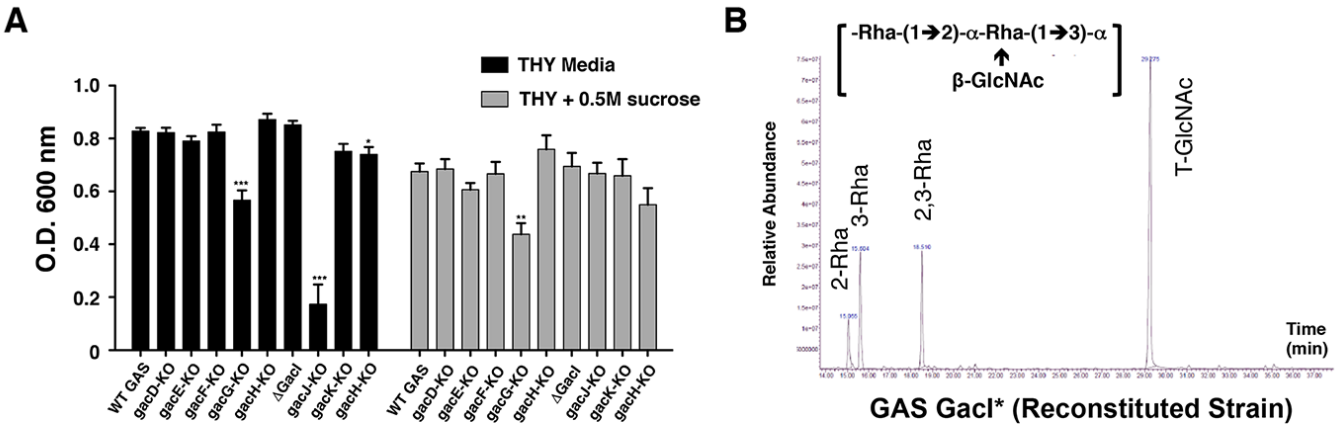

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	Composition Analysis					Linkage Analysis				
	Rha	Man	Glc	GlcNac	PS (µg)	T-Rha	2-Rha	3-Rha	2,3-Rha	T-GlcNac
Wild-Type M1 GAS	57.49	0.44	1.06	41.01	417.5	0.14	7.18	23.66	24.86	44.16
ΔGacI Mutant	94.85	0.43	1.46	3.27	137.4	0.88	68.47	27.84	1.05	1.76
GacI* Reconstituted	47.56	0.35	1.35	50.75	254.7	0.2	7.93	17.96	16.54	57.37

Figure S1, related to Figure 1. Supplemental Data on Growth and Polysaccharide Composition of GAC Mutants

(A) Growth of GAC insertional mutants in rich broth. Plasmid integrational mutants in the GAC gene cluster were generated in the presence of osmotic stabilization (0.5 M sucrose) and targeted plasmid insertion was confirmed by PCR. Mutants were grown overnight in regular rich broth (THY) or THY + 0.5 M sucrose and the optical density at 600 nm recorded to determine the effects of mutation on bacterial growth. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (B) HPLC tracing and linkage analysis with deduced schematic structure of the repeating unit of extracted GAC from the GacI* reconstituted mutant strain. (C) Carbohydrate composition analysis of GAC from GAS WT, ΔGacI, and reconstituted GacI* strains shows the mole percentage amount of individual sugars; the total amount of PS noted is that present in 1 ml of aqueous solution. Linkage analysis data represent the area percentages from the HPLC assay. Abbreviations: GAS = group A *Streptococcus*; PS = polysaccharide; Rha = Rhamnose; Man = Mannose; Glc = Glucose; GlcNAc = N-acetyl-D-glucosamine; T = terminally-linked.

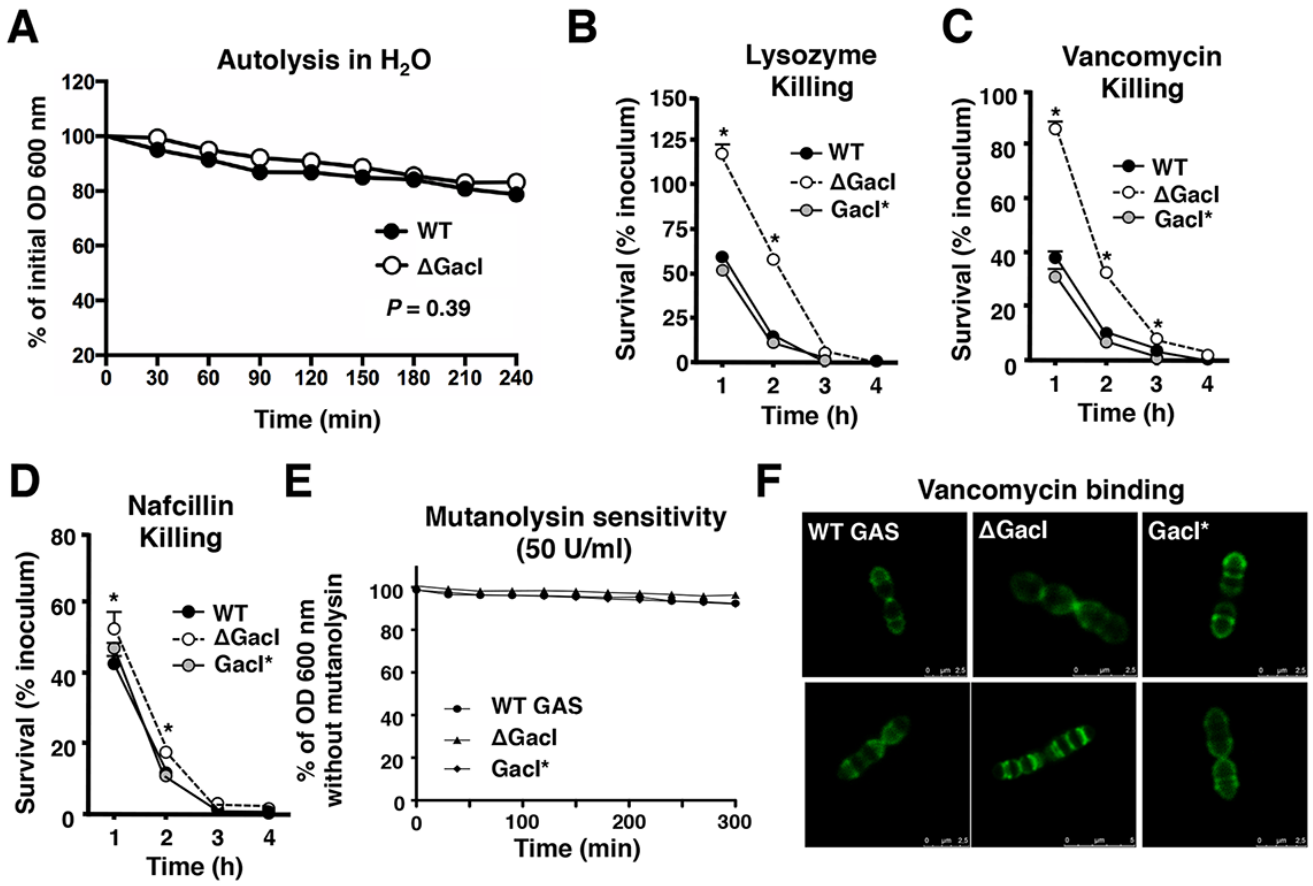

Figure S2, related to Figure 3. Supplemental Data on Cell Wall Integrity of ΔGacI Mutant GAS

(A) Sensitivity of GAS WT and ΔGacI to autolysis. Kinetics measured for (B) lysozyme-, (C) vancomycin-, and (D) nafcillin-mediated killing of GAS WT, ΔGacI, and GacI* strains. Pooled normalized data from three independent experiments are shown (mean ± SEM; two-way ANOVA). *$p <$ 0.05. (E) GAS WT, ΔGacI, and GacI* bacteria are equally resistant to lysis by mutanolysin (50 U/ml; pooled data from two independent experiments, mean ± SEM). (F) Fluorescent vancomycin staining of exponentially growing GAS WT, ΔGacI, and GacI* bacteria. Two representative pictures per strain are shown

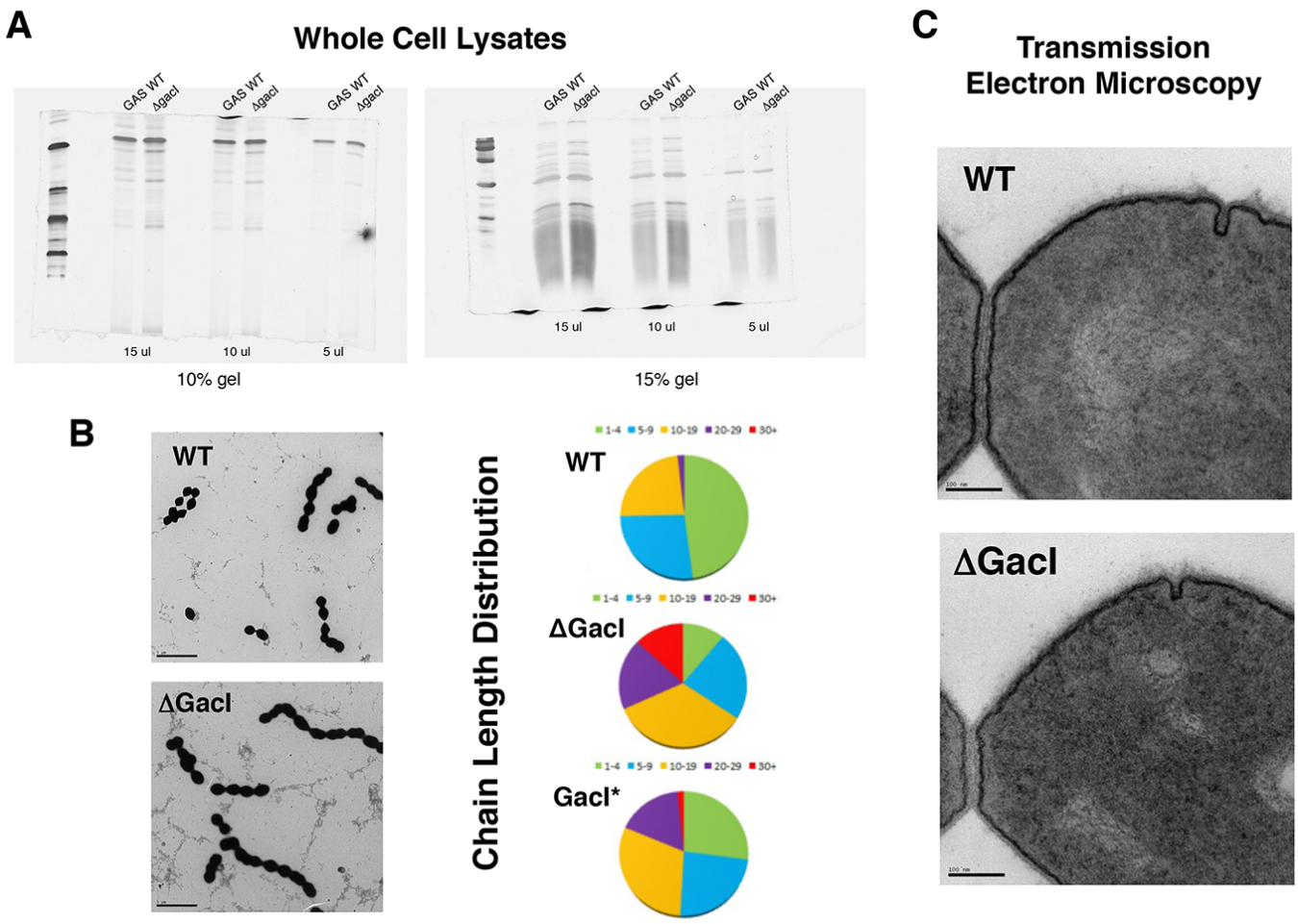

Figure S3, related to Figure 4. Supplemental Data on Protein Composition and Morphology of ΔGacI Mutant GAS

(A) Similar protein profiles of cell lysates prepared from WT and ΔGacI mutant GAS. WT and ΔGacI strains were grown to exponential phase, harvested, and washed in PBS. Equivalent amounts of bacteria were resuspended in Tris buffer containing mutanolysin and lysostaphin and incubated at 37°C. Preparations were boiled in sample buffer and different amounts of bacterial lysate were separated on 10% and 15% SDS-PAGE gels and silver stained to visualize the bacterial protein profile. (B) Deletion of the *gacI* gene affects cell separation as deduced from an observed increase in chain length by microscopy. Chain length was quantified by counting the number of segments in a chain from at least 200 chains. Chain length was categorized as follows: 1-4 segments, 5-9 segments, 10-19 segments, 20-29 segments, or more than 30 segments per streptococcal chain. (C) Cell wall appearance by transmission electron microscopy of GAS WT and ΔGacI mutant bacteria.

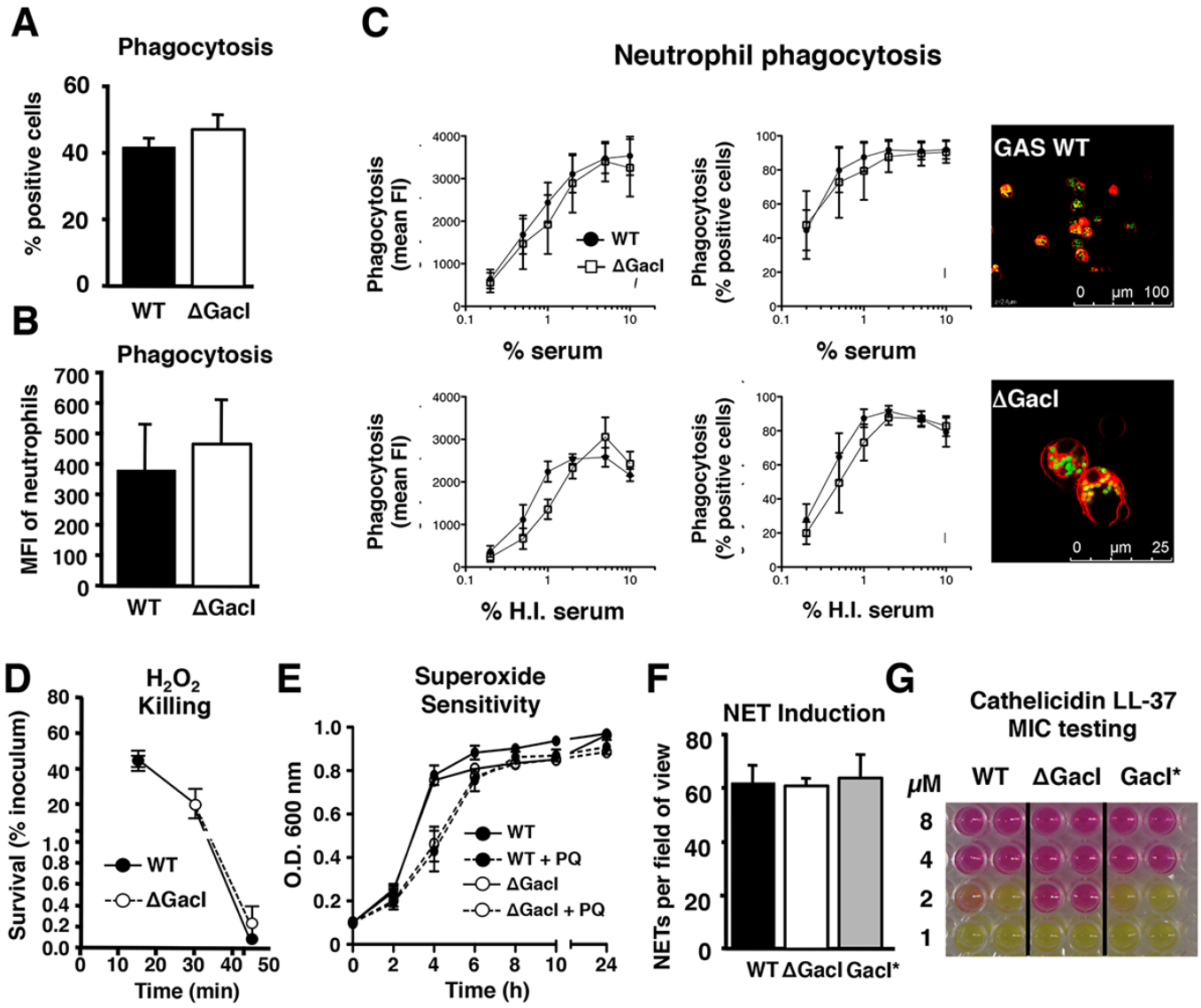

Figure S4, related to Figure 5. Supplemental Data on ΔGacI Mutant GAS Susceptibility to Neutrophil Phagocytosis, Reactive Oxygen Species, and Cathelicidin LL-37

(A) Phagocytosis by neutrophils of fluorescently (FITC)-labeled GAS WT and ΔGacI mutant bacteria in human whole blood. Data are presented as % FITC-positive neutrophils or (B), mean fluorescence intensity (MFI) on gated neutrophils. Pooled data from four independent experiments are shown (mean ± SEM). (C) Quantification of phagocytosis by isolated neutrophils of FITC-labeled GAS WT and ΔGacI mutant bacteria in the presence of different percentages of pooled active or heat-inactivated human serum. Data are presented as % FITC-positive neutrophils, and mean fluorescence intensity (MFI) on gated neutrophils. Pooled data from three independent experiments are shown (mean ± SEM). Representative confocal images demonstrate intracellular localization of fluorescent GAS WT (top) and ΔGacI mutant bacteria (bottom). Loss of the GlcNAc side chain does not affect resistance to oxidative stress including (D) hydrogen peroxide (pooled data from three independent experiments; mean ± SEM), and (E) paraquat (PQ)-generated superoxide (pooled data from three independent experiments; mean ± SEM). (F) Quantification of NET induction upon neutrophil incubation with the indicated bacterial strains (mean ± SEM, two independent experiments). (G) ΔGacI mutant bacteria are hypersensitive to human cathelicidin antimicrobial peptide LL-37 (MIC assay, $t = 24$ h).

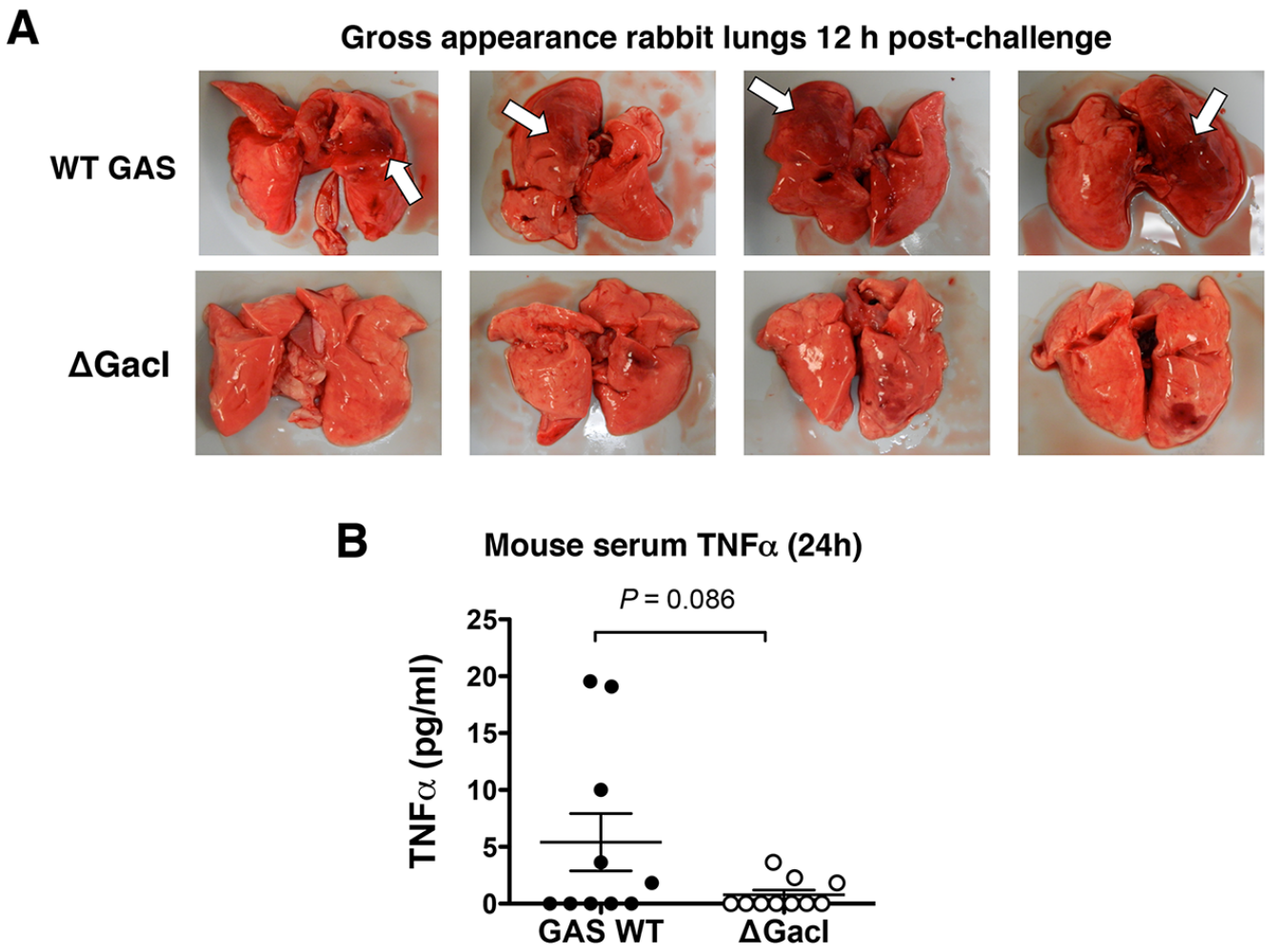

Figure S5, related to Figure 6. Supplemental Data from Animal Challenge Studies with WT and ΔGacI Mutant GAS

(A) Gross lung appearance of rabbit lungs 12 h after infection with GAS WT or ΔGacI mutant showing increased evidence of hemorrhagic necrosis in the WT-infected animals. (B) Trend toward lower serum TNF-α levels 24 h post intraperitioneal challenge in mice infected with ΔGacI mutant GAS.

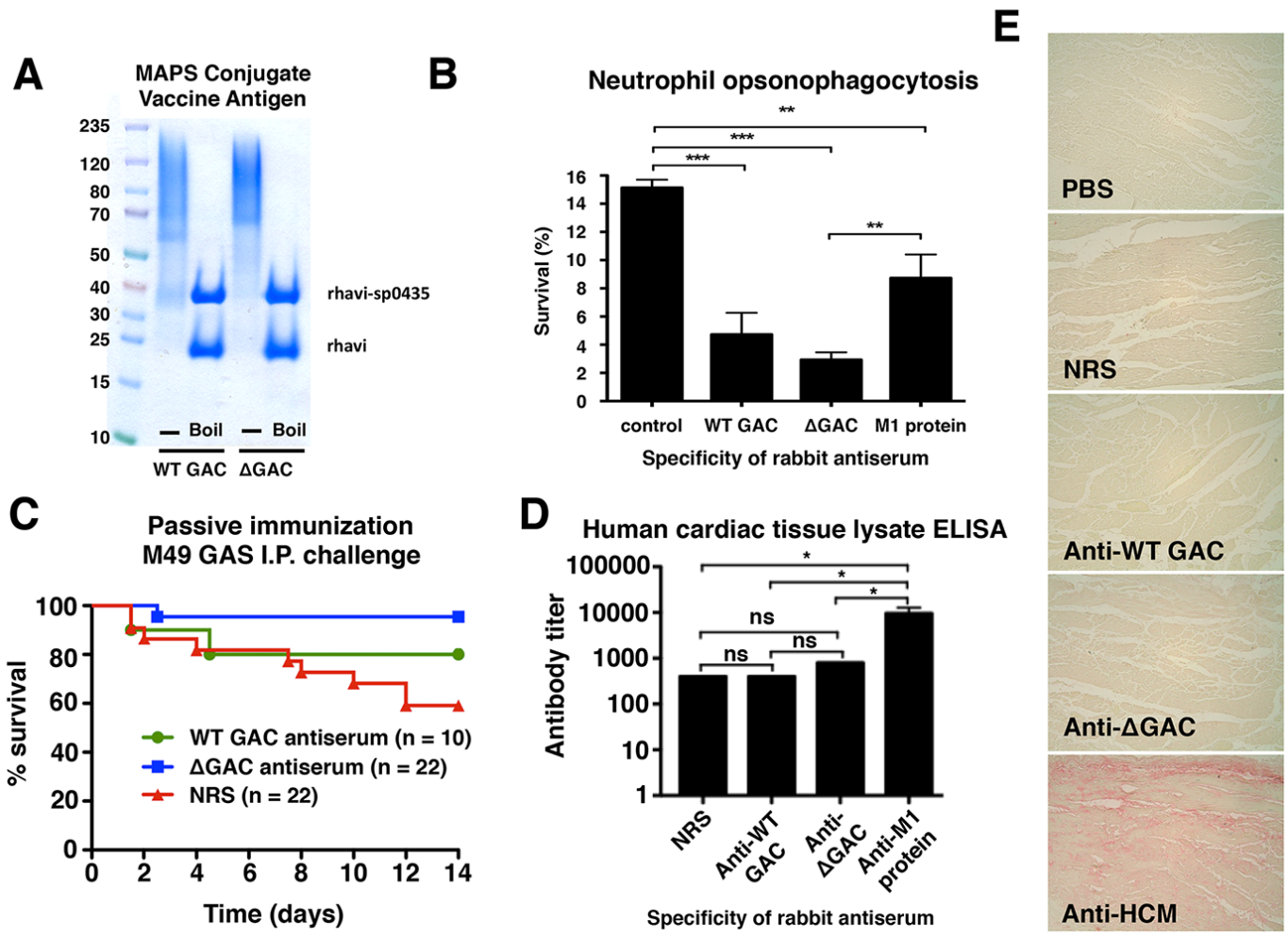

Figure S6, related to Figure 7. Comparison of Activities of Rabbit Antisera Raised Against MAPS Protein Conjugates of WT GAC and ΔGAC

(A) SDS-PAGE analysis of MAPS conjugate protein-GAC complexes prepared from WT GAC and ΔGAC carbohydrate and subjected to further gel-filtration purification. No exogenous protein contamination is appreciated in boiled, denatured samples. (B) Opsonophagocytic killing of serotype M1 GAS serotype upon addition of anti-ΔGAC antiserum, WT GAC antiserum, normal rabbit serum (NRS) and anti-M1 protein antiserum. (C) Mice are protected from infection with WT GAS M49 through passive immunization with ΔGAC antiserum or WT GAC antiserum compared to NRS. (D, E) Lack of cross-reactivity of WT GAC or ΔGAC rabbit antiserum against human cardiac tissue as assessed by (D) ELISA of human cardiac cell extract (anti-M1 protein positive control) and (E) direct immunohistochemistry of human cardiac tissue; 1:1,000 antibody dilution, anti-human cardiac myosin (HCM) used as positive control. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.