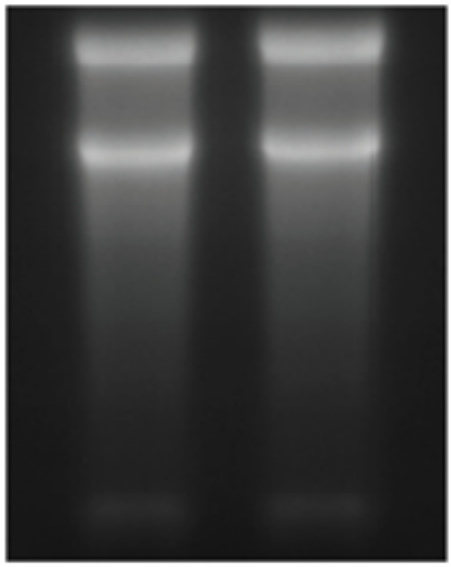

Figure S1 No degradation of RNA was observed by recombinant GAS DNase Sda1. RNA was isolated from GAS and co-incubated with either the DNase buffer alone or with 365 ng of the recombinant Sda1 in DNase buffer for 10 minutes at 37uC. Visualisation followed by 1.5% TBE agarose gel electrophoresis.

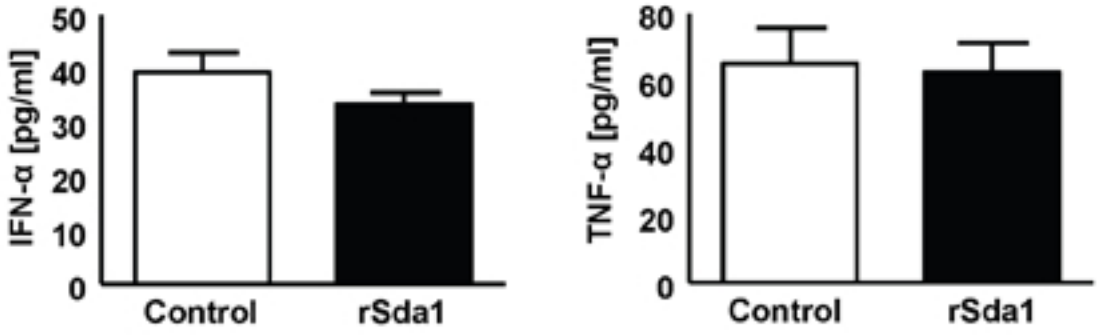

Figure S2 The recombinant GAS DNase Sda1 does not induce IFN-a and TNF-a secretion. rSda1 was co-incubated with murine macrophages and the cytokine response measured after 12 hours.

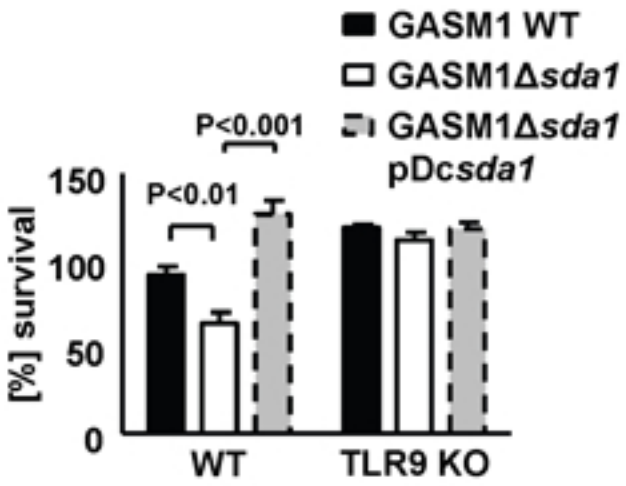

Figure S3 The GAS DNase Sda1 interferes with TLR9 activation. Stimulation for 12 hours of BMDMs with GAS strains expressing Sda1 (GASM1 WT and GASM1 Dsda1 pDcsda1) resulted in significantly less IFN-a and TNF-a secretion compared to matching strains lacking Sda1 (GASM1 Dsda1). Data were pooled from 3 experiments done in triplicates and presented as mean 6 SEM.

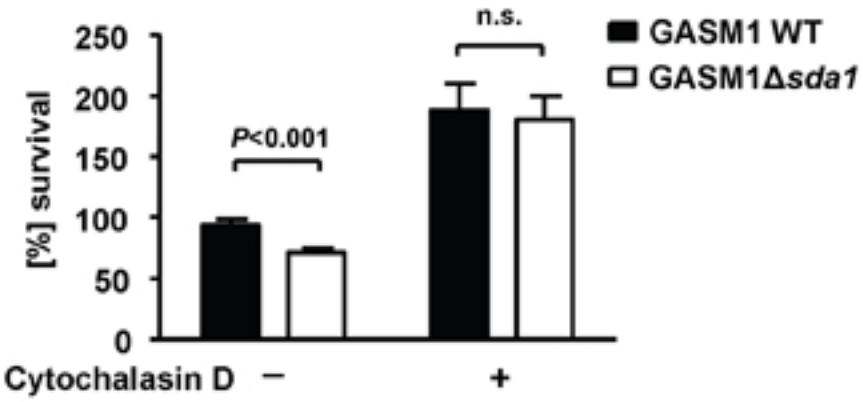

Figure S4 The GAS DNase Sda1 does not affect extracellular killing in macrophages.

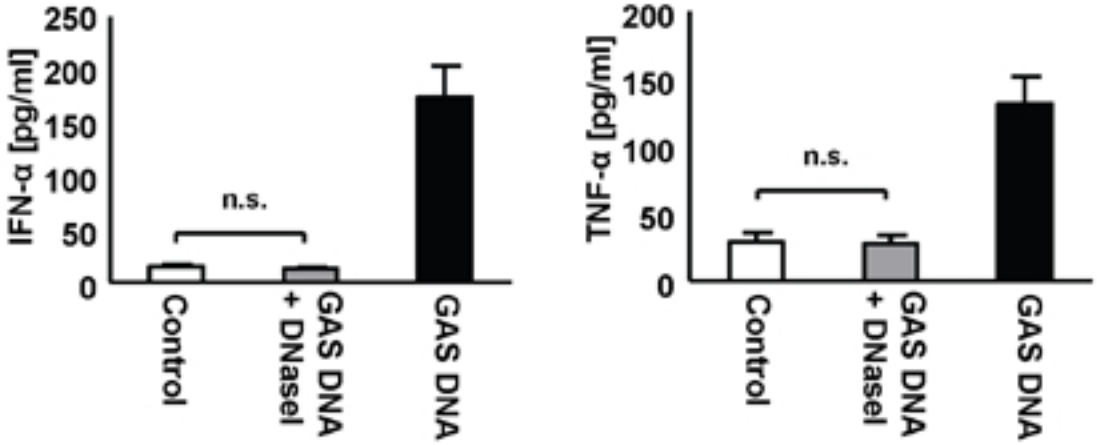

Figure S5 GAS DNA digested with DNaseI does not induce IFN-a and TNF-a secretion. In order to test for purity of our isolated bacterial DNA we co-incubated the bacterial DNA with and without DNaseI and stimulated BMDMs for 12 hours. Addition of DNaseI resulted in similarly low IFN-a and TNF-a secretion as observed for the controls.

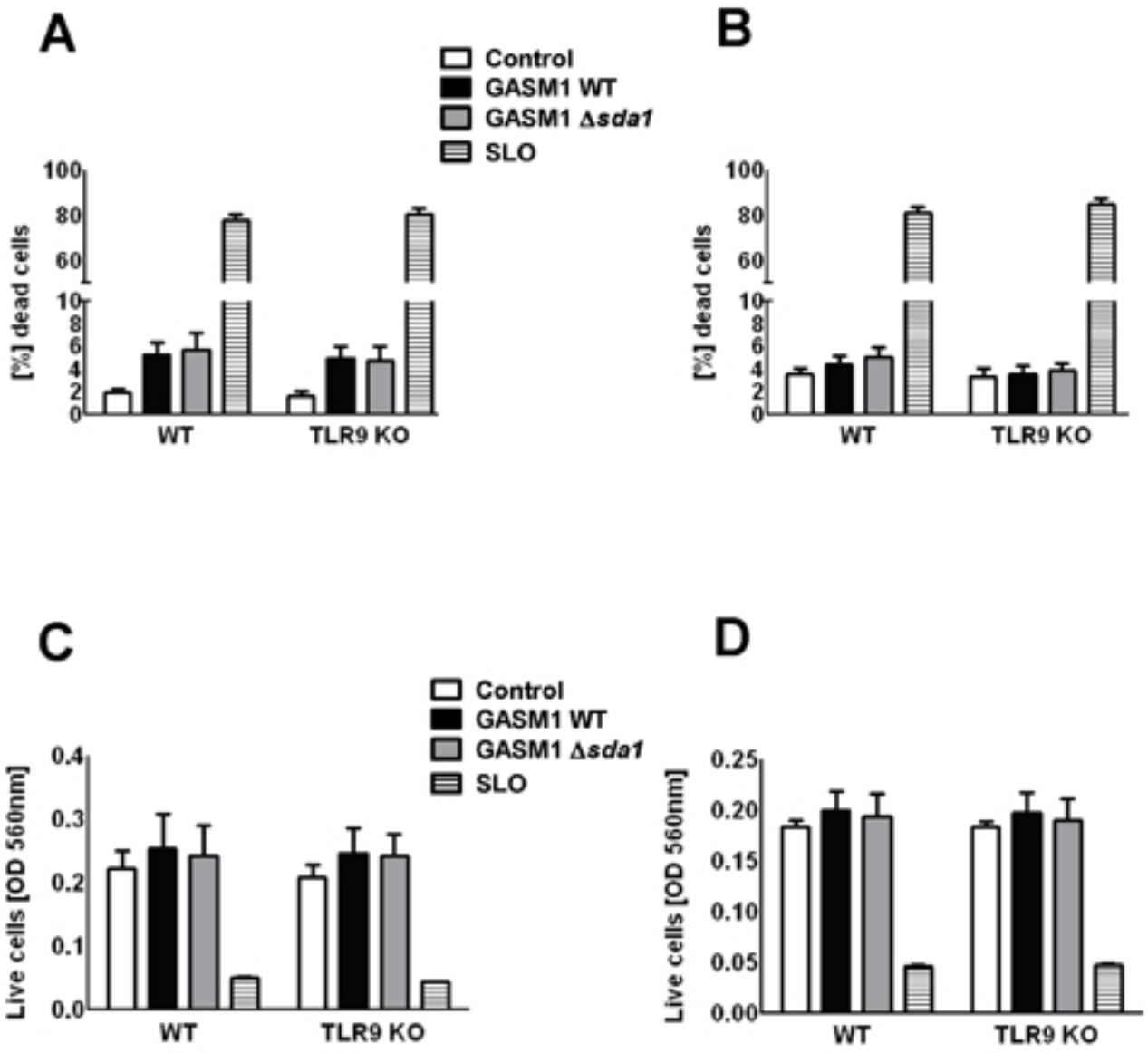

Figure S6 BMDMs viability assays. Logarithmic phase bacteria were added to BMDM at MOI 1. Recombinant streptolysin O (rSLO) was used as a positive control at a final concentration of 16 mg/ml. After 4 (A) and 12 hours (B) BMDMs were analysed using the mammalian cell live/dead staining kit or the MTT assay at 4 (C) and 12 hours (D) respectively. Data were pooled from 3 experiments done in triplicates and presented as mean 6 SEM.

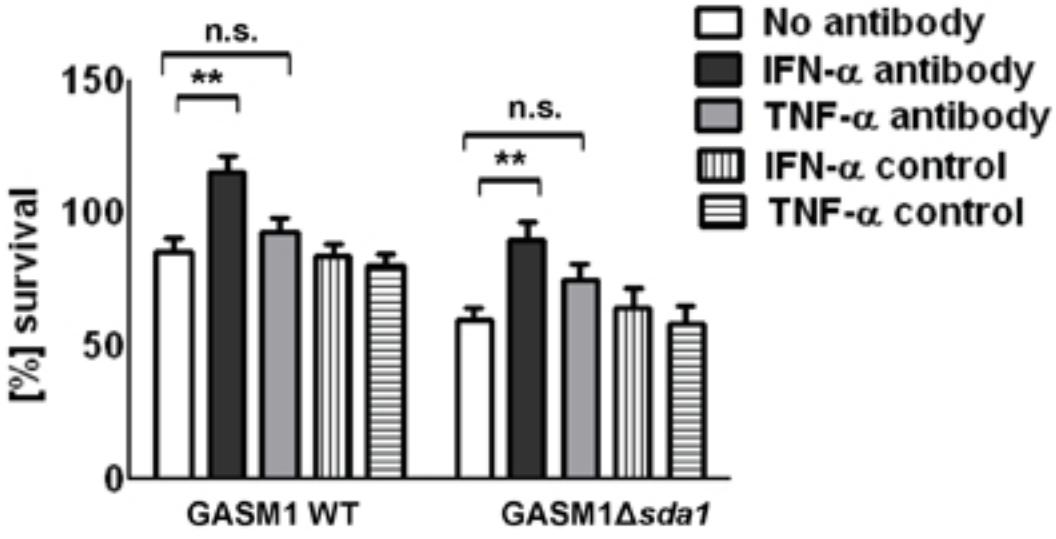

Figure S7 Addition of neutralizing antibodies against INF-a increases bacterial survival of GAS. In order to test if blocking IFN-a and TNF-a can prevent phagocytic killing mediated by GASDsda1 we repeated the BMDM killing assays using with WT BMDM challenged with GASWT M1 and GASDsda1 bacteria (MOI 1) after having pre-incubated the BMDM for 2 h with either the neutralizing antibodies against TNF-a or IFN-a or their respective controls. Data were pooled from 3 experiments done in triplicates and presented as mean 6 SEM. ** P,0.01.

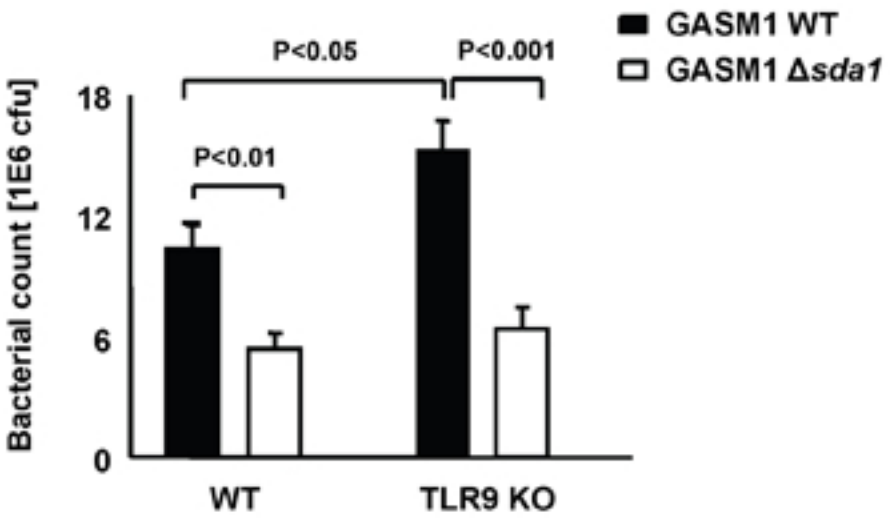

Figure S8 TLR9 is important for controlling GAS infection in vivo. WT and TLR9-deficient mice were injected subcutaneously with equivalent inocula of GASWT M1 and GASDsda1 and after 4 days bacteria were enumerated in the skin. N= 5 for the group of TLR9 mice injected with GAS Dsda1 and n =6 for the other groups. Data shown were pooled from two independent experiments and presented as mean 6 SEM.

Supplemental Materials and Methods

Pretreatment of RNA with recombinant Sda1

RNA was prepared from GAS using the Qiagen RNAeasy kit following the manufacturer's instructions. Five µg of GAS RNA were then incubated at 37°C for 10 minutes with either the DNase buffer alone or with 365 ng of the recombinant sda1 in DNase buffer . Visualisation followed by 1.5% TBE agarose gel electrophoresis.

Macrophage extracellular killing assays

For extracellular killing the BMDMs were incubated with 10 µg/ml cytochalasin D (Sigma) for 1 hour. After this incubation period, the total killing assay protocol was followed. Serial dilutions of the lysates were plated on THA for enumeration of surviving bacterial colony forming units (cfu). Reactive oxygen species were measured as described before [1]. BMDM killing assays were repeated with WT BMDM pretreated with the neutralizing antibodies against TNF-α or IFN-α. Ten µg/ml anti murine TNF-α antibody (clone XT3.11, BioXcell) or 1 µg/ml anti murine IFN-α antibody (PBL interferon source) were added to the BMDMs 2h before the GAS killing assay was performed as described in the methods section. Rat anti-human monoclonal antibody (Hycult biotech) for TNF-α and polyclonal rabbit IgG control (R&D) for IFN-α served as controls.

BMDMs viability assays

Macrophages were harvested as described above. Logarithmic phase bacteria were added to the wells

at final MOI of 1 and plates were centrifuged for 5 minutes at 1500 rpm. The same time points as for the assays above were used (i.e. 4 hours as for the bacterial killing assay and 12 hours as for the cytokine stimulation assays). Recombinant streptolysin O (rSLO) was used as a positive control [2] at a final concentration of 16 µg/ml. Since rSLO lyses the cells completely after 12 hours rSLO was used only for 4hours for both time points.

Live/dead mammalian cell staining: BMDMs were seeded at 1 x 10^5 cells/well into black 96 well plates with glass bottoms and challenged with bacteria as described above. After 4 and 12 hours, respectively, the BMDMs were washed with PBS 3 times and the mammalian cell live/dead staining (Invitrogen) was performed following the manufacturer's instructions. The BMDMs were visualised with the fluorescent IX71 microscope. At least 9 pictures per condition were randomly taken for analysis. For data analysis, the total number of BMDMs per picture was assessed as well as dead BMDMs and the ratio of dead cells/total cells (%) was calculated.

MTT assay: BMDMs were split into 96 well plates at 1 x 10^5 cells / well and challenged with bacteria as described above. After 4 and 12 hours respectively, the wells were washed 3 times with HBSS with Ca^{2+} and Mg^{2+} before 120 µl DMEM+10% FCS and 30µl of 5 mg/ml MTT solution (Sigma) were added to each wells. The plate was then incubated at 37^{o}C in 5% CO_2. The supernatant was removed and 100µl/well 0.04 M HCl in isopropanol was added to the wells and was incubated in RT for 15 minutes and the OD_{560} nm measured.

REFERENCES

1. Zinkernagel AS, Hruz P, Uchiyama S, von Kockritz-Blickwede M, Schuepbach RA, et al. (2011) Importance of Toll-Like Receptor 9 in Host Defense against M1T1 Group A Streptococcus Infections. J Innate Immun.
2. Timmer AM, Timmer JC, Pence MA, Hsu LC, Ghochani M, et al. (2009) Streptolysin O promotes group A Streptococcus immune evasion by accelerated macrophage apoptosis. J Biol Chem 284: 862-871.