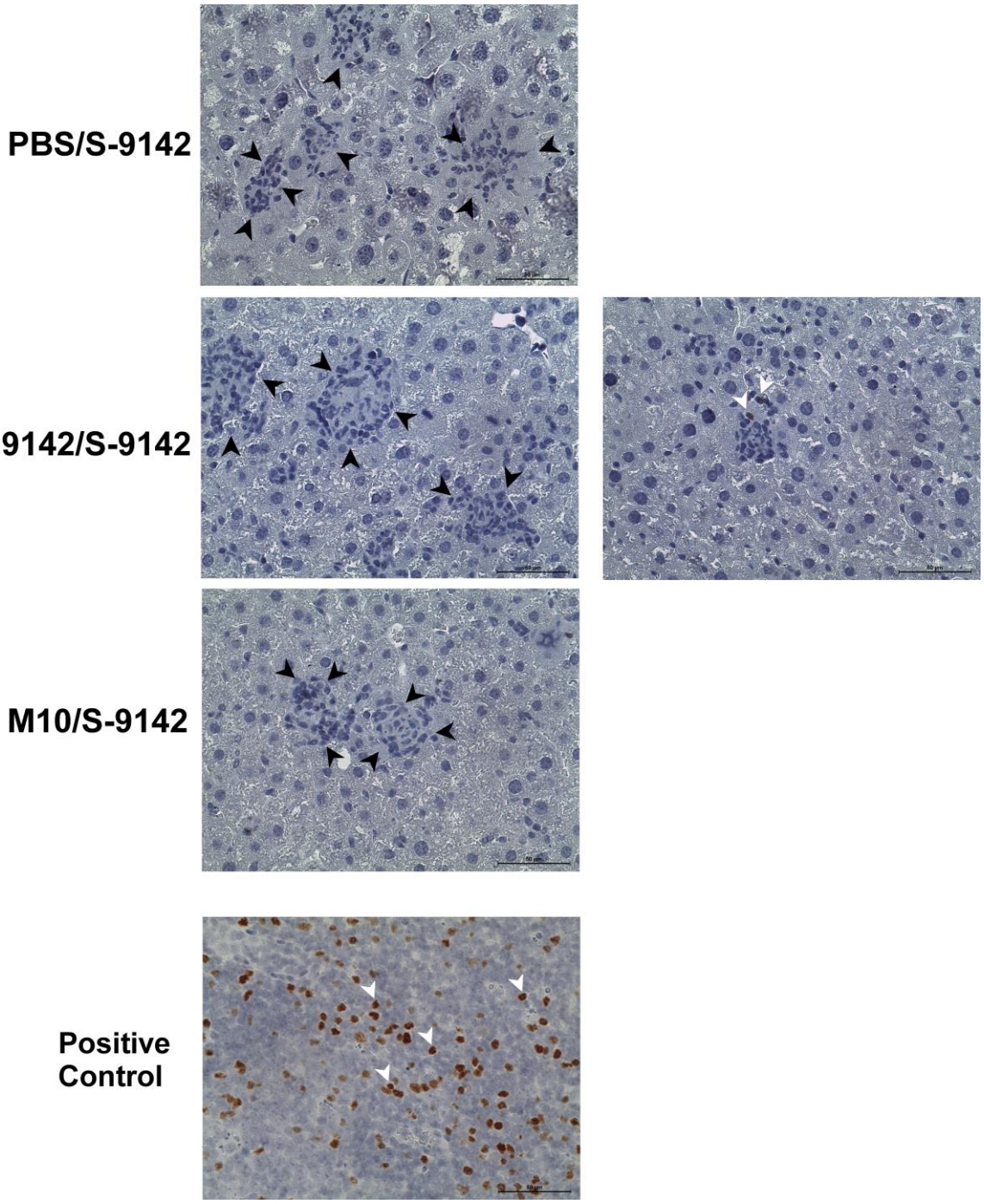

FIG S9. Representative micrographs taken at 400× magnification of Foxp3-specific staining of thin liver sections of 9142/S-9142, M10/S-9142 and PBS/S-9142 mouse groups, 4 days following i.v. infection. A positive control corresponding to a mesenteric lymph node section was included. Black arrows indicate cell infiltrate clusters and white arrows indicate $Foxp3^{+}$ cells. Bar = 50µm.

Table S1. Log_{10} of the CFU determined 4 days after i.v. S-9142 infection in the liver of WT mice of the indicated groups. Each value represents one individual mouse

log_{10} CFU counts		
PBS/S-9142	9142/S-9142	M10/S-9142
2,70	7,93	> 8
Undetectable	6,79	7,90
2,48	7,04	> 8
2,30	5,61	> 8
	5,72	> 8
	> 8	> 8
	7,86	4,53

SUPPLEMENTAL REFERENCES

S1. **Cerca F, Franca A, Guimaraes R, Hinzmann M, Cerca N, Lobo da Cunha A, et al.** 2011. Modulation of poly-N-acetylglucosamine accumulation within mature Staphylococcus epidermidis biofilms grown in excess glucose. Microbiology and immunology. **55**:673-82.

S2. **Kelly-Quintos C, Cavacini LA, Posner MR, Goldmann D, Pier GB.** 2006. Characterization of the opsonic and protective activity against Staphylococcus aureus of fully human monoclonal antibodies specific for the bacterial surface polysaccharide poly-N-acetylglucosamine. Infect Immun. **74**:2742-50.

S3. **Cerca F, Trigo G, Correia A, Cerca N, Azeredo J, Vilanova M**. 2011. SYBR green as a fluorescent probe to evaluate the biofilm physiological state of Staphylococcus epidermidis, using flow cytometry. Can J Microbiol; **57**:850-6