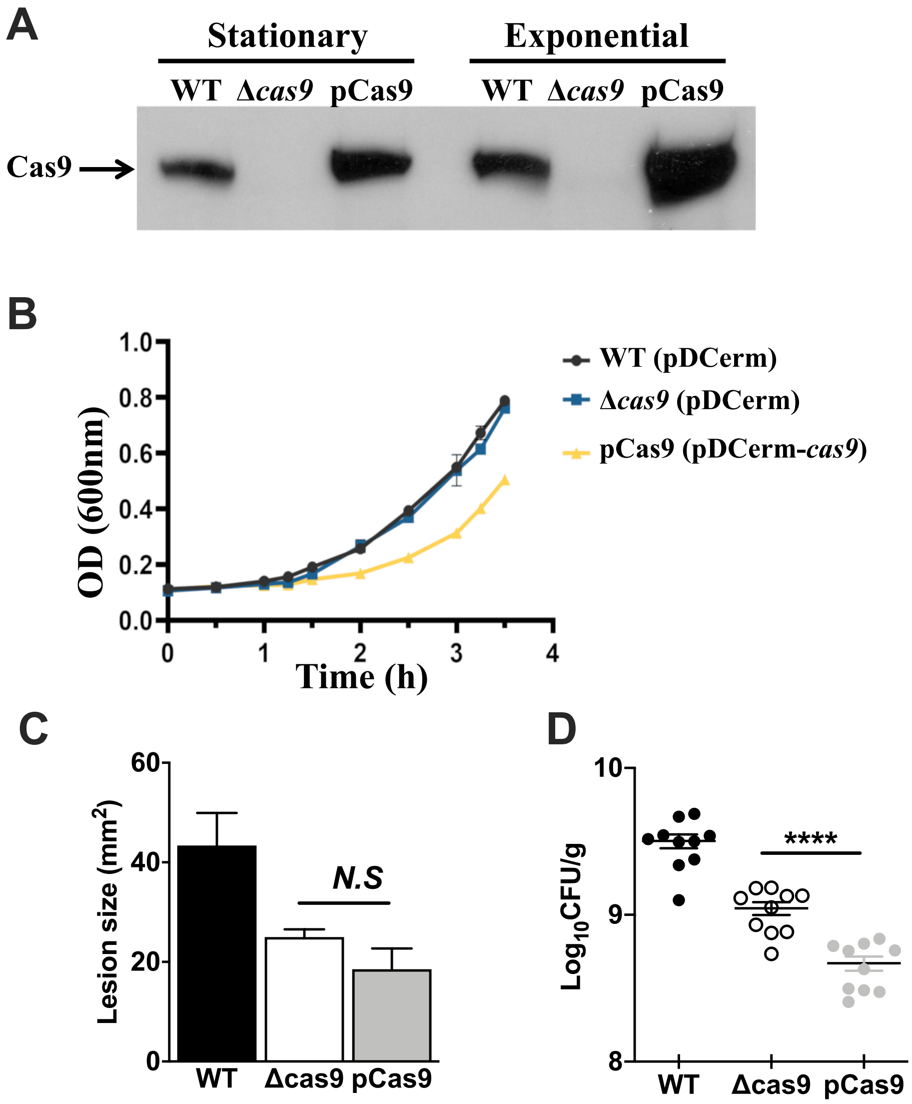
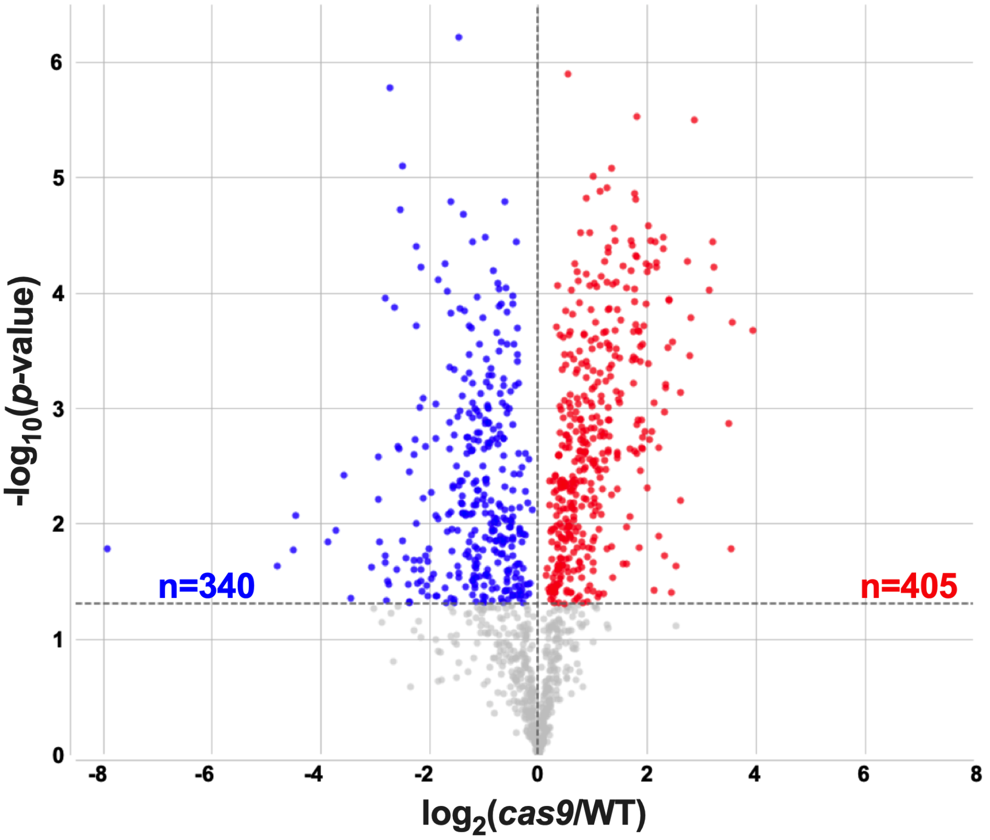
Supplementary Material

Supplementary Figures



Supplementary Figure 1. Genetic complementation of Δ*cas9* GAS M1T1 5448 strain leads to marked growth defect. (**A**) Western blot analysis of Cas9 protein expression in wild type (WT), cas9-deficient (Δ*cas*9) and *cas9*-plasmid complemented (pCas9) GAS strains from cells grown at stationary or exponential growth phases. (**B**) Cellular growth curves of WT, Δ*cas*9 and pCas9 GAS strains in THB media at 37°C. GAS strains in (**B**) carried out either the empty plasmid control (pDCerm) or the *cas9* expressing plasmid (pDCerm-*cas9*). (**C and D**) Subcutaneous infection of C57BL/6 mice with GAS WT, Δ*cas9* or Cas9-complemented (pCas9) strains. Average lesion sizes (**C**) and enumeration of CFUs recovered from excised lesions (**D**) 48 h post-infection. Data in **C** and **D** are plotted as the mean ± s.e.m and are pooled from two independent experiments and analyzed by Student's t test. N.S, (non significant) (p>0.05); ****P<0.001.



Supplementary Figure 2. Abundance of proteins detected by tandem mass tag proteomics in WT and Δ*cas9* GAS M1T1 5448 strains. Volcano plot shows proteins significantly more abundant in the WT (blue dots, n=340) or in the Δ*cas9* strains (red dots, n=405). Significant proteins were initially identified by Student's t test with $p < 0.05$.

Supplementary Tables

Supplementary Table 1. Minimal Inhibitory Concentration (MIC) of cell wall synthesis inhibitory antibiotics against WT or Δ*cas*9 GAS M1T1 5448 strains †.

Antibiotic	WT	Δ*cas9*
penicillin G, μg/ml	0.015-0.031	0.015-0.031
vancomycin, μg/ml	1.0-2.0	1.0-2.0

† MIC performed in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Todd-Hewitt broth

Supplementary Table 2. TMTproteomics.xlsx Normalized abundance of all proteins detected from WT and Δ*cas9* GAS 5448 strains by tandem mass tag proteomics. Peptide reads were searched against database derived from the MGAS5005 M1 serotype *S. pyogenes* genome (GenBank: CP000017.2). Columns include: GenBank protein ID, protein description and full-raw data from three independent biological samples corresponding to each protein identified. F test, *P* value and pi score statistical analysis are also listed in the corresponding column. #, p<0.05 (proteins statistically significant by Student's t test). *, level alpha<0.05 (proteins statistically significant by pi score).

Supplementary Table 3. RAST_significant.xlsx Normalized protein abundance of proteins that were identified to be significantly enriched either in WT or in Δ*cas9* GAS 5448 strains. Significant proteins were grouped in subcategories using RAST. Out of the 745 significantly differentially quantified proteins between both strains, 474 proteins were found annotated by RAST server (RAST annotation +). Some proteins annotated by RAST in different categories (RAST annotation +/-) or not annotated (RAST annotation -) were manually added into the corresponding functional category base on reported literature. Columns include: GenBank protein ID, corresponding gene name, protein description, RAST category and subcategory, as well as full-raw data from three independent biological samples corresponding to each protein identified as significant. F test, *P* value and pi score statistical analysis are also listed in the corresponding column. #, p<0.05 (proteins statistically significant by Student's t test). *, level alpha<0.05 (proteins statistically significant by pi score).