Supplementary Table S1. The *emm* type, the phosphorylation CovR (CovR~P), and the expression level of SpeB, SLO, and PepO in GAS isolates.

SPY	***emm* type**	**CovR~P***	**SpeB#**	**SLO#**	**PepO#**
301	1	+	+	+	+
303	1	+	+	+	+
304	1	+	+	+	+
143	1	-	-	++	++
252	1	-	-	++	++
261	1	-	-	++	++
306	12	+	+	+	-
322	12	+	+	-	-
235	12	-	-	++	++
201	22	+	+	-	-
328	22	+	+	-	-
338	22	+	+	-	-
363	22	+	+	+	+
111	22	-	-	++	++
138	22	-	-	++	++
173	22	-	-	++	++
205	22	-	-	++	++

343	25	-	-	++	++
213	44	+	+	-	+
127	44	-	-	++	++
132	44	-	-	++	++
380	44	-	-	++	++
331	49	+	+	+	-
362	49	+	+	++	++
337	49	-	-	++	++
348	49	-	-	++	++
139	73	+	+	+	-
141	73	+	+	+	-
369	73	-	-	++	++
315	78	-	-	++	++
177	81	+	+	-	-
179	81	+	+	-	-
163	81	-	-	++	++
267	87	+	+	+	-
309	87	+	+	-	-
228	87	-	-	+	++

136	89	+	-	+	-
161	89	+	+	+	-
168	89	+	+	-	-
178	89	+	+	-	-
230	89	+	+	-	-
212	89	-	-	++	++
215	90	+	+	++	+
218	90	+	+	+	+
367	90	-	-	++	++
373	90	-	-	+	++
272	92	+	-	+	-
273	92	+	+	+	+
276	92	-	-	++	++
320	92	-	-	++	++
317	102	+	+	-	+
319	102	+	+	-	+
323	102	+	+	-	+
116	102	-	-	++	++
122	102	-	-	+	++

233	102	-	-	+	++
326	102	-	-	+	++
340	113	+	+	-	-
344	113	+	+	-	-
121	113	-	-	+	++
365	113	-	-	+	++

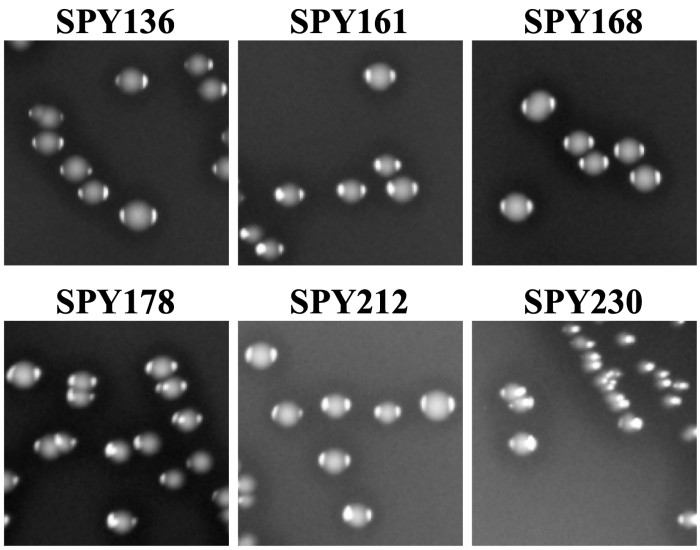
3 *: +, CovR~P positive; –, CovR~P negative

4 #: –, negative; +, low-level expression; ++, high level expression

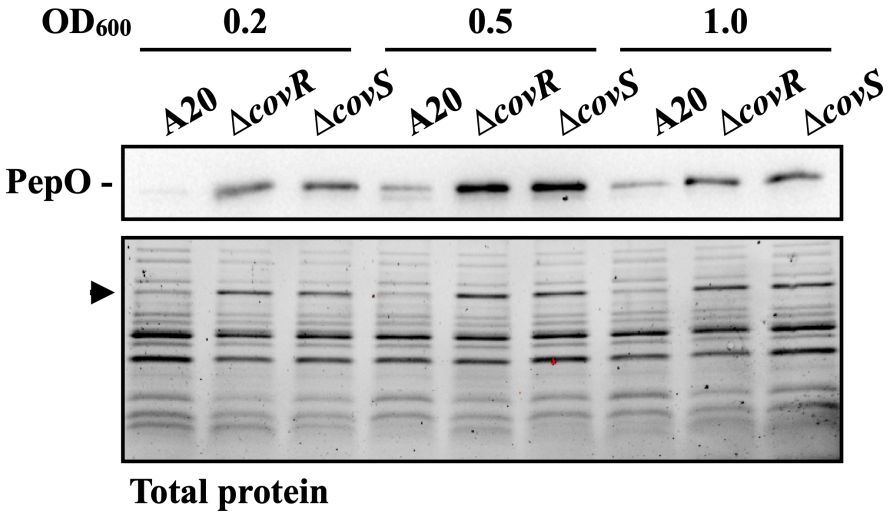
Supplementary Table S2. Nucleotide sequence of *covR/covS*, *rocA*, *hasABC* operon, *speB*, *slo*, and *pepO* in SPY362.

Gene	Nucleotide change	Amino acid change
covR	G108T, A118G	G33V, K40E
covS	A634T	N212I
rocA	A66G	-
ropB	-	-
hasA	C96A, C192A	P33T, P68T
hasB	-	-
hasC	C147T, A539G	D180G
speB	C115A, C378A, C392A, T404A, C406A, C408T, C409A, T410C, T413A	Q39K, D126E, T131N, V135D, P136T, L137T, F138Y
slo	-	-
pepO	-	-

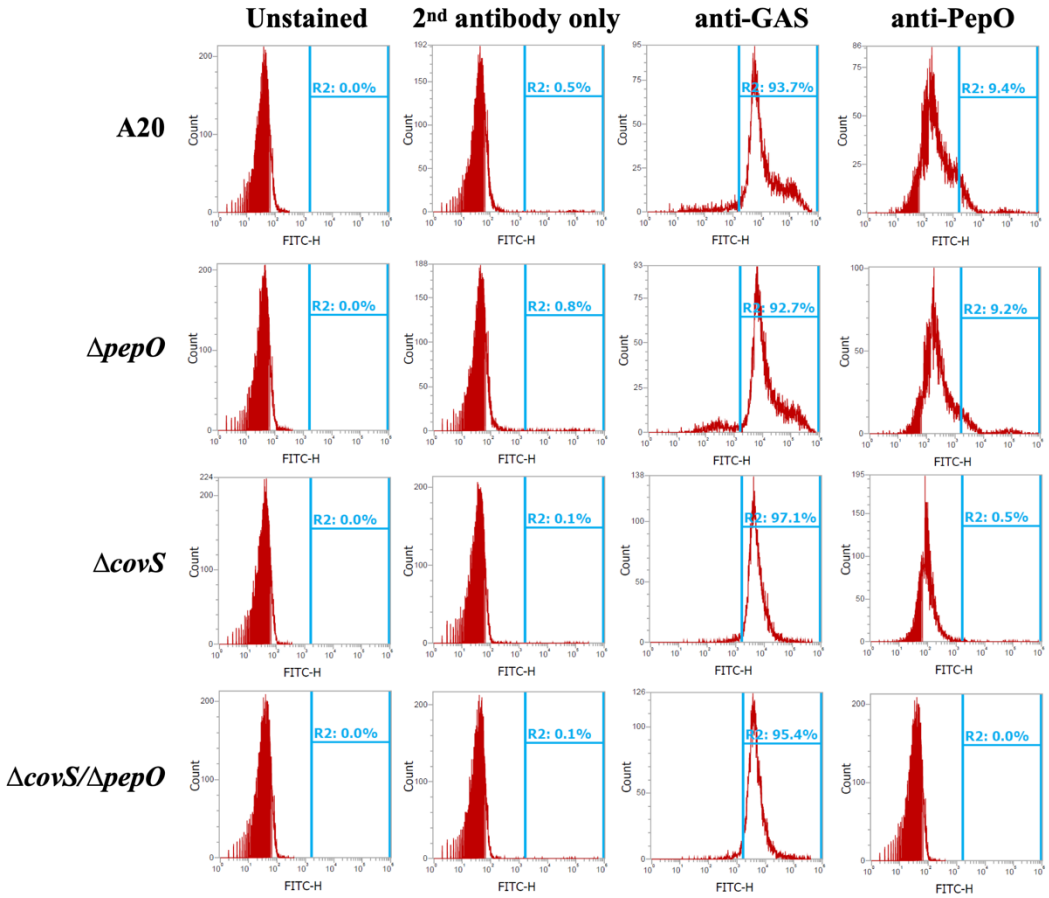
Reference strain: NZ131 (NCBI accession No. CP000829.1)



Supplementary Figure S1. The colony morphology of the *emm*89 isolates. The *emm*89 isolates were inoculated on the blood agar plate and incubated at 37ºC with 5% CO_2 for 16 h. The image was recorded by the Gel Doc XR+ system (Bio-Rad).



Supplementary Figure S2. The PepO expression in the wild-type A20 strain, the *covR* mutant (Δ*covR*), and *covS* mutant (Δ*covS*) at different growth phases. GAS strains were cultured to the log phase (OD_{600} = 0.2), exponential phase (OD_{600} = 0.5), and stationary phase (OD_{600} = 1.0) and the total proteins were extracted for western blot hybridization. The lower panel of images shows total protein as the internal loading control. An arrow indicates when a signal from only the CovR/CovS-inactivated mutants was identified.



Supplementary Figure S3. Detection of the bacterial surface PepO by using flow cytometry. The exponential phase (OD600 = 0.6) wild-type A20 strain, its *pepO* mutant (Δ*pepO*), the *covS* mutant (Δ*covS*), and the Δ*covS*/Δ*pepO* mutant were collected and fixed by 3% paraformaldehyde at room temperature for 10 min. The fixed bacterial cells were incubated with the primary antibody (anti-GAS antibody 1.25 μg/mL or anti-PepO antibody 15 μg/mL) at room temperature for 1 h. After removing the primary antibodies, the bacterial cells were incubated with the secondary antibody (anti-rabbit IgG-Alexa-488, 1.875 μg/mL) at room temperature for 0.5 h. The signal of PepO on the bacterial surface (total 10,000 bacterial cells) was analyzed by Attune NxT Flow Cytometer (Thermo Fisher Scientific). Unstained and the secondary antibody (2nd antibody only) groups were utilized as the negative controls. Bacteria that incubated with anti-GAS antibody was utilized as the positive control.