Figure S1

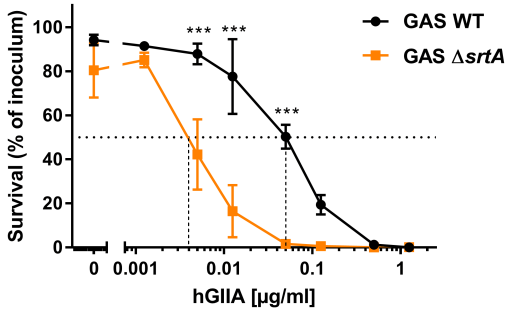
Survival (% of inoculum)
100
80
60
40
20
0
***
***
***
GAS WT
GAS ΔsrtA
0
0.001
0.01
0.1
1
hGIIA [µg/ml]

Figure S2

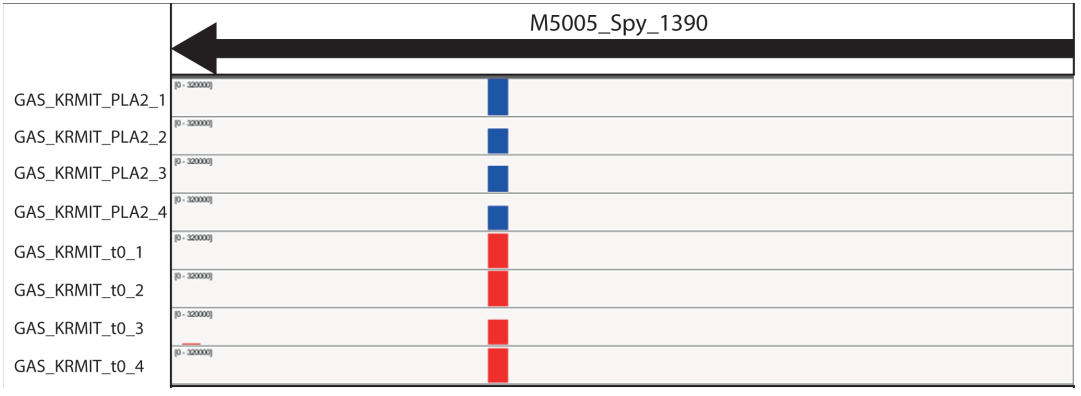
M5005_Spy_1390
GAS_KRMIT_PLA2_1
GAS_KRMIT_PLA2_2
GAS_KRMIT_PLA2_3
GAS_KRMIT_PLA2_4
GAS_KRMIT_t0_1
GAS_KRMIT_t0_2
GAS_KRMIT_t0_3
GAS_KRMIT_t0_4

Figure S3

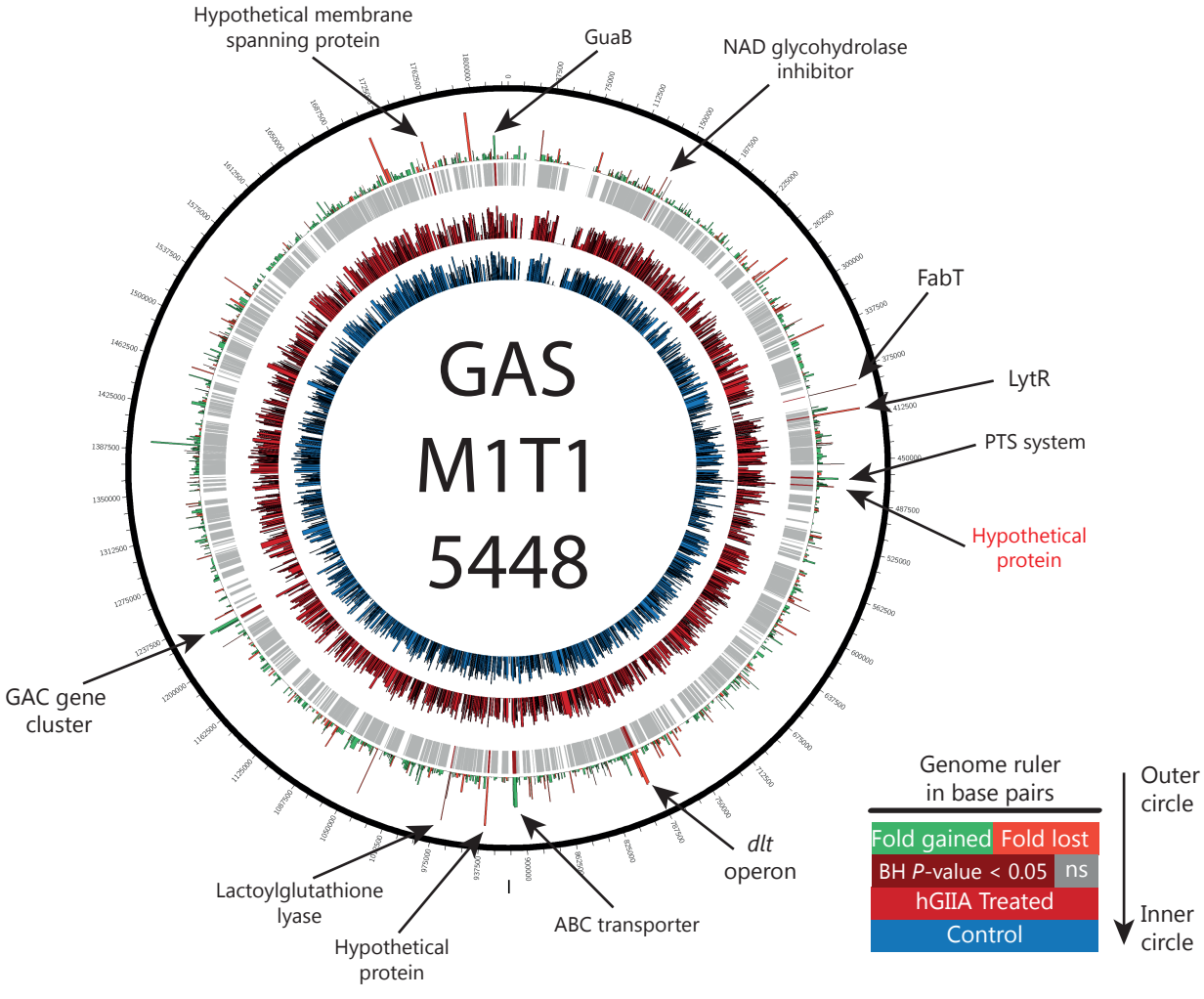
Hypothetical membrane spanning protein
GuaB
NAD glycohydrolase inhibitor
FabT
LytR
PTS system
Hypothetical protein
GAS M1T1 5448
GAC gene cluster
dlt operon
ABC transporter
Hypothetical protein
Lactoylglutathione lyase
Genome ruler in base pairs
Fold gained
Fold lost
BH P-value < 0.05
ns
hGIIA Treated
Control
Outer circle
Inner circle

Figure S4

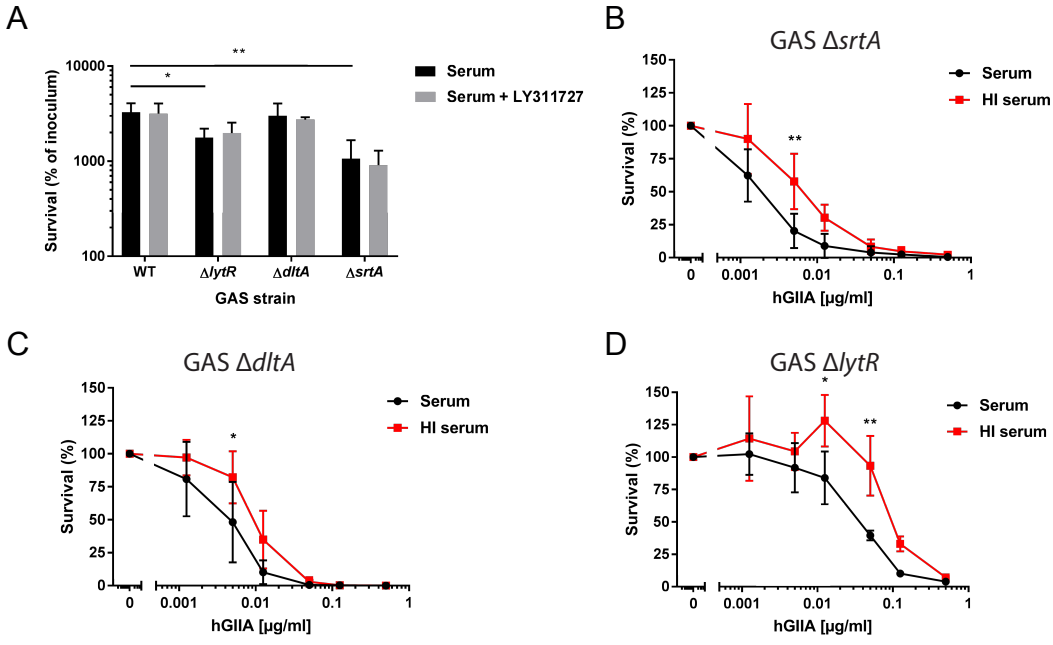
A
B
C
D
Survival (% of inoculum)
10000
1000
100
*
**
WT
ΔlytR
ΔdltA
ΔsrtA
GAS strain
Serum
Serum + LY311727
GAS ΔsrtA
Survival (%)
150
125
100
75
50
25
0
0
0.001
0.01
0.1
1
hGIIA [µg/ml]
Serum
HI serum
GAS ΔdltA
GAS ΔlytR

Figure S5

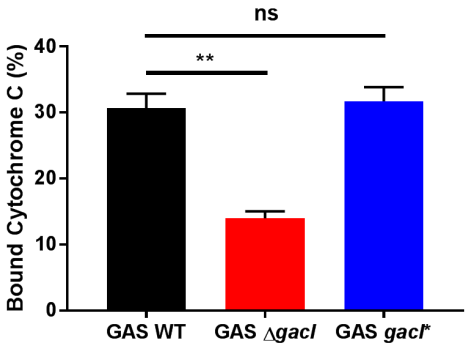

Figure S6

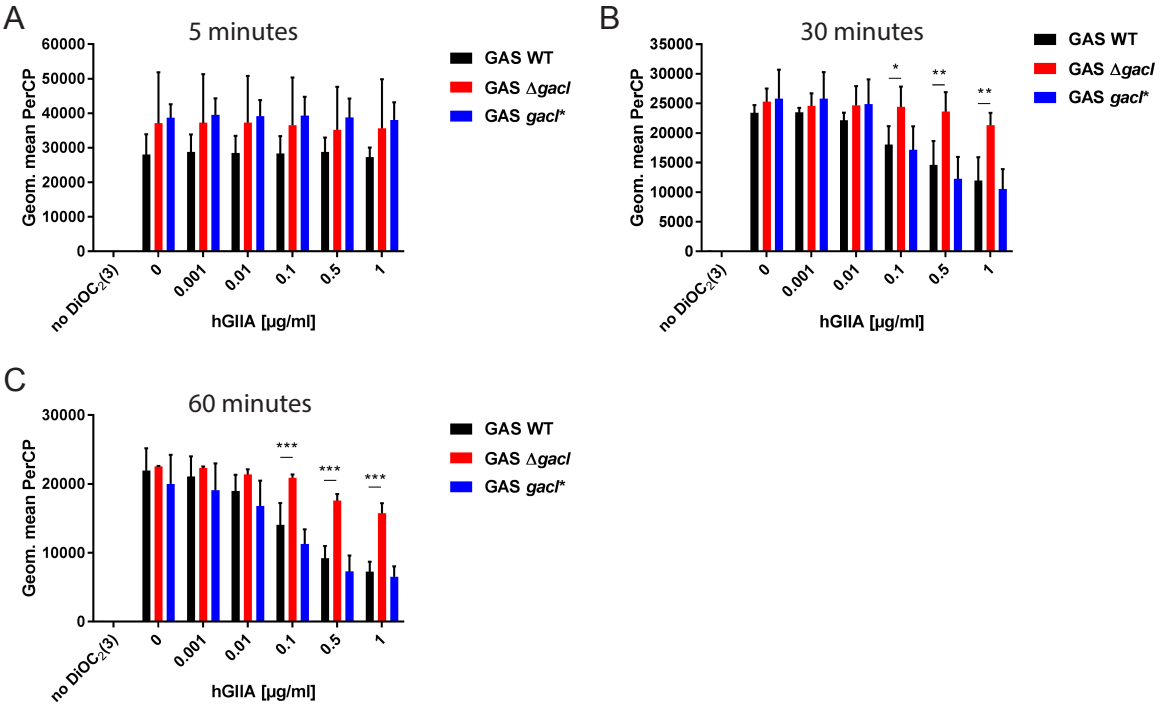
A
5 minutes
Geom. mean PerCP
60000
50000
40000
30000
20000
10000
0
no DIOC2(3)
0
0.001
0.01
0.1
0.5
1
hGIIA [µg/ml]
GAS WT
GAS Δgacl
GAS gacl*
B
30 minutes
Geom. mean PerCP
35000
30000
25000
20000
15000
10000
5000
0
*
**
**
no DIOC2(3)
0
0.001
0.01
0.1
0.5
1
hGIIA [µg/ml]
GAS WT
GAS Δgacl
GAS gacl*
C
60 minutes
Geom. mean PerCP
30000
20000
10000
0
***
***
***
no DIOC2(3)
0
0.001
0.01
0.1
0.5
1
hGIIA [µg/ml]
GAS WT
GAS Δgacl
GAS gacl*

Figure S7

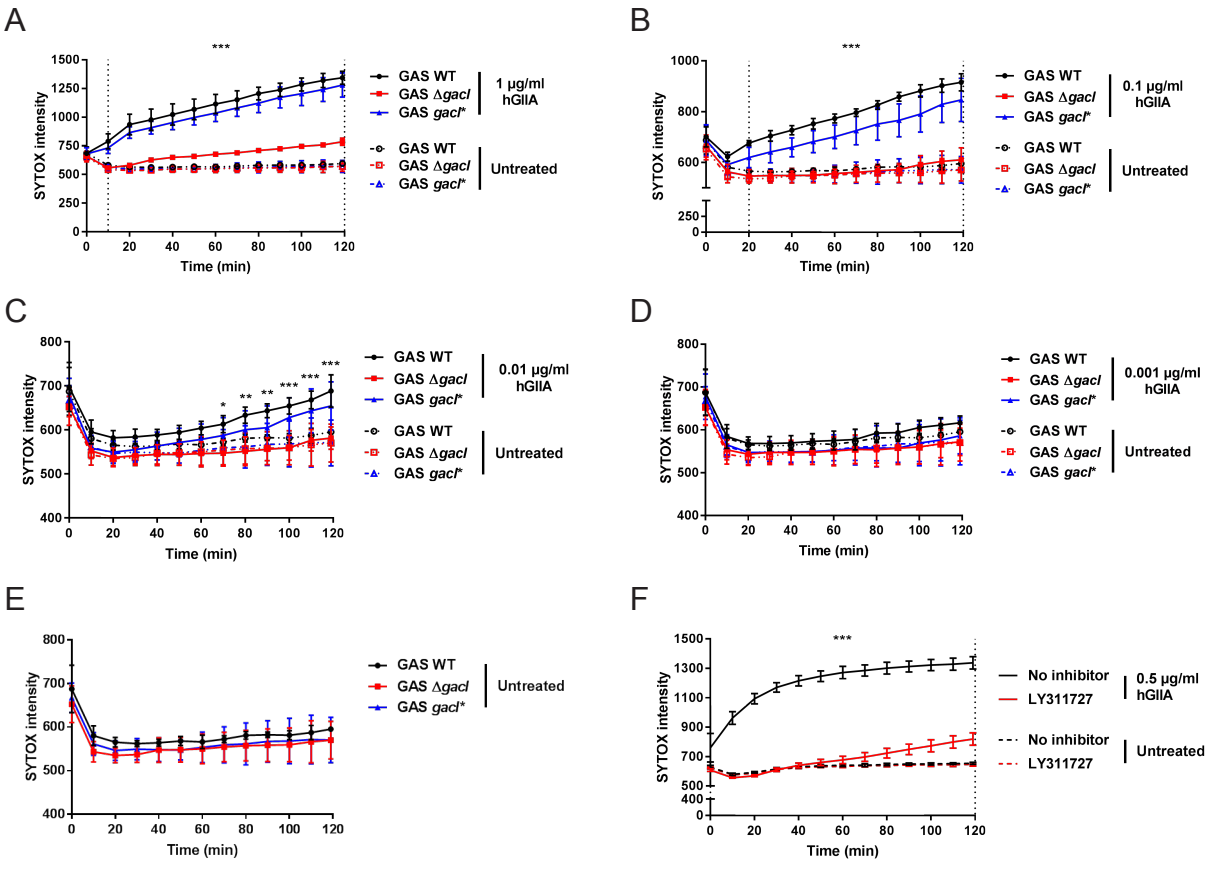
A
B
C
D
E
F
SYTOX intensity
Time (min)
GAS WT
GAS Δgacl
GAS gacl*
1 µg/ml hGIIA
0.1 µg/ml hGIIA
0.01 µg/ml hGIIA
0.001 µg/ml hGIIA
0.5 µg/ml hGIIA
Untreated
No inhibitor
LY311727

Figure S8

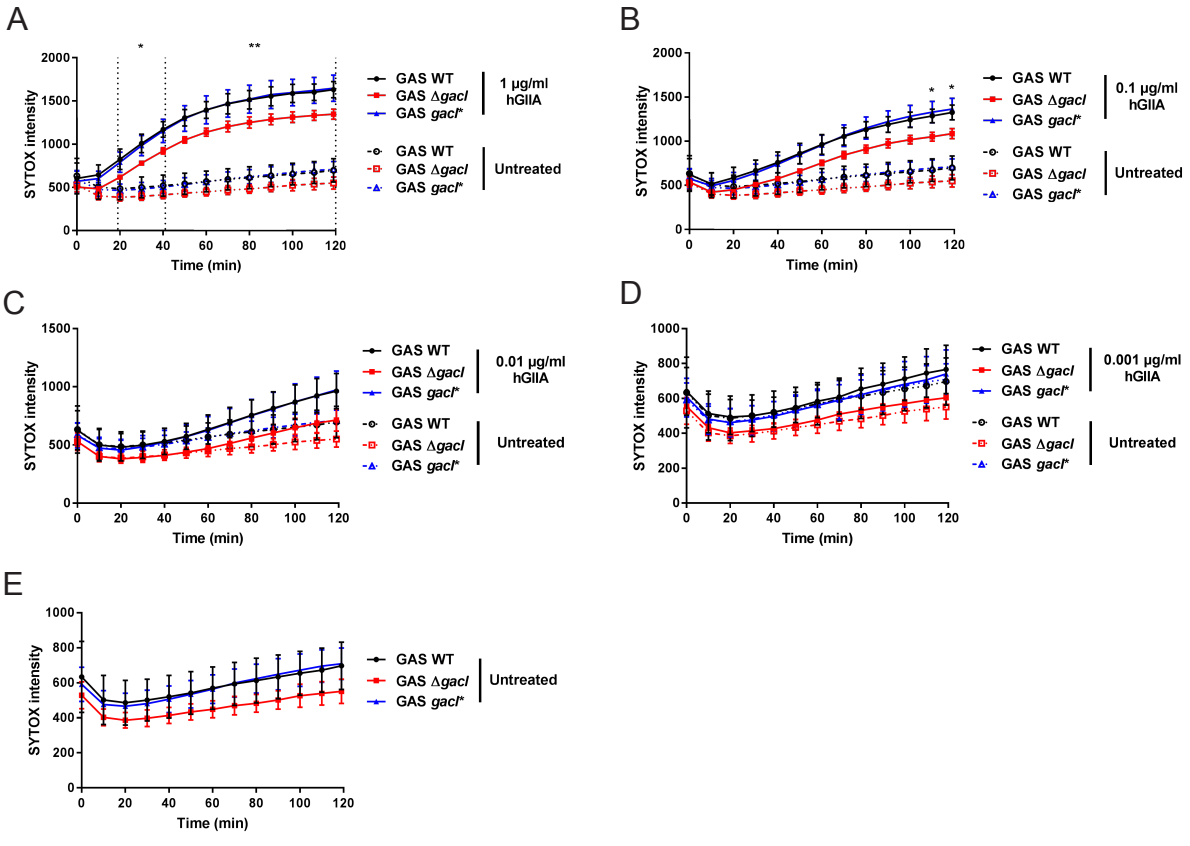
A
B
C
D
E
SYTOX intensity
Time (min)
GAS WT
GAS Δgacl
GAS gacl*
1 µg/ml hGIIA
0.1 µg/ml hGIIA
0.01 µg/ml hGIIA
0.001 µg/ml hGIIA
Untreated
*
**

Figure S9

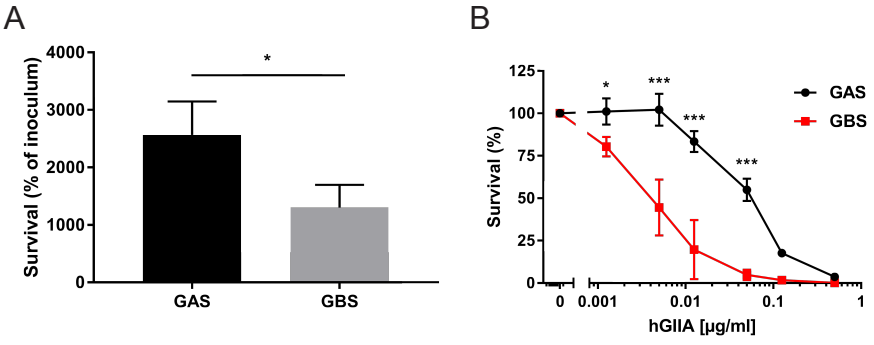
A
B
Survival (% of inoculum)
4000
3000
2000
1000
0
*
GAS
GBS
Survival (%)
125
100
75
50
25
0
*
***
***
***
GAS
GBS
0
0.001
0.01
0.1
1
hGIIA [µg/ml]

Figure S10

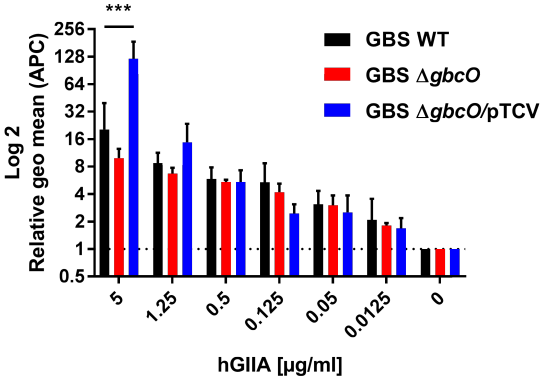
***
GBS WT
GBS ΔgbcO
GBS ΔgbcO/pTCV
Log 2
Relative geo mean (APC)
256
128
64
32
16
8
4
2
1
0.5
5
1.25
0.5
0.125
0.05
0.0125
0
hGIIA [µg/ml]

Fig S1. GAS M1T1 5448 is killed by hGIIA in a dose-dependent manner. Mutation of *srtA* renders 5448 more susceptible to hGIIA killing. Data represent mean +/- SD of three independent experiments. ***, $p > 0.001$.

Fig S2. Biased transposon insertions in M5005_Spy_1390. Unusual high number of transposon insertions at one location in the gene M5005_Spy_1390.

Fig S3. Tn-seq results. Circos respresentation of the Tn-seq data. Each bar in the inner two circles, where blue is control and red the hGIIA treated, represent the average RKPM value of a gene. The following to circles represent the BH corrected *p*-value and the fold change in log of the hGIIA treated samples vs control samples. Red bars indicate a fold a respective fold decrease and green bars a respective fold increase of transposon insertions. The gene highlighted in red is M5005_Spy_1390, which showed significant fold change due to unusual high transposon insertions at one specific point in the gene.

Fig S4. Serum contains a heat-labile factor that increases hGIIA efficacy. (A) Endogenous hGIIA in serum does not affect growth of GAS. Mutation of lytR and srtA does attenuate GAS growth independent of hGIIA. This heat-labile factor also affects killing of the (B) *srtA,* (C) *dltA,* and (D) *lytR* mutants. Data represent mean +/- SD of three independent experiments, *, $p > 0.05$; **, $p > 0.01$..

Fig S5. Surface charge of GAS WT and GAS Δ*gacI*. Deletion of *gacI* affects surface charge of GAS as determined in cationic cytochrome c binding assay. Data represent mean +/- SD of three independent experiments. ns = not significant, **, $p > 0.01$.

Fig S6. Three additional time points for $DiOC_2(3)$ measurements. The effect of hGIIA stress on GAS membrane potential after (A) 5 minutes, (B) 30 minutes and (C) 60 minutes. Data represent mean +/- SD of three independent experiments. *, $p > 0.05$; **, $p > 0.01$; ***, $p > 0.001$.

Fig S7. Kinetics of the SYTOX influx in intact GAS strains. SYTOX influx measured over

120 minutes when GAS strains are incubated with , (A) 1, (B) 0.1, (C) 0.01, (D) 0.001, and (E) 0 µg/ml hGIIA. (F) Addition of 500 µM LY311727 to 0.5 µg/ml hGIIA prevents SYTOX influx. Data represent mean +/- SD of three independent experiments. *, $p > 0.05$; **, $p > 0.01$; ***, $p > 0.001$.

Fig S8. Kinetics of the SYTOX influx in protoplast GAS strains. SYTOX influx measured over 120 minutes when protoplast GAS strains are incubated with (A) 1, (B) 0.1, (C) 0.01, (D) 0.001, and (E) 0 µg/ml hGIIA. Data represents mean +/- SD of three independent experiments. *, $p > 0.05$; **, $p > 0.01$.

Fig S9. Gas and GBS are differently affected by human serum. (A) GAS grows faster in human serum compared to GBS. (B) GBS is more susceptible to hGIIA-spiked in serum compared to GAS. Data represent mean +/- SD of three independent experiments. *, $p > 0.05$; ***, $p > 0.001$.

Fig S10. HGIIA surface binding to GBS. No significant difference in relative hGIIA surface binding of GBS WT and GBS Δ*gbcO*. Data represent mean +/- SD of three independent experiments. ***, $p > 0.001$.