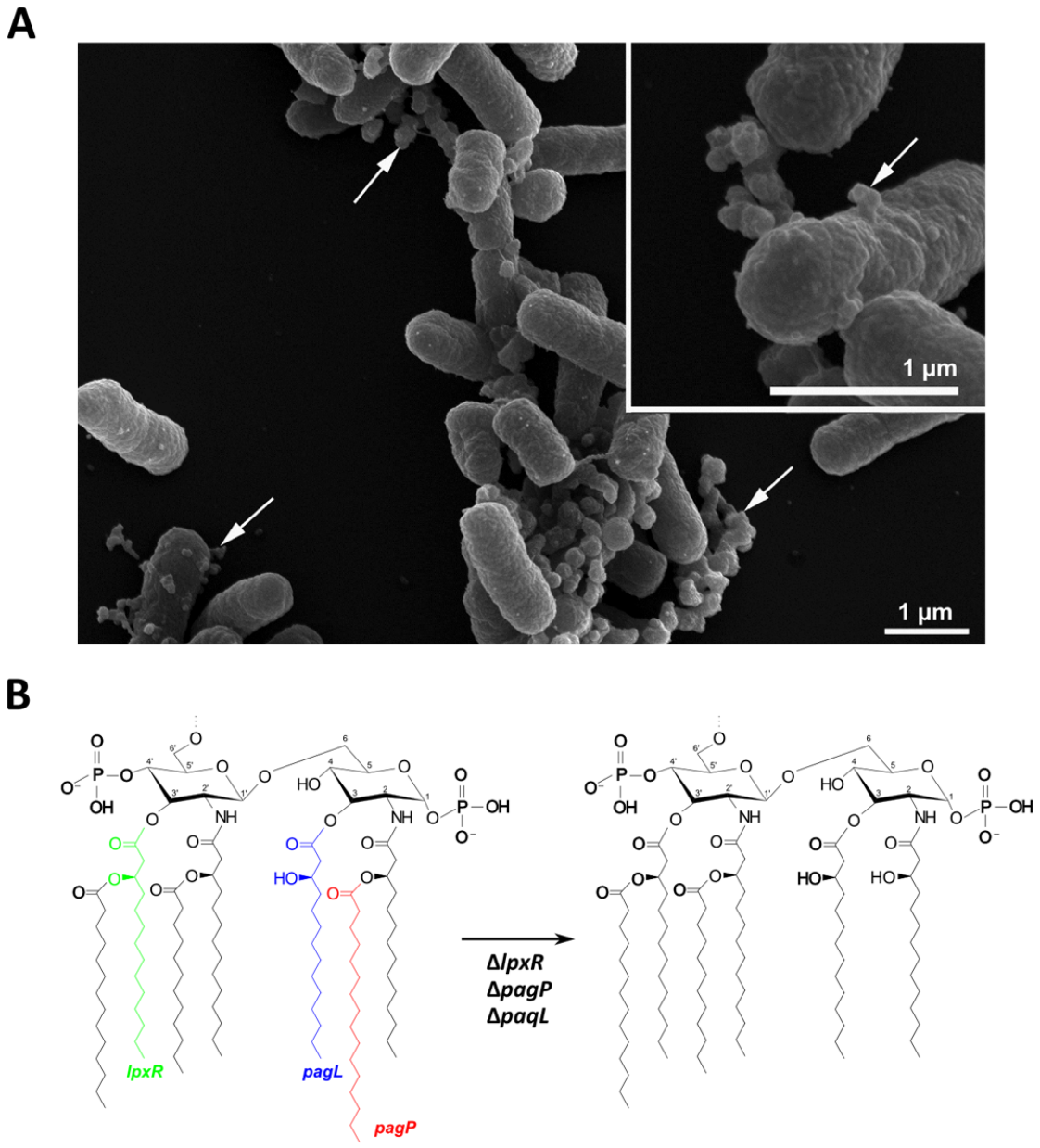
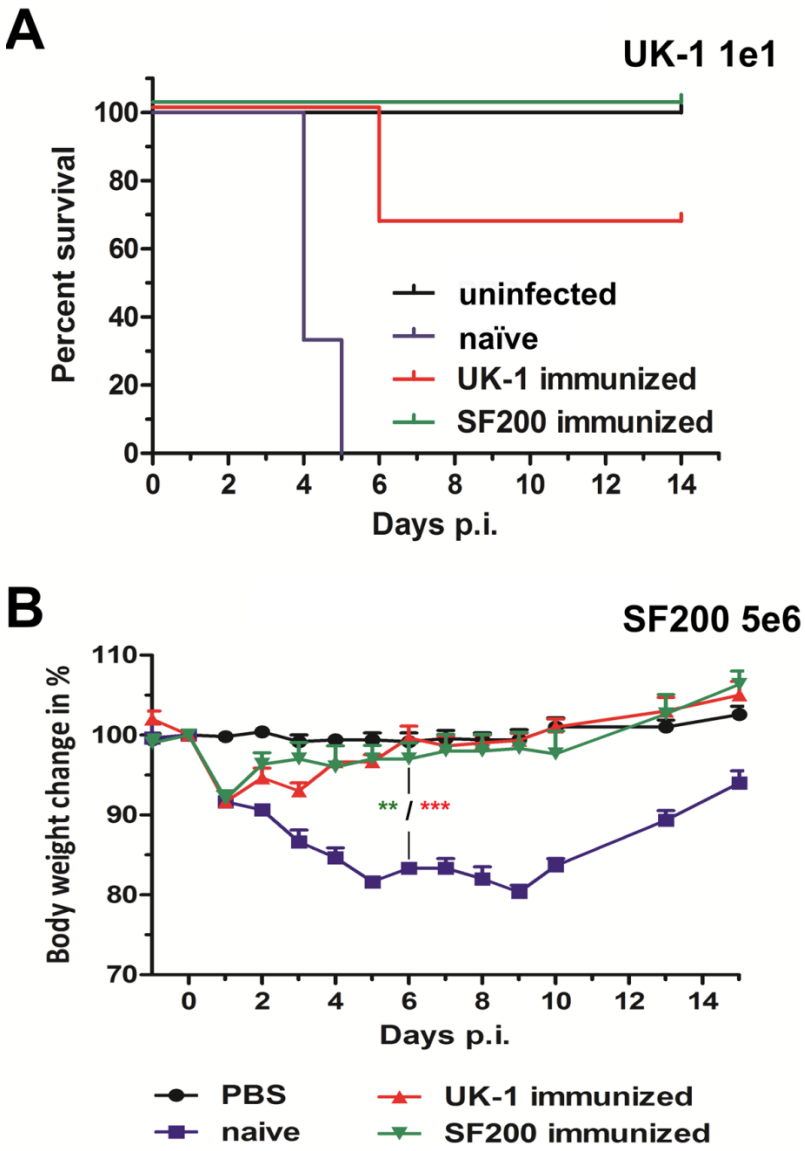
Supplementary Figures

Supplementary Figure S1:



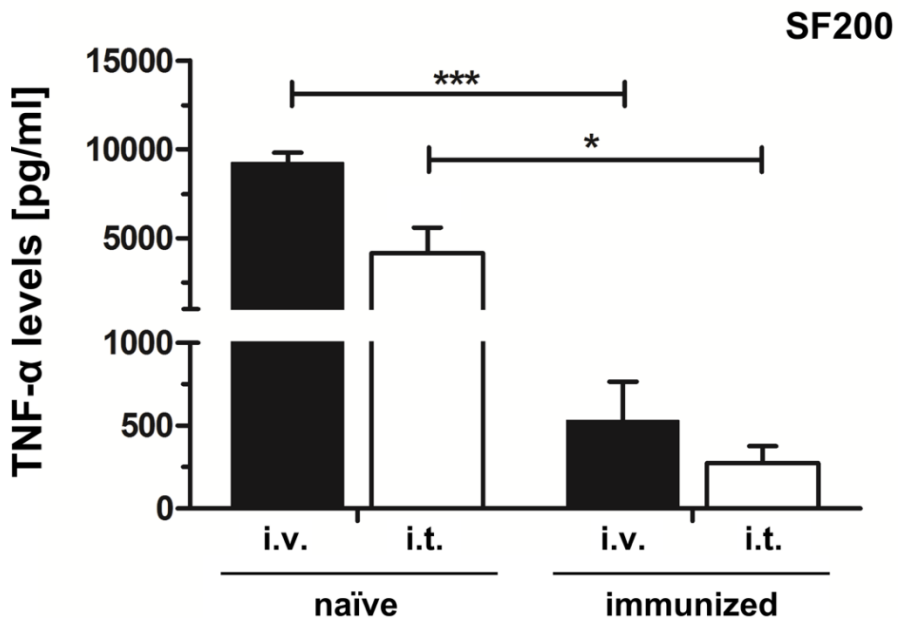
Supplementary Figure S1: Phenotype of SF200 (*ΔlpxR9 ΔpagL7 ΔpagP8 ΔaroA ΔydiV ΔfliF*). (**A**) Scanning electron microscopy of SF200. *ΔfliF* results in a non-flagellated phenotype. White arrows indicate putative OMV formation; insert showing OMV formation at higher magnification on the bacterial surface. (**B**) Schematic representation of the Lipid A molecule. Left: Wt *Salmonella* is able to modify the Lipid A structure by means of *pagP*, *pagL* and *lpxR* resulting in heterogeneous mixtures of Lipid A molecules. Right: Deletion of such genes resulted in the homogeneous hexa-acylated structure of SF200.

Supplementary Figure S2:



Supplementary Figure S2: Effect of UK-1 and SF200 (*ΔlpxR9 ΔpagL7 ΔpagP8 ΔaroA ΔydiV ΔfliF*) immunization on mouse survival upon secondary infection. Mice were pre-treated with PBS (naïve), heat-inactivated UK-1 (UK-1 immunized) or heat-inactivated SF200 (SF200 immunized). Pre-exposed mice were intravenously infected with 10^1 UK-1 (**A**) and $5*10^6$ SF200 (**B**). Mouse survival was monitored for 14 days. Uninfected mice served as control. Displayed are values of mean ± SD of four replicates in each group.

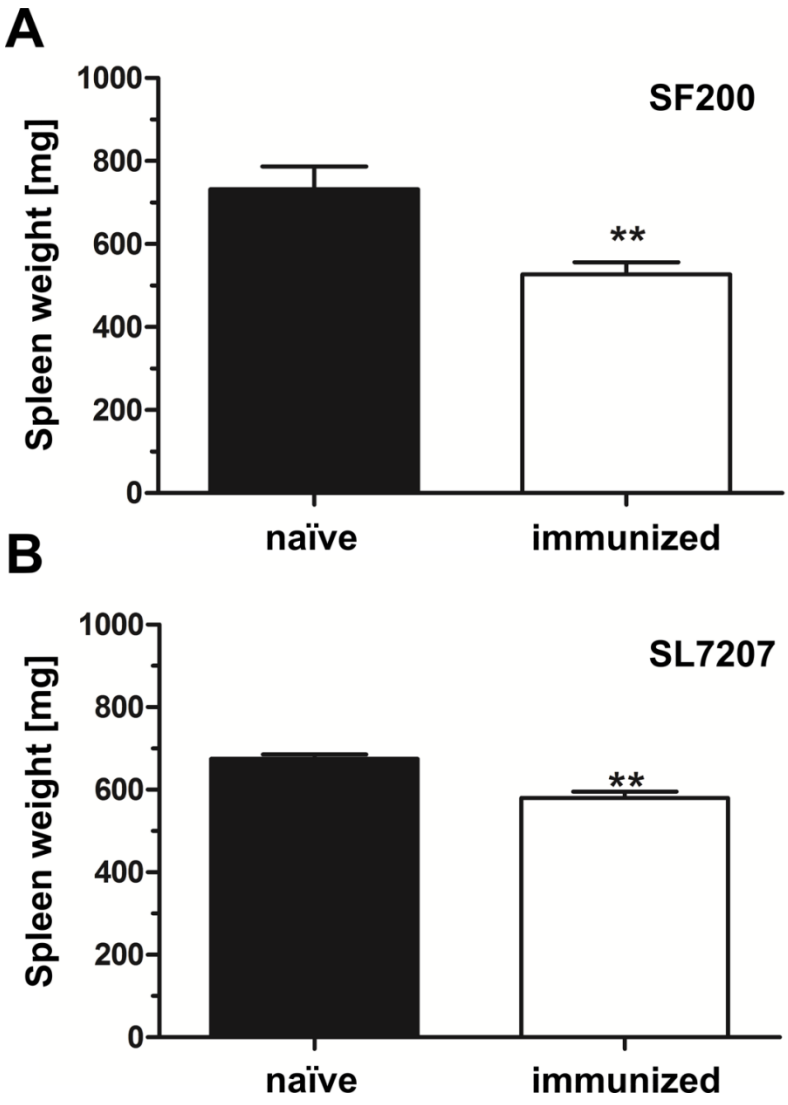
Supplementary Figure S3:



Supplementary Figure S3: TNF-α induction of *Salmonella* in naïve and immunized mice upon infection. Naïve and immunized CT26 tumor-bearing mice were infected intravenously and intratumorally with $5*10^6$ SF200 (*ΔlpxR9 ΔpagL7 ΔpagP8 ΔaroA ΔydiV ΔfliF*). TNF-α levels were measured in sera of CT26 tumor bearing mice isolated 1.5 h upon infection with SF200. Displayed are values of mean ± SD. Results are representative of two independent experiments with six replicates in each group. *, $p<0.05$; ***, $p<0.001$.

Supplementary Figures

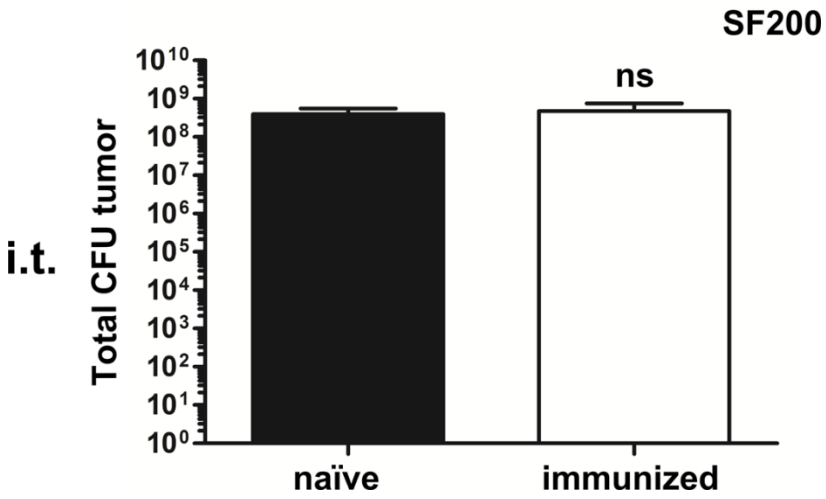
Supplementary Figure S4:



Supplementary Figure S4: Spleen phenotype of naïve and immunized mice upon infection with *Salmonella*. Naïve and immunized CT26 tumor-bearing mice were infected intravenously $5*10^6$ SF200 (*ΔlpxR9 ΔpagL7 ΔpagP8 ΔaroA ΔydiV ΔfliF)* (**A**) or SL7207 (**B**). Spleen weight was measured with a scale 6 dpi and used as indicator for splenomegaly. Displayed are values of mean ± SD. Results are representative of two independent experiments with six replicates in each group. *, $p<0.05$; **, $p<0.01$.

Supplementary Figures

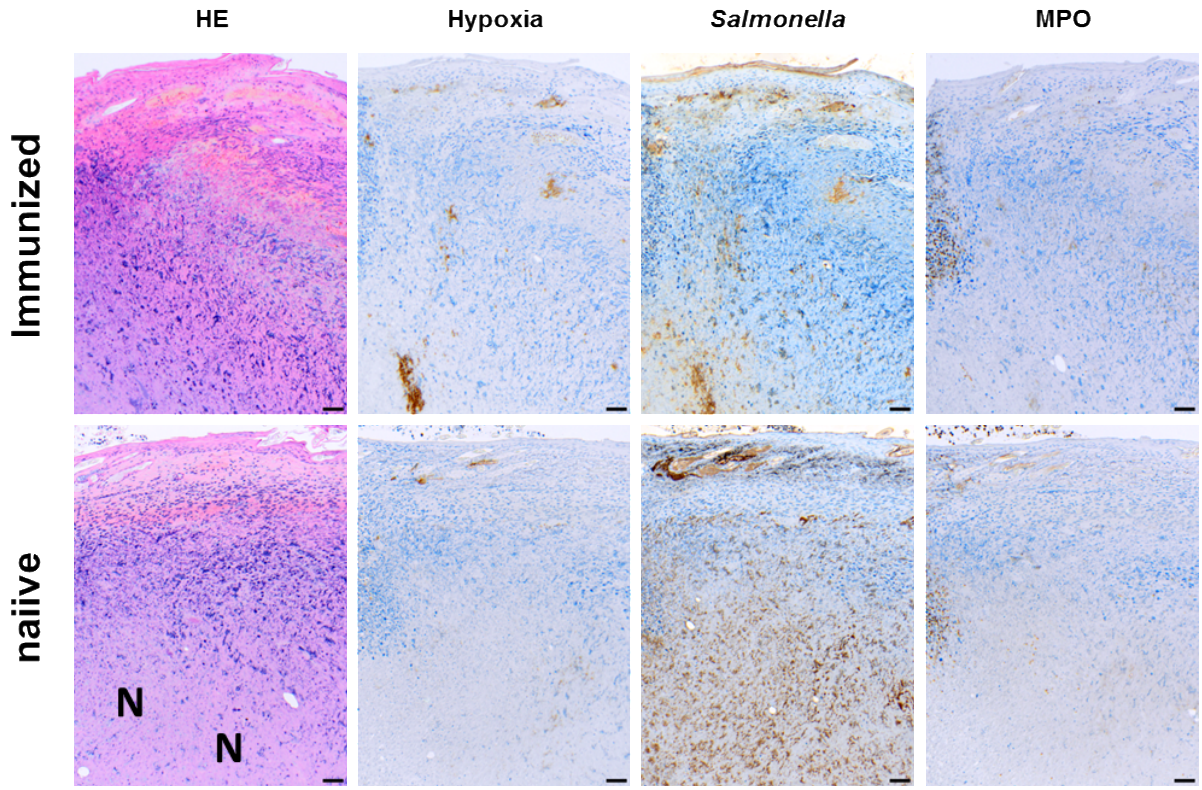
Supplementary Figure S5:



Supplementary Figure S5: Tumor colonization of naïve and immunized mice upon infection with *Salmonella*. Naïve and immunized CT26 tumor-bearing mice were infected intratumorally with $5*10^6$ SF200 (*ΔlpxR9 ΔpagL7 ΔpagP8 ΔaroA ΔydiV ΔfliF*). Total CFU values were determined by serial plating at 4 dpi. Displayed are values of mean ± SD. Results are representative of two independent experiments with six replicates in each group.

Supplementary Figures

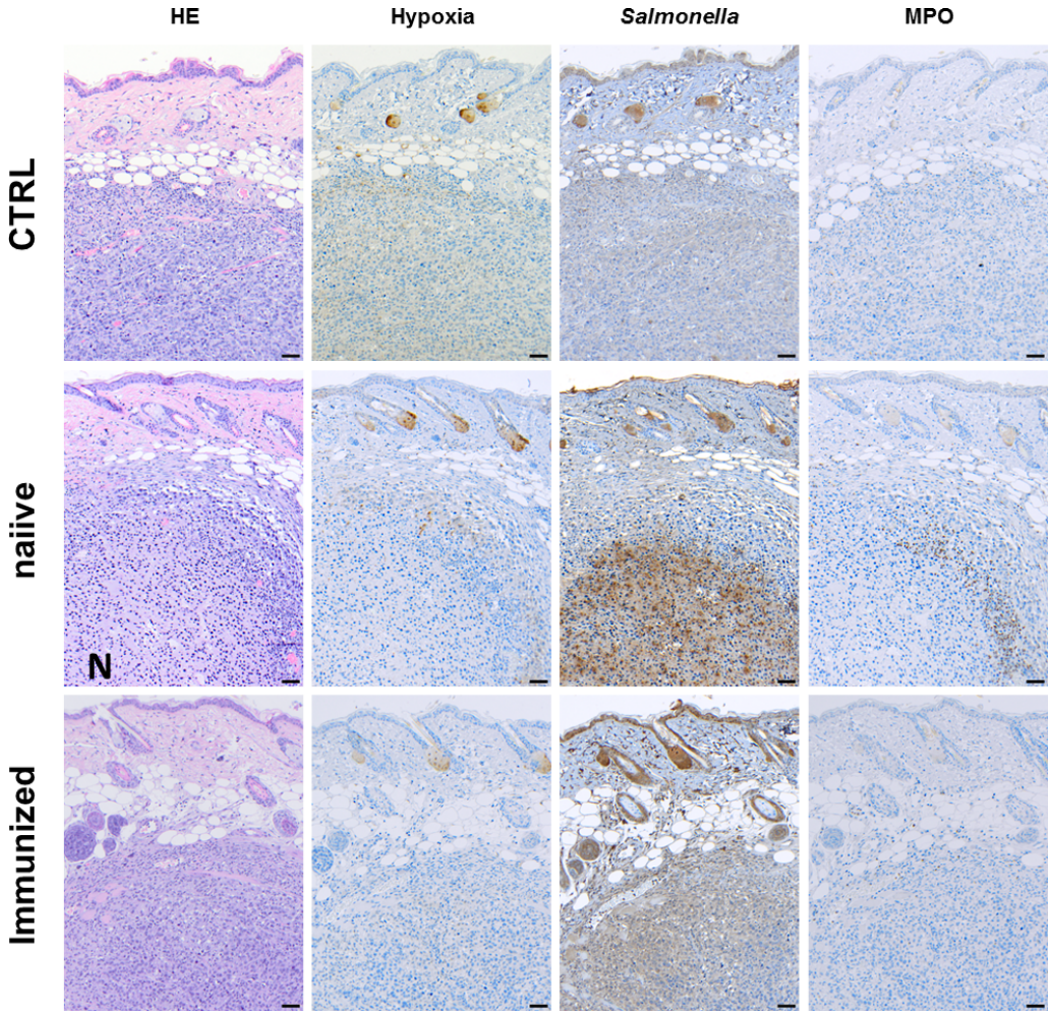
Supplementary Figure S6:



Supplementary Figure S6: Pre-exposure reduces the formation of necrosis in the early stages of infection upon intratumoral infection with *Salmonella*. CT26 tumor-bearing mice were infected with $5*10^6$ SF200 (*ΔlpxR9 ΔpagL7 ΔpagP8 ΔaroA ΔydiV ΔfliF*) via intratumoral infection. 48 hpi, tumors were isolated and prepared for immune histochemical staining. Immunized mice are less prone to necrosis formation and hypoxia. Dispersion of salmonellae in and beyond necrotic center, and presence of neutrophils in immediate proximity to the salmonellae was only clearly visible in naïve mice. "N" denotes areas necrosis**.** Hypoxia was stained with antibodies against metabolites of pimonidazole-HCl, otherwise administered i.v. 30 mins prior to isolation. Myeloperoxidase (MPO) denotes presence of neutrophilic granulocytes, and *Salmonella* was stained using a specific antibody. Differential staining was performed on consecutive sections. Scale bar corresponds to 100 µm. Images representative of at least 3 replicates are displayed.

Supplementary Figures

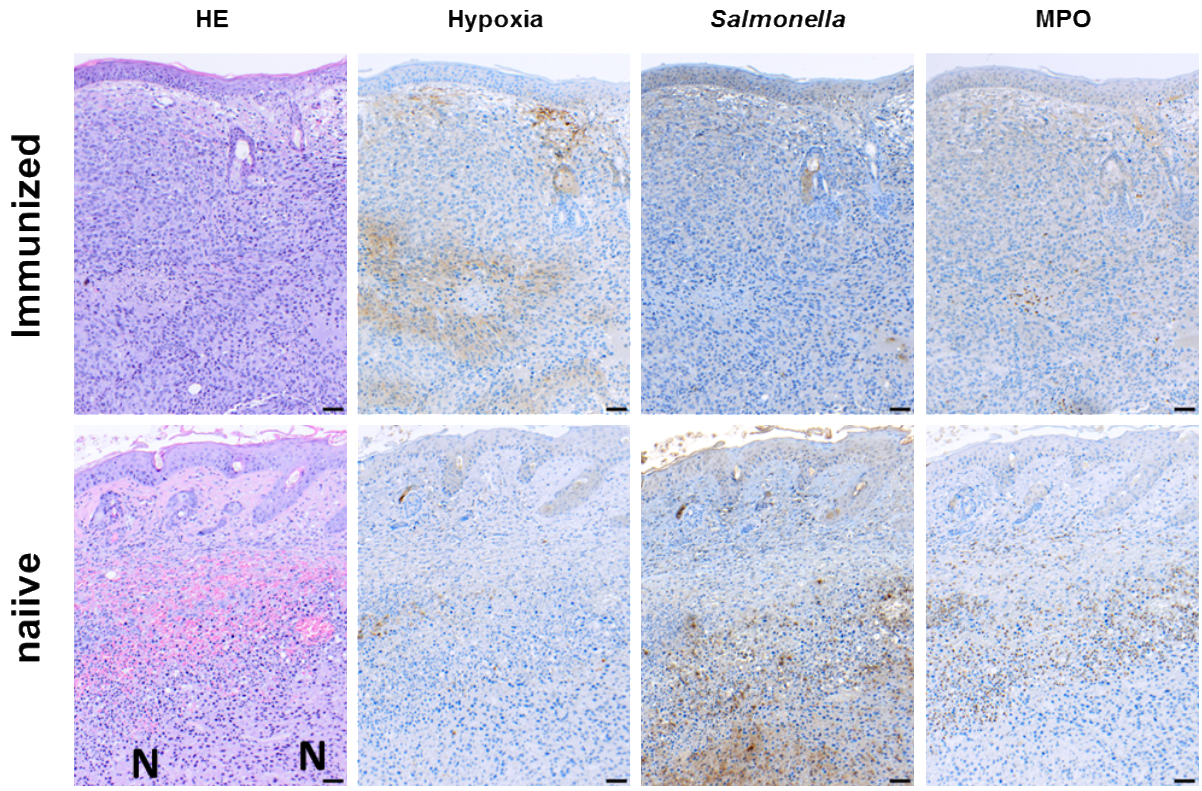
Supplementary Figure S7:



Supplementary Figure S7: Pre-exposure reduces the formation of necrosis in the early stages of infection upon intravenous infection with *Salmonella*. CT26 tumor-bearing mice were infected with $5*10^6$ SF200 (*ΔlpxR9 ΔpagL7 ΔpagP8 ΔaroA ΔydiV ΔfliF*) via intravenous infection. 24 hpi, tumors were isolated and prepared for immune histochemical staining. Immunized mice are less prone to necrosis formation and hypoxia. Dispersion of salmonellae in and beyond necrotic center, and presence of neutrophils in immediate proximity to the salmonellae was only clearly visible in naïve mice. "N" denotes areas necrosis**.** Hypoxia was stained with antibodies against metabolites of pimonidazole-HCl, otherwise administered i.v. 30 mins prior to isolation. Myeloperoxidase (MPO) denotes presence of neutrophilic granulocytes, and *Salmonella* was stained using a specific antibody. Differential staining was performed on consecutive sections. Scale bar corresponds to 100 µm. Images representative of at least 3 replicates are displayed.

Supplementary Figures

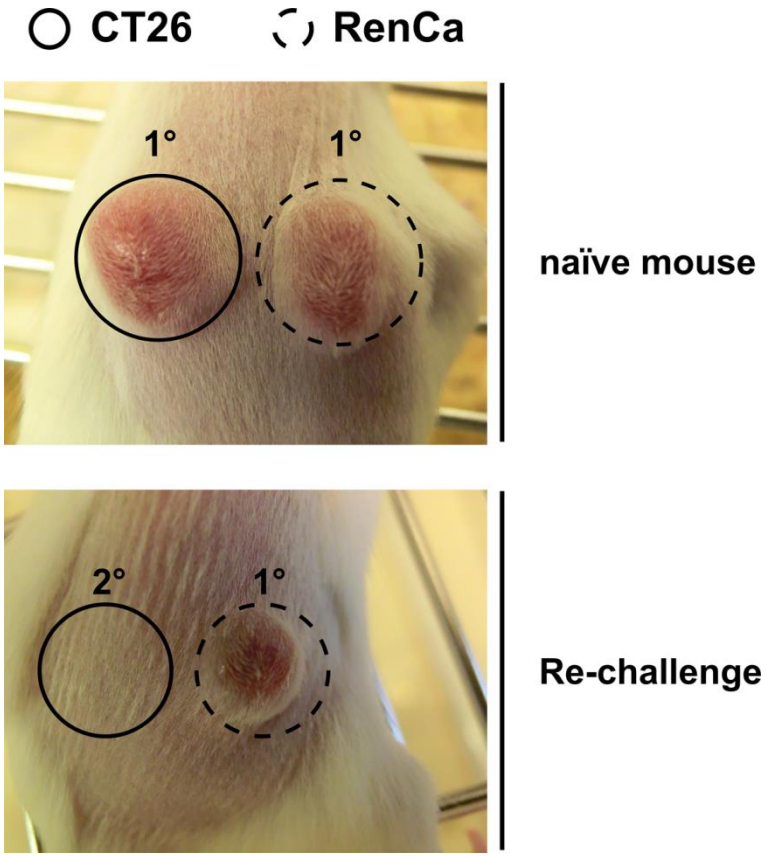
Supplementary Figure S8:



Supplementary Figure S8: Pre-exposure reduces the formation of necrosis in the early stages of infection upon intratumoral infection with *Salmonella*. CT26 tumor-bearing mice were infected with $5*10^6$ SF200 (*ΔlpxR9 ΔpagL7 ΔpagP8 ΔaroA ΔydiV ΔfliF*) via intratumoral infection. 24 hpi, tumors were isolated and prepared for immune histochemical staining. Immunized mice are less prone to necrosis formation and hypoxia. Dispersion of salmonellae in and beyond necrotic center, and presence of neutrophils in immediate proximity to the salmonellae was only clearly visible in naïve mice. "N" denotes areas necrosis**.** Hypoxia was stained with antibodies against metabolites of pimonidazole-HCl, otherwise administered i.v. 30 mins prior to isolation. Myeloperoxidase (MPO) denotes presence of neutrophilic granulocytes, and *Salmonella* was stained using a specific antibody. Differential staining was performed on consecutive sections. Scale bar corresponds to 100 µm. Images representative of at least 3 replicates are displayed.

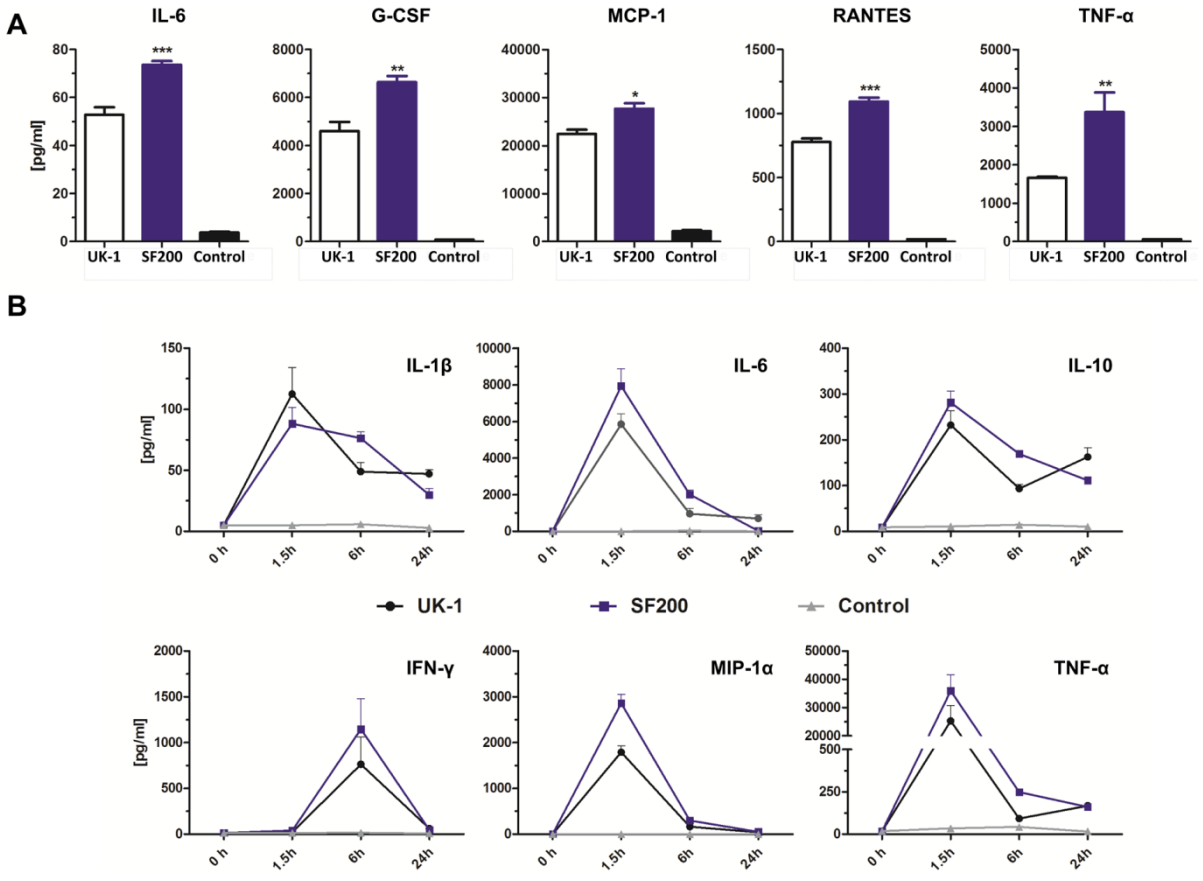
Supplementary Figures

Supplementary Figure S9:



Supplementary Figure S9: Re-challenge (2°) experiment with CT26. Upon successful CT26 tumor clearance in response to SF200 (*ΔlpxR9 ΔpagL7 ΔpagP8 ΔaroA ΔydiV ΔfliF*), mice were re-inoculated with CT26 (2°). Concurrent primary inoculation (1°) of RenCa and inoculation on naïve mice served as control. Pictures display tumors 14 days post inoculation.

Supplementary Figure S10:



Supplementary Figure S10: Cytokine, chemokine and growth factor detection in supernatants, sera and lysates upon infection with SF200 and Wt. Cytokine, chemokine and growth factor concentrations in supernatants of (**A**) 264.7 RAW macrophages cells (6 hpi with MOI 10) and (**B**) sera (1.5 hpi, 6 hpi and 24 hpi; infection: $5*10^6$ SF200 or $5*10^6$ Wt) were quantified. The cytokine levels were analyzed using a Bio-PlexPro™ kit and compared to Wt infections. Displayed are values of mean ± SD of four replicates in each group. Uninfected macrophages and mice served as control.

Supplementary Table

Supplementary Table S1: Bacterial strains and plasmids used in this study

Strain	Description	Source	Ref.
***Salmonella* Typhimurium strains**			
SL7207	*hisG*⁻, *ΔaroA*	Lab stock	(1)
SF200	*ΔlpxR9 ΔpagL7 ΔpagP8 ΔaroA ΔydiV ΔfliF*	This study	-
***E. coli* strains**			
Symbioflor-2	*Escherichia coli* Symbioflor-2 (G1/2, G3/10, G4/9, G5, G6/7 and G8, pooled 1:1)	SymbioPharm	-

1. Hoiseth SK and Stocker BA. Aromatic-dependent *Salmomella* Typhimurium are non-virulent and effective as live vaccines. Nature. 1981; 291:238-9.