Sumitomo *et al.* Supplemental table 1

Table S1. Oligonucleotides used in this study.

Primer	Sequence (5'-3')	Purpose
slskoF1	GCGAATTCACGATGACTCATTTAGTTATG	deletion of the *sagA* gene
slskoR1	ATAGAGATGCTAAATAGATTACATAAGGTTTACCTCCTTATC	deletion of the *sagA* gene
slskoF2	GATAAGGAGGTAAACCTTATGTAATCTATTTAGCATCTCTAT	deletion of the *sagA* gene
slskoR2	GCGGATCCGATGCTTGTGAACTAACACCA	deletion of the *sagA* gene
sagCheckF	CCAAGAACGGAGTGTATTGA	confirmation of the *sagA* deletion
sagCheckR	GGTATAAACCTCCACCTGAA	confirmation of the *sagA* deletion
hasCheckF	TAAAATAATTTATAACAATTCAATTATCC	confirmation of the *hasA* mutation
hasCheckR	AAATTACTCCTTCTCTAACTACTCG	confirmation of the *hasA* mutation
SqTn916UP1	GACCTTGATAAAGTGTGATAAGTCC	determination of Tn*916* insertion site
SqTn916UP2	GGAGTTTTAGCTCATGTTGATGC	determination of Tn*916* insertion site
SqTn916DW1	CAATTGGAATTCCTCTCTGACTGACGTTC	determination of Tn*916* insertion site
SqTn916DW2	CTTCCTGCAGTAAAAATACTCGAAAGCAC	determination of Tn*916* insertion site
M13F (-20)	GTAAAACGACGGCCAG	determination of Tn*916* insertion site
M13R	CAGGAAACAGCTATGAC	determination of Tn*916* insertion site
emmRTF	TCGCTGTGGCTGTTTTAGGA	real-time RT-PCR
emmRTR	CTCCGCAGCCTTAACTTCTGTT	real-time RT-PCR
mgaRTF	GACAGCTTGCCACTAGACAAACC	real-time RT-PCR
mgaRTR	GGAAGATAACCTCTTTGCGATGA	real-time RT-PCR
speBRTF	TTTGGCTACAACCAATCTGTTC	real-time RT-PCR
speBRTR	TGCTTCCCAATCTTGTTTGCT	real-time RT-PCR
sloRTF	TTGATTTACCAGGTATGGGAGACA	real-time RT-PCR
sloRTR	AAACATTGGCATAGGTAGGGTCAT	real-time RT-PCR

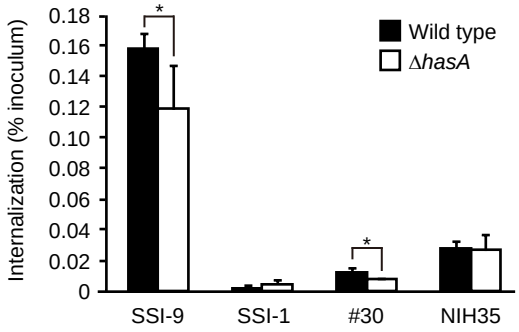

Fig. S1. Internalization of GAS strains into Caco-2 cells.

Caco-2 cells were grown using a Millicell filter system, then infected with GAS clinical isolates and isogenic acapsular mutants at an MOI of 10. At 6 h after infection, extracellular GAS was killed by addition of penicillin and gentamicin, then intracellular GAS was recovered following lysis of the cells. Three experiments were performed and data are presented as the mean ± SD of sextuplet samples from a representative experiment. *$P < 0.01$.

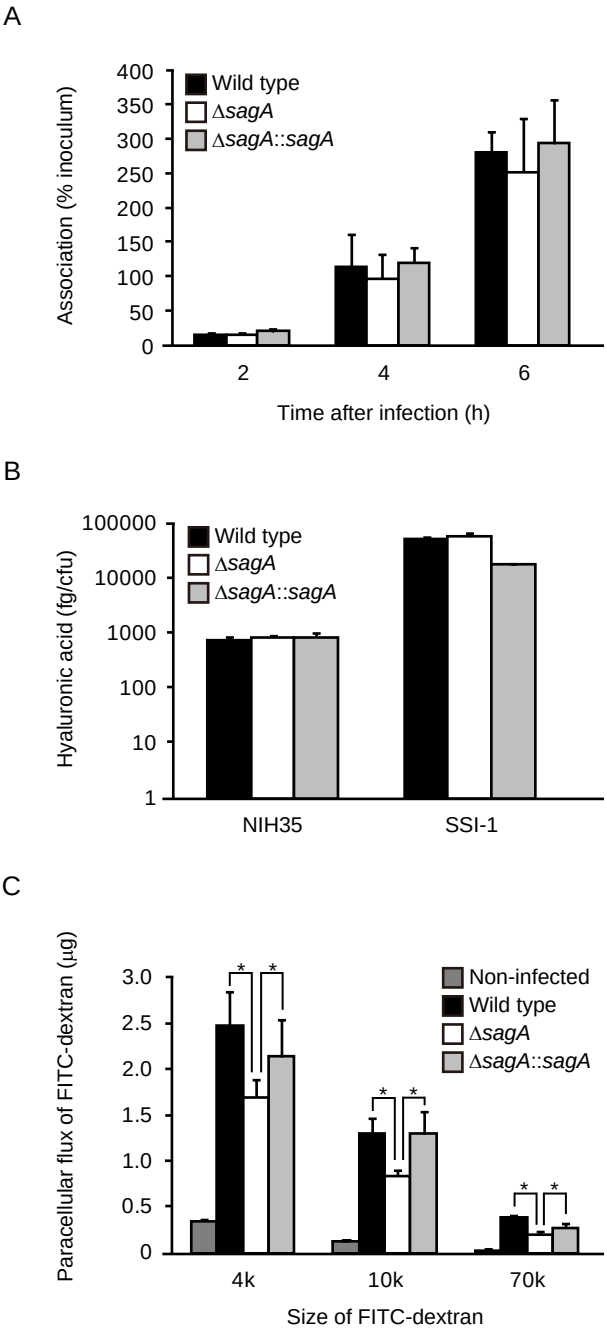

Fig. S2. Analysis of effects of the *sagA* deletion on other phenotypes.

A, Caco-2 cells were grown on Millicell filters, then infected with strain NIH35, the *sagA* deletion mutant, and the complemented strain at an MOI of 10. At the indicated time points after infection, cell-associated GAS was recovered after lysis of the cells.

B, the effects of the *sagA* deletion on the level of capsule production was analyzed using an enzyme-linked immunosorbent assay.

C, Caco-2 cells were grown on Millicell filters, then infected with strain NIH35, the *sagA* deletion mutant, and the complemented strain at an MOI of 10 for 2 h. After removing non-adherent bacteria, FITC-dextran was added to the apical surface of the cell monolayers. After 6 h of infection, the amounts of FITC-dextran in basolateral media were measured. All experiments were performed in sextuplet with 3 technical repeats. Data shown are presented as the mean ± SD of sextuplet samples from a representative experiment. *$P < 0.01$.

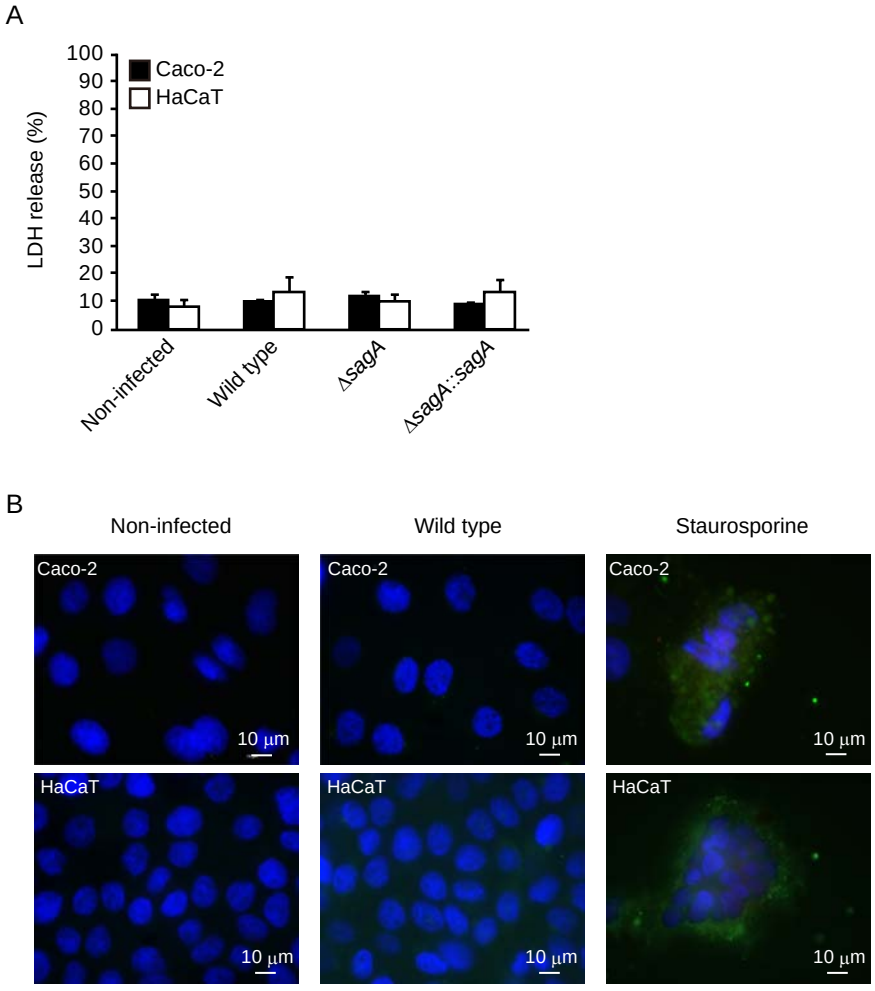

Fig. S3. Analysis of cytotoxic effect of GAS strains against human epithelial cells.

A, Caco-2 and HaCaT cells were infected with strain NIH35, the *sagA* deletion mutant, or the complemented strain at an MOI of 10 for 6 or 8 h, respectively. After infection, cytotoxicity was determined by measuring the release of LDH into the culture supernatant. Data are represented as a percentage of the total amount of LDH released by complete lysis of uninfected cells. The bar represents the SD from three independent experiments.

B, Caco-2 and HaCaT cells were infected with strain NIH35 at an MOI of 10 for 6 or 8 h, respectively. Annexin V-FITC reagent and propidium iodide were used as a probe for detecting apoptosis and necrosis. Annexin V-FITC-bound cells show green staining on the plasma membrane. Cell nuclei were labeled with 4,6-diamidino-2-phenylindole as blue images. As a positive control, Caco-2 and HaCaT cells were treated with 10 μM staurosporine for 6 h. Data shown are representative of at least 3 separate experiments.