

```

M1 rocA MLEDFLQFLGFIFLDIIEIMLTLKLSFVSAIPLRLKNIFYLSLSMVLQVVFWAFFPDHFILDVVMLAQFLFFALIALY
M3 rocA MLEDFLQFLGFIFLDIIEIMLTLKLSFVSAIPLRLKNIFYLSLSMVLQVVFWAFFPDHFILDVVMLAQFLFFALIALY
*****

M1 rocA YGKSIKAKFLMFYAFFPLVSI SLVKRFIVFFVMPLFGMPYSVVKHNTLLIYSITCFSIFLIYRCIQVFHFDSTWRQYFQ
M3 rocA YGKSIKAKFLMFYAFFPLVSI SLVKRFIVFFVMPLFGMPYSVVKHNTLLIYSITCFSIFLIYRCIQVFHFDSTWRQYFQ
*****

M1 rocA SHRASKLLVFTNSSMALYYLCVQGDVMSPSLSGLATTARSIIVLFYFILFLTLIHLERYVKQNSIEAIVQQKEYREL
M3 rocA SHRASKLLVFTNSSMALYYLCVQGDVMSPSLSGLATTARSNIVLFYFILFLTLIHLERYVKQNSIEAIVQQKEYREL
*****

M1 rocA INYSQHGLLQDIQELRRLTTVSSRLKIGIEQNDISIVRLTYEGILNAEKNNAKDDRDLTCLDKLQVEAIRHIVLAK
M3 rocA INYSQHGLLQDIQELRQLTTVSSRLKIGIEQNDISIVRLTYEGILNAEKNNAKDDRDLTCLDKLQVEAIRHIVLAK
*****

M1 rocA LIEAKNKKLVEVSI PNCIATFFLEVVDFTKLLSFLLDNAIEMSLETKQPCLSI AFLDQNHKLVIVIQSSTKQGQDSDS
M3 rocA LIEAKNKKLVEVSI PNCIATFFLEVVDFTKLLSFLLDNAIEMSLETKQPCLSI AFLDQNHKLVIVIQSSTKQGQDSDS
*****

M1 rocA VFAIPALKKRDDWQFDRNVTTILNRYDYLTISSQIHDGILTQLIEIAKPD
M3 rocA VFATPALKKEMTGNLT-----
*** *****. :

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Figure S1
RocA sequence alignment from representative serotype M1 and M3 GAS isolates. Red asterisks highlight amino acid identity, gaps highlight dissimilar amino acids, periods highlight weakly similar amino acids, and colons highlight strongly similar amino acids.

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M1 rocA CTTATGATAAAAATAAGAATATGATTTAAATGAAAAGCTCAGTTTATCTATTACGGAGTTCGAAATGAAAAGAAAATCG
M3 rocA CTTATGATAAAAATAAGAATATGATTTAAATGAAAAGCTCAGTTTATCTATTACGGAGTTCGAAATGAAAAGAAAATCG
*****

M1 rocA AGATGTGATAACATATTTTGAACGAGAAGGAGAAGGATAAATGTTAGAAGATTTTCTCAATTTTATAGGATTTATATTTT
M3 rocA AGATGTGATAACATATTTTGAACGAGAAGGAGAAGGATAAATGTTAGAAGATTTTCTCAATTTTATAGGATTTATATTTT
*****

M1 rocA TAGATATTATTGAAATATGTTAACGTTAAAGCTTTTGTAGCTTTGTAAGTGAATTCGCTTCGCCTGAAAAATATTTTT
M3 rocA TAGATATTATTGAAATATGTTAACGTTAAAGCTTTTGTAGCTTTGTAAGTGAATTCGCTTCGCCTGAAAAATATTTTT
*****

M1 rocA TATTTATCACTTAGTATGGTTTTGTTTCAAGTAGTTTTTTGGGCTTTCCTTCCAGACCATTTCATTCCTGATGTTGTGAT
M3 rocA TATTTATCACTTAGTATGGTTTTGTTTCAAGTAGTTTTTTGGGCTTTCCTTCCAGACCATTTCATTCCTGATGTTGTGAT
*****

M1 rocA GTTGGCTCAGTTTCTTTTCTTGGCCTTATAGCCCTATACTATGGTAAATCAATAAAAGCTAAGTTTTTAATGTTTTATG
M3 rocA GTTGGCTCAGTTTCTTTTCTTGGCCTTATAGCCCTATACTATGGTAAATCAATAAAAGCTAAGTTTTTAATGTTTTATG
*****

M1 rocA CCTTTTTCCACTAGTGTCTATTAGTTGGTTAAGCGATTTATTGTATTTTTTGTATGCCATTGTTGGTATGCCTTAT
M3 rocA CCTTTTTCCACTAGTGTCTATTAGTTGGTTAAGCGATTTATTGTATTTTTTGTATGCCATTGTTGGTATGCCTTAT
*****

M1 rocA TCAGTTGTTAAACATAATACTACTGATTTACAGCATTACTTGTTTTAGTATTTTTTTGATTTATCGCTGCATTCAAGT
M3 rocA TCAGTTGTTAAACATAATACTACTGATTTACAGCATTACTTGTTTTAGTATTTTTTTGATTTATCGCTGCATTCAAGT
*****

M1 rocA TTTTCATTTTCGATTTTCAACCTGGCGCCAATATTTTCAATCACATAGAGCTAGTAAACTATTAGTGTTTACTAACTCTT
M3 rocA TTTTCATTTTCGATTTTCAACCTGGCGCCAATATTTTCAATCACATAGAGCTAGTAAACTATTAGTGTTTACTAACTCTT
*****

M1 rocA CGATGGCTCTATATTACTTGTGTGTCCAAGGATAGATGTGATGTCGCCTTCTTTATCAGGACTTGCTACAACGACTGCT
M3 rocA CGATGGCTCTATATTACTTGTGTGTCCAAGGATAGATGTGATGTCGCCTTCTTTATCAGGACTTGCTACAACGACTGCT
*****

M1 rocA CGTTCAATCATAGTGTCTTTTATTTTCATTTTGTTCCTACTTTATTAATTCATTTAGAGCGTTATGTAACAAAATC
M3 rocA CGTTCAACATAGTGTCTTTTATTTTCATTTTGTTCCTACTTTATTAATTCATTTAGAGCGTTATGTAACAAAATC
*****

M1 rocA TATTGAGGCAATGTGCAACAAAAGAATATCGTGAGCTGATTAATATAGTCAGCACCTGGATTGCTGTATCAAGATA
M3 rocA TATTGAGGCAATGTGCAACAAAAGAATATCGTGAGCTGATTAATATAGTCAGCACCTGGATTGCTGTATCAAGATA
*****

M1 rocA TTCAAGAGCTTAGGCGGCTATTAACCTACCGTGTCTAGTCGTCTTAAGATTGGAATCGAACAAAATGATATCTCTATTGTG
M3 rocA TTCAAGAGCTTAGGCGGCTATTAACCTACCGTGTCTAGTCGTCTTAAGATTGGAATCGAACAAAATGATATCTCTATTGTG
*****

M1 rocA AGACTTACTTATGAAGGTATCTTGAATGCTGAAAAGAATAATGCTAAAGATGACAGACTTGATTAACTTGTTTAGATAA
M3 rocA AGACTTACTTATGAAGGTATCTTGAATGCTGAAAAGAATAATGCTAAAGATGACAGACTTGATTAACTTGTTTAGATAA
*****

M1 rocA ATTACAAGTTGAAGCAATCAGACATATTGTTTGTAGCTAAATTAATGAGGCAAAAATAAGAGCTTAAGGTTGAGGTAT
M3 rocA ATTACAAGTTGAAGCAATCAGACATATTGTTTGTAGCTAAATTAATGAGGCAAAAATAAGAGCTTAAGGTTGAGGTAT
*****

M1 rocA CGATCCCTAATGTATTGCAACGTTTTTCTAGAAGTAGTAGATTTCACTAAGCTATTGTCATTTTGTAGATAATGCT
M3 rocA CGATCCCTAATGTATTGCAACGTTTTTCTAGAAGTAGTAGATTTCACTAAGCTATTGTCATTTTGTAGATAATGCT
*****

M1 rocA ATAGAAATGAGTTGGAGACAAAGCAACCTTGCTTATCAATAGCATTCTGGATCAAAACCATAAACTTGTCATAGTCAT
M3 rocA ATAGAAATGAGTTGGAGACAAAGCAACCTTGCTTATCAATAGCATTCTGGATCAAAACCATAAACTTGTCATAGTCAT
*****

M1 rocA TCAAAGCAGTACTAAACAAGGACAAGATGATAGTCAAAGCGTGTGCTATACCGGCTTTGAAAAAAGAGATGACTGGC
M3 rocA TCAAAGCAGTACTAAACAAGGACAAGATGATAGTCAAAGCGTGTGCTATACCGGCTTTGAAAAAAGAGATGACTGGC
*****

M1 rocA AATTTGACTTAAGGAATGTCACGACCATTTTAAATCGTTATGACTATCTCACAATTAGCTCGCAGATTTCATGATGGCATT
M3 rocA AATTTGACTTAAGGAATGTCACGACCATTTTAAATCGTTATGACTATCTCACAATTAGCTCGCAGATTTCATGATGGCATT
*****

M1 rocA TTAACCCAGTTAATAGAAATAGCTAAGCCTGACTGATGTTAAAGGTATGAATAACTCACTCAAATCAACTTAAGAGTCTG
M3 rocA TTAACCCAGTTAATAGAAATAGCTAAGCCTGACTGATGTTAAAGGTATGAATAACTCACTCAAATCAACTTAAGAGTCTG
*****

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Figure S2
Alignment of the rocA genes from representative serotype M1 and M3 GAS isolates. Red asterisks highlight nucleotide identity while gaps highlight differences. The start codons (ATG) are colored green while the stop codons (TAA or TGA) are colored red.

Strain	Serotype	Isolation year	Isolation location	<i>rocA</i> allele
MGAS2221	M1	1988	Australia	Wild-type
MGAS1251	M3	1920s	UK	mutant
MGAS1254	M3	1937	New York, USA	mutant
MGAS182	M3	1940s	Ottawa, Canada	mutant
MGAS1428	M3	1974	Cottbus, Germany	mutant
MGAS315	M3	1980s	Texas, USA	mutant
MGAS159	M3	Late 1980s	Utah, USA	mutant
MGAS491	M3	Pre-1991	UK	mutant
SSI-1	M3	1994	Japan	mutant
MGAS9056	M3	1998	Illinois, USA	mutant
MGAS9507	M3	2001	Texas, USA	mutant
MGAS10870	M3	2002	Ontario, Canada	mutant
MGAS15049	M3	2006	Ontario, Canada	mutant
MGAS22283	M3	2010	Alberta, Canada	mutant
PGAS9429	M12	2001	Texas, USA	Wild-type
MGAS8232	M18	Prior to 2002	Utah, USA	mutant
MGAS6180	M28	1990s	Texas, USA	Wild-type

Table S1

Clinical GAS isolates used in this study.

Strain	Serotype	Information	Reference
10870::rocA ^{M1}	M3	Derivative of clinical M3 GAS isolate MGAS10870 in which the mutant <i>rocA</i> allele has been replaced with a functional allele from M1 GAS	This study
10870::rocA ^{M1-RV-M3}	M3	Derivative of M3 GAS isolate 10870::rocA ^{M1} in which the functional <i>rocA</i> allele has been reverted back to the M3 GAS allele	This study
10870ΔrocA	M3	Derivative of clinical M3 GAS isolate MGAS10870 in which the mutant <i>rocA</i> allele has been replaced with a spectinomycin resistance cassette	This study
2221ΔrocA	M1	Derivative of clinical M1 GAS isolate MGAS2221 in which the functional <i>rocA</i> allele has been replaced with a spectinomycin resistance cassette	This study
2221rocA ^{Comp}	M1	Derivative of M1 GAS isolate 2221ΔrocA in which a functional <i>rocA</i> allele has been reintroduced by replacing the spectinomycin cassette	This study
2221ΔcovR	M1	Derivative of clinical M1 GAS isolate MGAS2221 in which the <i>covR</i> gene has been replaced with a kanamycin resistance cassette	Treviño <i>et al.</i> , 2009
2221ΔrocAΔcovR	M1	Derivative of 2221ΔcovR in which the <i>rocA</i> gene has been replaced with a spectinomycin resistance cassette	This study

Table S2
Derivatives of clinical GAS isolates used in this study.

Primer name	Sequence (5' - 3')	Role
UNR26	TGTACAATTGCTAGCGTACGTAGAAAAGTTACTTAATCAAGCTTTTCG	Used with UNR27, with M1 GAS gDNA as template, to PCR a region of <i>rocA</i> used in the creation of the plasmid to introduce a wild-type <i>rocA</i> into M3 GAS
UNR27	CTATTATCATTAGTAATAGATTAAC	Used with UNR26, with M1 GAS gDNA as template, to PCR a region of <i>rocA</i> used in the creation of the plasmid to introduce a wild-type <i>rocA</i> into M3 GAS
UNR28	GTTAATCTATTACTAATGATAATAG	Used with UNR29, with M3 GAS gDNA as template, to PCR a region of <i>rocA</i> used in the creation of the plasmid to introduce a wild-type <i>rocA</i> into M3 GAS
UNR29	CAAAATTTTCATCAAGCTCTAGTTCGAATGCTTTGTGAGTCTCTTTGATGGTG	Used with UNR28, with M3 GAS gDNA as template, to PCR a region of <i>rocA</i> used in the creation of the plasmid to introduce a wild-type <i>rocA</i> into M3 GAS
UNR30	CGAAAAGCTTGATTAACTAACTTTTCTACGTACGCTAGCAATTGTACA	Used with UNR31, with pBBL740 DNA as template, to PCR the plasmid. Was used in Gibson assembly to join with the PCR products made with UNR26/27 and UNR28/29
UNR31	CACCATCAAGAGACTCACAAGACTTCGAACACTAGAGCTTGATGAAAATTTG	Used with UNR30, with pBBL740 DNA as template, to PCR the plasmid. Was used in Gibson assembly to join with the PCR products made with UNR26/27 and UNR28/29
UNR32	CCAGCCAATCATGCCAACCC	Used with UNR39 to check that the <i>rocA</i> gene has been replaced with a <i>specR</i> cassette in strain 10870Δ <i>rocA</i>
UNR33	GCCATTCTATCTCCGCAGATAAGCTC	Used with UNR39 to check that the <i>rocA</i> gene has been replaced with a <i>specR</i> cassette in strain 10870Δ <i>rocA</i>
UNR34	CTATTTAAATAACAGATTAAAAAATATAACagtttaagaaatagctagcctgac	Used with UNR33, with M3 GAS gDNA as template, to amplify one flank of the <i>rocA</i> gene for construction of the M3 <i>rocA</i> inactivating plasmid (replacement with <i>Spec</i> cassette)
UNR35	gtcagcttagctatttctataactgTTATAATTTTTAACTGTTATTTAAATAG	Used with UNR33, with M3 GAS gDNA as template, to amplify one flank of the <i>rocA</i> gene for construction of the M3 <i>rocA</i> inactivating plasmid (replacement with <i>Spec</i> cassette)
UNR36	CGTTATTAGTTAGTATTATAACATGATTtctcaaaaatgaagaaatcttc	Used with UNR38, with M3 GAS gDNA as template, to amplify one flank of the <i>rocA</i> gene for construction of the inactivating plasmid (replacement with <i>Spec</i> cassette)
UNR37	gaagatttctcaatttttaggaAATACATGTTATAAATACTATAACTAATAACG	Used with UNR35, with pS160 as template, to PCR the <i>specR</i> cassette and use in construction of the M3 <i>rocA</i> inactivating plasmid
UNR38	GCTTTGACAAGCTTACTATGAGG	Used with UNR38, with M3 GAS gDNA as template, to amplify one flank of the <i>rocA</i> gene for construction of the inactivating plasmid (replacement with <i>Spec</i> cassette)
UNR39	GTGGGGTTGGCTGATTGGCTTTCG	Used with UNR36, with M3 GAS gDNA as template, to amplify one flank of the <i>rocA</i> gene for construction of the inactivating plasmid (replacement with <i>Spec</i> cassette)
UNR26	TGTACAATTGCTAGCGTACGTAGAAAAGTTACTTAATCAAGCTTTTCG	Used with UNR29, with M3 GAS gDNA as template, to PCR <i>rocA</i> and use in the creation of the plasmid to revert the wild-type <i>rocA</i> in 10870::rocAM1 to the mutant M3 <i>rocA</i> allele
UNR29	CAAAATTTTCATCAAGCTCTAGTTCGAATGCTTTGTGAGTCTCTTTGATGGTG	Used with UNR26, with M3 GAS gDNA as template, to PCR <i>rocA</i> and use in the creation of the plasmid to revert the wild-type <i>rocA</i> in 10870::rocAM1 to the mutant M3 <i>rocA</i> allele
HASATMF	ATGATCGATGTTTAAACAATTATGCTATTG	Taqman primer for <i>hasA</i>
HASATMR	TTAAATAACTTTTAAATTGGAAAGGTACATCAG	Taqman primer for <i>hasA</i>
HASATMP	ACGCACTGTCTACCAATCAACAGCTAGATGTG	Taqman probe for <i>hasA</i>
PROSTMF	TACCACTGGCAATCGTACC	Taqman primer for <i>proS</i>
PROSTM	CATTTCAACAGCACCAGTCT	Taqman primer for <i>proS</i>
PROSTMP	CACGCATGATGGCTTGAATTTCTCA	Taqman probe for <i>proS</i>
SCPCTMF	AAGGAGCTGGGCAAGGGATA	Taqman primer for <i>scpC</i>
SCPCTMR	TGATGGCCGGATCGA	Taqman primer for <i>scpC</i>
SCPCTMP	CAATAACTGCGACACCTTGCCTTGTCTCT	Taqman probe for <i>scpC</i>
SLOTMF	GACCTTTAAAGAGTTGCAACGAAAA	Taqman primer for <i>slo</i>
SLOTMR	GACCATAAGCTACGTTACTCACAAGA	Taqman primer for <i>slo</i>
SLOTMP	TGTCAGCAATGAAGCCCGCC	Taqman probe for <i>slo</i>
SPNTMF	AACGCACTCTCTAAAGAAGACA	Taqman primer for <i>spn</i>
SPNTMR	TGAATTTTCTCCGAGAAGTACTC	Taqman primer for <i>spn</i>
SPNTMP	TCATGTCATGCACACATTAGACGGCTCA	Taqman probe for <i>spn</i>
SKATMF	CGGCTCTTTGAGTCAATGATT	Taqman primer for <i>ska</i>
SKATMR	CCGAACCATTTGTCCAGCAA	Taqman primer for <i>ska</i>
SKATMP	CAAGCGATGCAACCACTTAGTATGATGAAAC	Taqman probe for <i>ska</i>
ROCATMF	AGGGCTATAGCGAAAGAA	Taqman primer for <i>rocA</i>
ROCATMR	GGCTTCTTCCAGACCAAT	Taqman primer for <i>rocA</i>
ROCATMP	TGAGCCAACATCACAACCAAGAAATG	Taqman probe for <i>rocA</i>

Table S3
Table of primers and probes used in this study.

Gene	Expression ratio (10870::rocA ^{M1} / MGAS10870)
<i>hasA</i>	0.004
<i>hasB</i>	0.006
<i>hasC</i>	0.011
<i>spyCEP</i>	0.011
<i>mac</i>	0.021
<i>SpyM3_1493</i>	0.043
<i>SpyM3_0305</i>	0.050
<i>SpyM3_0105</i>	0.068
<i>ska</i>	0.071
<i>SpyM3_0129</i>	0.079
<i>nga</i>	0.084
<i>slo</i>	0.096
<i>sclB</i>	0.126
<i>scpA</i>	0.127
<i>arcC</i>	0.146
<i>SpyM3_1195</i>	0.168
<i>mf4</i>	0.182
<i>SpyM3_1583</i>	0.201
<i>sla</i>	0.205
<i>arcB</i>	0.205
<i>sagP</i>	0.220
<i>SpyM3_1193</i>	0.226
<i>SpyM3_1192</i>	0.232
<i>SpyM3_0157</i>	0.236
<i>speA3</i>	0.252
<i>SpyM3_0158</i>	0.260
<i>SpyM3_1203</i>	0.261
<i>SpyM3_0307</i>	0.263
<i>SpyM3_0742</i>	0.282
<i>SpyM3_0408</i>	0.330
<i>SpyM3_1759</i>	0.333
<i>SpyM3_1161</i>	3.083673469
<i>SpyM3_1509</i>	3.105280528
<i>ropB</i>	3.123188406
<i>SpyM3_1020</i>	3.204597701
<i>SpyM3_0025</i>	3.234782609
<i>purK</i>	3.394444444
<i>SpyM3_0467</i>	3.419230769
<i>SpyM3_1061</i>	3.521705426
<i>SpyM3_0402</i>	3.543801653
<i>speB</i>	3.61
<i>purM</i>	3.744444444
<i>agaS</i>	4.072222222
<i>SpyM3_0793</i>	4.11
<i>purN</i>	4.180952381
<i>purF</i>	4.627184466
<i>grab</i>	4.75907208
<i>purC</i>	5.075384615
<i>asnA</i>	5.156716418
<i>SpyM3_0591</i>	5.709134615
<i>purD</i>	5.711176471
<i>purL</i>	6.199264706
<i>rpsU</i>	7.663265306
<i>purH</i>	8.196551724
<i>metB</i>	11.35205479
<i>kup</i>	12.95283019

Table S4

Genes whose transcript levels differ three-fold or greater between parental M3 isolate MGAS10870 and the *rocA* complemented derivative 10870::rocA^{M1}.