Supplemental Methods

TCA precipitation of secreted proteins

GAS from an overnight culture were inoculated 1:20 into 10 ml THY supplemented with appropriate antibiotics and grown to stationary phase (Klett 100-120). Cells were then pelleted and culture supernatants were prepared by centrifugation at 8,000 g for 15 min at 4ºC. Bovine Serum Albumin (BSA) was added to a final concentration of 50 µg/ml and trichloroacetic acid (TCA) to a final concentration of 15%. The resulting solution was incubated on ice for 30 min and centrifuged for 15 min at 12,000 g. The pellet was washed twice with 500 µl of ice-cold acetone, centrifuged for 15 min at 12,000 g, and the resulting pellet was resuspended in 50 µl of 1X β-mercaptoethanol.

Western Blot

GAS proteins were separated on 10% SDS-PAGE, transferred to nitrocellulose membranes, and probed with α-SpeB antiserum at a 1:500 dilution for 2 h at room temperature. After three 10 min washes with PBS-Tween, blots were incubated with goat α-rabbit horseradish peroxidase-conjugated secondary antibody (Sigma) at a 1:12,500 dilution for 1 h. The blots were then washed three times with PBS-Tween for 20 min. Blots were developed using the Western Lightning chemiluminescence system (Femto) and visualized using a Fuji LAS3000 imager (GE Healthcare).

Biofilm formation assay

Overnight cultures of GAS grown at 37ºC (5% CO_2) in THY were harvested and used to inoculate fresh THY (1:50), and allowed to grow to an OD_{600} of 0.5. Coated polystyrene

6-well cell culture plates (Corning) were seeded with 3 ml of culture per well. Plates were incubated for 24 h at 37°C, 5% CO_2. Medium was removed without disturbing the biofilm, wells were washed three times with distilled H_2O, and 1 ml aliquots of 0.1 % crystal violet (Sigma-Aldrich) dissolved in dH_2O were dispensed to each well. Surface-attached bacteria were allowed to stain for 15 minutes at room temperature and then washed three times with dH_2O, after which 1 ml ethanol was added to each well to solubilize the crystal violet. Absorbance was measured at 600 nm for each sample.

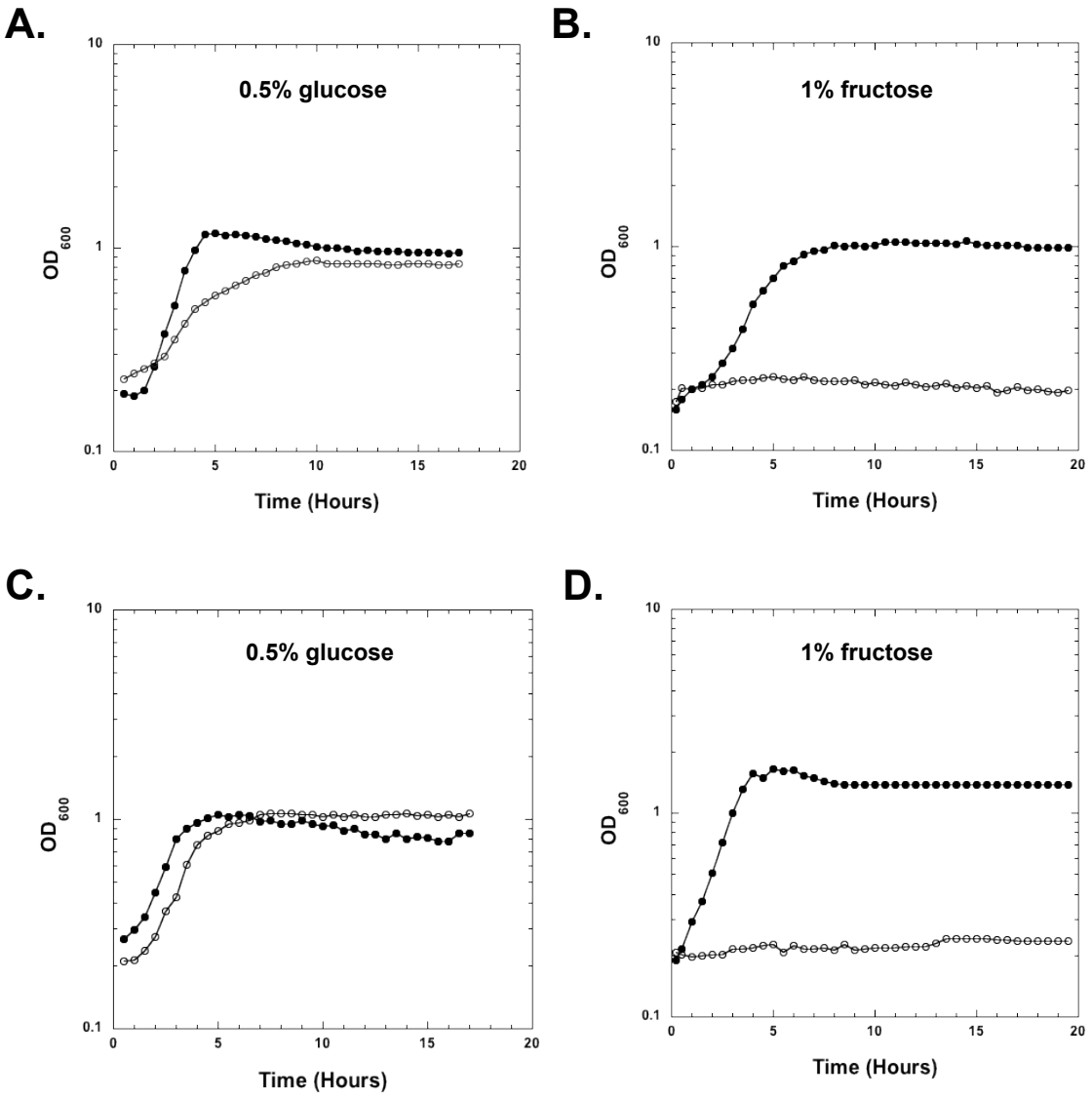

Figure S1: Growth curve of additional M1T1 wild type GAS (closed circles) and their corresponding isogenic Δ*pts1* mutant (open circles) in M1T1 5448 **(A, B)** and 5448AP **(C, D).** GAS cells were grown in CDM supplemented with either 0.5% (w/v) glucose (left) or 1% (w/v) fructose (right). Data is representative of three independent experiments.

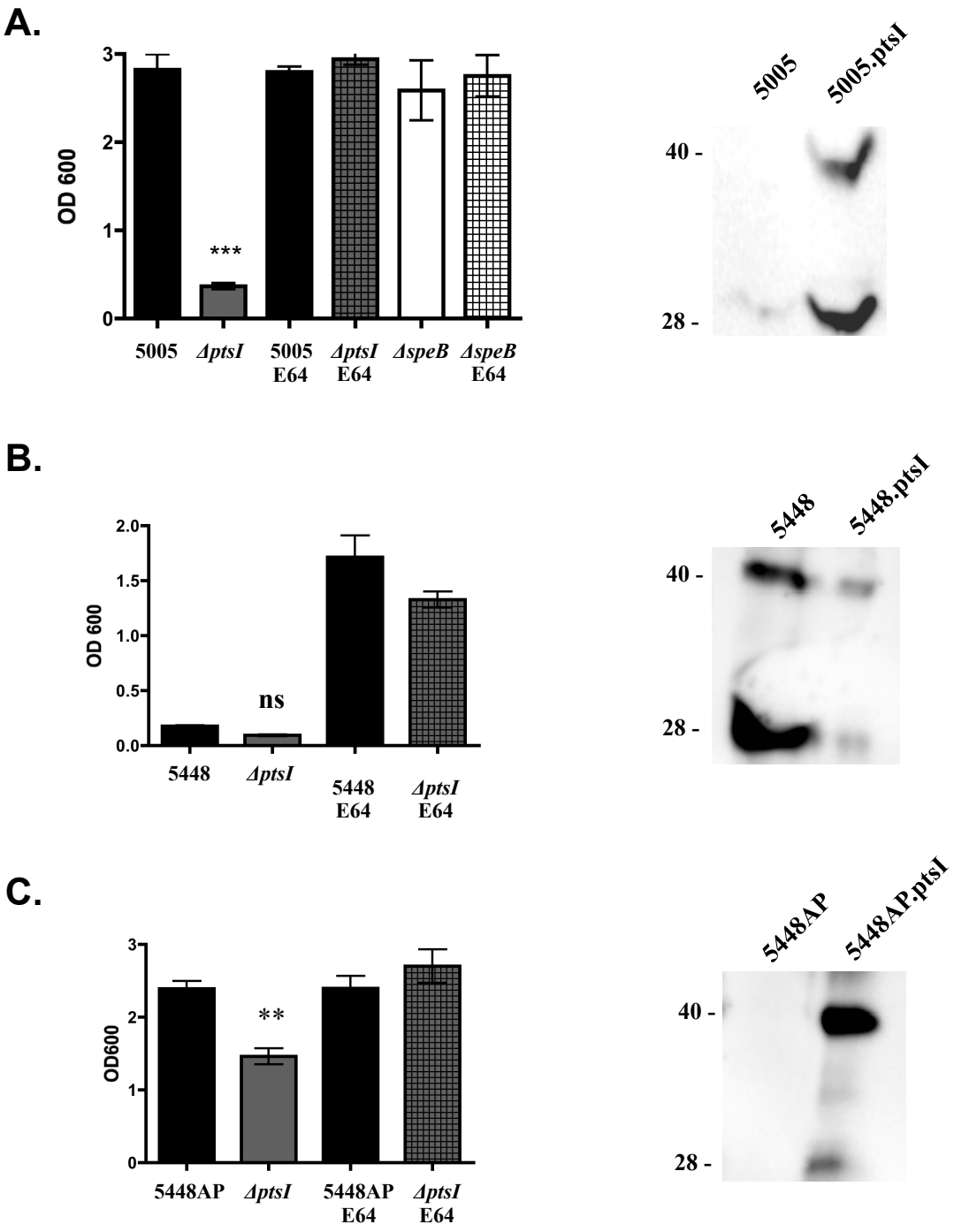

Figure S2: Effect of *ptsI* mutant on biofilm formation and SpeB secretion for MGAS5005 (A), 5448 (B), and 5448AP (C). Quantification of biofilm formation (left column) was determined by crystal violet staining with and without the cysteine protease inhibitor E64 (333μm). P values were determined using an unpaired two-tailed t test. **p≤0.01, ***$p\leq$. 001; NS, not significant. SpeB secretion (right column) was assayed Western blot for SpeB in cell-free culture supernatants collected at early stationary phase. Blot was probed with anti-SpeB. The 40 kDa band is the inactive zymogen and the 28 kDa band is the cleaved active mature protein.