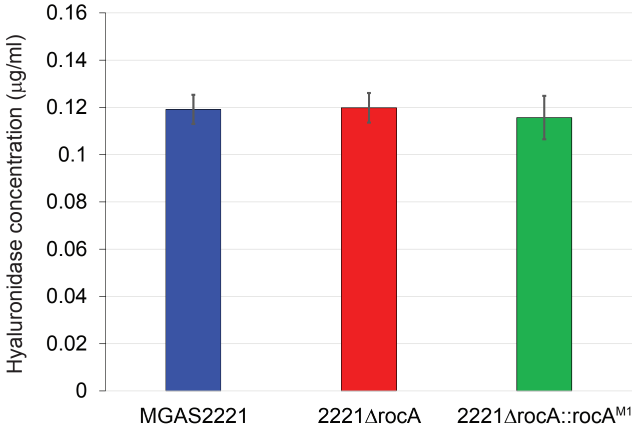

Figure S1

Hyaluronidase production is unaffected by *rocA* allele status.
Parental isolate MGAS2221, *rocA* deletion mutant derivative 2221ΔrocA, and complemented mutant strain 2221ΔrocA::rocAM1 were compared in a plate assay. Shown are the averages ((± standard deviation) of duplicate samples ran in duplicate.

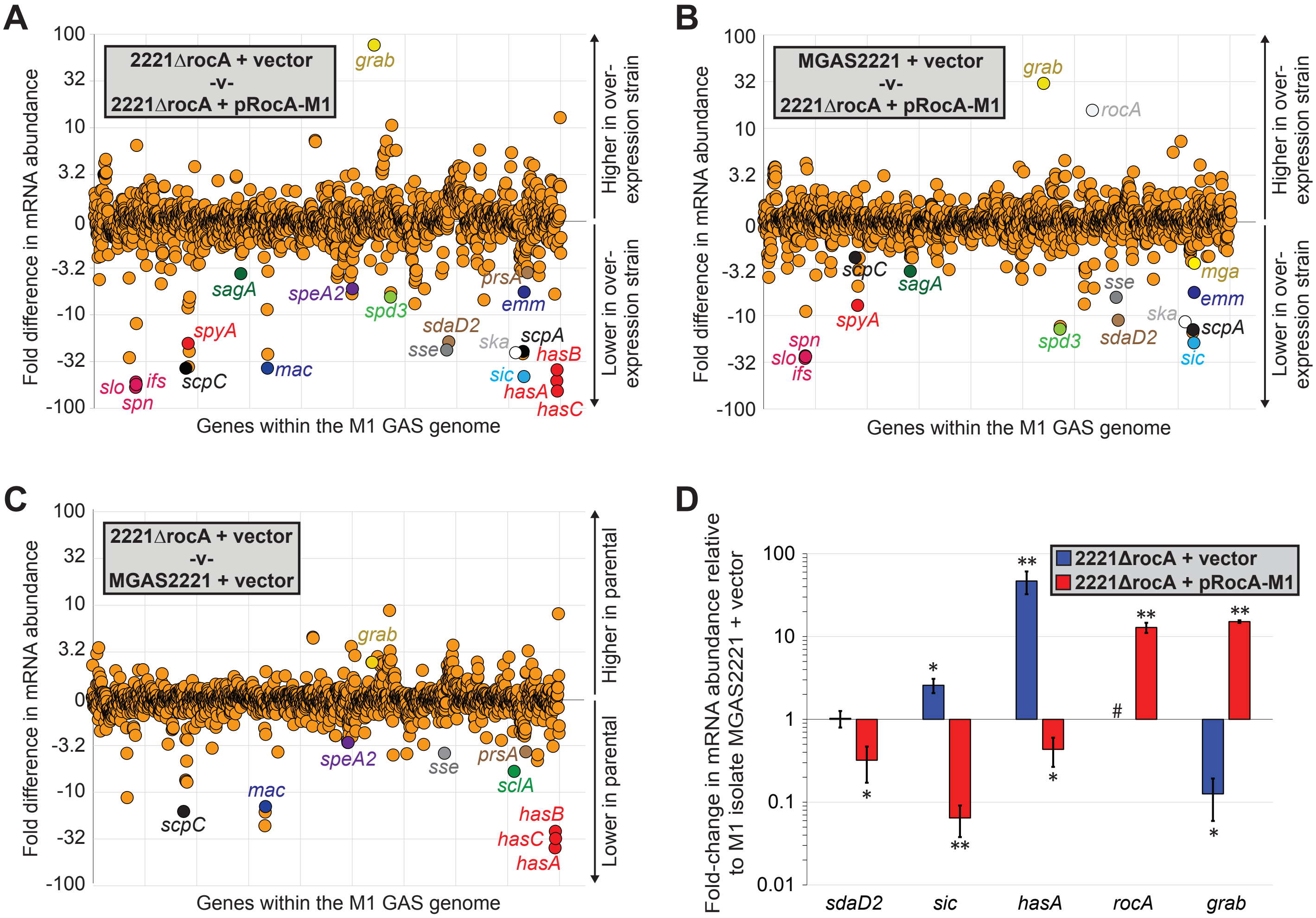

Figure S2

RocA overexpression selectively modifies transcript abundance.

(**A-C**) Summary of RNAseq data. Shown are pair-wise comparisons of the transcriptomes between the *rocA* deletion mutant strain containing either empty vector (pDCBB) or *rocA*-expressing plasmid (pRocA-M1) (**A**), between vector-containing MGAS2221 and 2221ΔrocA containing pRocA-M1 (**B**), and between vector-containing derivatives of MGAS2221 and 2221ΔrocA (**C**). The relative expression levels of all genes are graphed, with each represented by a circle. Select mRNAs of interest have been colored and labeled. (**D**) Taqman-based quantitative RT-PCR data confirming that RocA regulates the abundance of virulence factor-encoding mRNAs in GAS. The abundance of *sdaD2*, *sic*, *hasA*, *rocA*, and *grab* mRNAs were determined from triplicate exponential phase GAS cultures, with the mean (± standard deviation) shown. The hashtag highlights the lack of *rocA* transcript in the *rocA* deletion mutant strain. The asterisks highlight statistical significance relative to the parental M1 isolate (*t*-test, **P* < 0.05, ***P* < 0.01).

Feature ID	**Fold change in transcript level in MGAS2221 relative to 2221ΔrocA**
M5005_Spy1541	7.33
scrK	6.75
spd3	6.49
M5005_Spy1746	6.33
endoS	5.88
M5005_Spy0859	5.78
M5005_Spy1170	5.41
scrA	5.22
M5005_Spy0028	5.16
nagB	4.96
xpt	4.49
M5005_Spy1668	4.39
salB	4.30
M5005_Spy1152	4.22
M5005_Spy1745	4.19
purD	4.05
M5005_Spy0169	3.96
M5005_Spy0023	3.85
M5005_Spy1750	3.71
purE	3.61
purM	3.40
purK	3.40
M5005_Spy0022	3.30
purF	3.15
sagD	3.07
smf	3.00
M5005_Spy0357	-3.00
M5005_Spy1018	-3.17
M5005_Spy1556	-3.35
M5005_Spy1015	-3.63
M5005_Spy0665	-4.41
speA2	-4.81
spyCEP	-6.39
M5005_Spy0355	-6.41
sclA	-6.56
M5005_Spy0354	-7.23
M5005_Spy0669	-7.59
M5005_Spy0115	-11.27
hasC	-12.49
M5005_Spy0352	-13.16
M5005_Spy0143	-13.47
hasA	-13.80
hasB	-14.11
M5005_Spy0666	-18.93
mac	-23.12
M5005_Spy0667	-27.10
M5005_Spy0144	-58.73

Table S1

Transcripts that are differentially regulated between strains MGAS2221 and 2221ΔrocA.

Feature ID	**Fold change in transcript level in MGAS2221 relative to 2221ΔrocA::rocA^{M1}**
M5005_Spy0143	3.07

Table S2

Transcripts that are differentially regulated between strains MGAS2221 and 2221ΔrocA::rocA^{M1}.

Feature ID	**Fold change in transcript level in 2221ΔrocA::rocA^{M1} relative to 2221ΔrocA**
spd3	5.79
M5005_Spy1541	5.50
M5005_Spy1170	5.46
M5005_Spy1152	5.09
M5005_Spy0859	4.95
scrK	4.84
M5005_Spy0028	4.72
nagB	4.59
M5005_Spy1746	4.42
xpt	4.41
endoS	4.01
M5005_Spy1668	3.80
scrA	3.43
purD	3.26
grab	3.23
metB	3.21
purE	3.04
M5005_Spy0023	3.00
sic1.01	-3.19
M5005_Spy0665	-3.38
speJ	-3.52
M5005_Spy1407	-3.57
M5005_Spy1556	-3.70
M5005_Spy0113	-3.88
M5005_Spy0357	-4.36
M5005_Spy0114	-5.14
speA2	-5.38
M5005_Spy0354	-9.54
M5005_Spy0669	-9.72
M5005_Spy0355	-12.28
spyCEP	-13.61
sclA	-17.98
M5005_Spy0115	-20.67
M5005_Spy0666	-20.75
M5005_Spy0352	-24.01
hasC	-24.76
hasA	-27.20
hasB	-27.36
M5005_Spy0667	-38.63
M5005_Spy0143	-41.30
mac	-41.79
M5005_Spy0144	-58.73

Table S3

Transcripts that are differentially regulated between strains 2221ΔrocA and 2221ΔrocA::rocA^{M1}.

Feature ID	Fold change in transcript level in MGAS2221 + vector relative to 2221ΔrocA + vector
M5005_Spy_1178	9.07
M5005_Spy_T0067	8.35
xpt	4.70
M5005_Spy_0859	4.58
M5005_Spy_1440	3.86
M5005_Spy_1025	3.73
M5005_Spy_T0020	3.62
M5005_Spy_1189	3.35
M5005_Spy_1744	3.35
M5005_Spy_T0005	3.12
M5005_Spy_1437	3.04
M5005_Spy_1843	-3.13
M5005_Spy_0459	-3.28
prsA_2	-3.58
M5005_Spy_T0048	-3.60
sse	-3.71
M5005_Spy_0114	-3.73
M5005_Spy_1263	-3.76
M5005_Spy_1784	-3.86
M5005_Spy_1206	-3.88
dexS	-4.45
M5005_Spy_0354	-4.51
sclA	-5.80
M5005_Spy_0350	-7.20
M5005_Spy_0352	-7.42
M5005_Spy_0355	-7.57
M5005_Spy_0115	-11.08
mac	-13.80
scpC	-15.57
M5005_Spy_0667	-15.81
M5005_Spy_0666	-22.13
hasB	-27.00
hasC	-32.28
hasA	-40.54

Table S4

Transcripts that are differentially regulated between strains MGAS2221 (+ vector) and 2221ΔrocA (+ vector).

Feature ID	**Fold change in transcript level in 2221ΔrocA + pRocA relative to MGAS2221 + vector**
grab	30.28
rocA	15.64
M5005_Spy_1668	7.28
salX	5.56
M5005_Spy_1206	4.54
metB	4.23
M5005_Spy_1746	4.07
M5005_Spy_0028	3.87
M5005_Spy_0995	3.85
purD	3.82
M5005_Spy_0152	3.68
purF	3.67
purM	3.51
purE	3.44
M5005_Spy_1029	3.43
M5005_Spy_1477	3.42
M5005_Spy_1263	3.41
M5005_Spy_1152	3.24
M5005_Spy_0023	3.19
M5005_Spy_0459	3.12
purH	3.12
M5005_Spy_1210	3.07
M5005_Spy_1204	3.04
purK	3.02
coaA	-3.02
M5005_Spy_1023	-3.06
M5005_Spy_1291	-3.08
M5005_Spy_1287	-3.11
M5005_Spy_T0034	-3.12
M5005_Spy_1008	-3.14
M5005_Spy_T0047	-3.24
M5005_Spy_1091	-3.24
M5005_Spy_0355	-3.26
M5005_Spy_1288	-3.27
sagA	-3.37
M5005_Spy_0247	-3.37
M5005_Spy_1289	-3.38
M5005_Spy_1290	-3.52
M5005_Spy_0189	-3.70
M5005_Spy_T0008	-3.92
M5005_Spy_1556	-4.10
M5005_Spy_1018	-4.12
M5005_Spy_0352	-4.86
M5005_Spy_1286	-4.95

M5005_Spy_T0053	-5.10
emm1.0	-5.68
Sse	-6.41
M5005_Spy_1285	-7.56
spyA	-7.81
M5005_Spy_0142	-9.06
sdaD2	-11.26
ska	-11.70
M5005_Spy_1170	-13.14
spd3	-14.02
scpA_2	-14.23
M5005_Spy_1714	-15.12
sic1.01	-19.63
ifs	-27.07
slo	-27.61
nga	-28.80

Table S5

Transcripts that are differentially regulated between strains MGAS2221 (+ vector) and 2221ΔrocA (+ pRocA).

Feature ID	Fold change in transcript level in 2221ΔrocA + pRocA relative to 2221ΔrocA + vector
grab	76.61
M5005_Spy_T0067	12.82
M5005_Spy_1178	10.63
xpt	7.34
M5005_Spy_1153	7.18
M5005_Spy_0859	7.09
metB	6.47
M5005_Spy_1152	5.91
scrK	5.78
M5005_Spy_1210	5.73
M5005_Spy_1174	5.69
M5005_Spy_1668	5.41
M5005_Spy_1143	5.30
M5005_Spy_1144	5.08
purD	4.60
purM	4.20
M5005_Spy_1744	4.19
M5005_Spy_1142	4.06
M5005_Spy_1760	3.97
M5005_Spy_1457	3.91
M5005_Spy_0376	3.84
M5005_Spy_1664	3.82
M5005_Spy_1477	3.71
scrA	3.62
M5005_Spy_1459	3.62
nagB	3.57
M5005_Spy_1135	3.54
endoS	3.53
M5005_Spy_1437	3.46
M5005_Spy_1440	3.36
purK	3.33
purE	3.30
M5005_Spy_0028	3.25
M5005_Spy_1137	3.22
M5005_Spy_1025	3.18
M5005_Spy_0022	3.18
purN	3.16
purF	3.15
M5005_Spy_1541	3.13
M5005_Spy_0023	3.06
M5005_Spy_1463	3.05
agaS	3.05
cfa	3.03
M5005_Spy_1746	3.01
M5005_Spy_1731	-3.06
M5005_Spy_1784	-3.09
dacA2	-3.12
M5005_Spy_1391	-3.15
dppA	-3.17
prsA_1	-3.24
M5005_Spy_0281	-3.29
mutY	-3.31
M5005_Spy_1288	-3.32
M5005_Spy_1291	-3.36

ciaR	-3.36
M5005_Spy_1709	-3.38
M5005_Spy_0669	-3.43
M5005_Spy_1007	-3.47
prsA_2	-3.55
ciaH	-3.62
M5005_Spy_1386	-3.63
sagA	-3.63
M5005_Spy_1289	-3.70
M5005_Spy_1290	-3.80
pepO	-3.81
speA2	-3.96
coaA	-3.98
M5005_Spy_0114	-4.05
M5005_Spy_T0008	-4.22
M5005_Spy_1287	-4.26
M5005_Spy_1286	-4.52
M5005_Spy_0247	-4.67
M5005_Spy_1018	-5.27
M5005_Spy_T0053	-5.28
emm1.0	-5.72
M5005_Spy_1170	-5.82
M5005_Spy_1008	-5.97
M5005_Spy_1843	-6.02
spd3	-6.46
M5005_Spy_0357	-6.50
M5005_Spy_1556	-6.93
M5005_Spy_0354	-7.32
M5005_Spy_1285	-7.62
M5005_Spy_0350	-8.44
M5005_Spy_0666	-8.79
M5005_Spy_0142	-12.43
sdaD2	-19.48
spyA	-20.27
Sse	-23.79
M5005_Spy_0355	-24.64
scpA_2	-24.69
ska	-25.47
M5005_Spy_1714	-26.11
mac	-26.45
M5005_Spy_0115	-28.82
M5005_Spy_0352	-36.11
M5005_Spy_0667	-37.29
spyCEP	-37.35
hasB	-38.83
sic1.01	-45.90
hasA	-50.89
ifs	-52.48
slo	-55.79
nga	-59.74
hasC	-65.58

Table S6

Transcripts that are differentially regulated between strains 2221ΔrocA (+ vector) and 2221ΔrocA (+ pRocA).

Primer name	Sequence (5' - 3')	Use
HASATMF	ATGATCGATGTTTAACAAATTATGCTATTG	Taqman primer for *hasA*
HASATMR	TTAAATAACTTTTTAATTGGAAAGGTACATCAG	Taqman primer for *hasA*
HASATMP	ACGCACTGTCTACCAATCAACAGCTAGATGTG	Taqman probe for *hasA*
PROSTMF	TACCACTGGCAAATCGTACC	Taqman primer for *proS*
PROSTMR	CATTTCAACAGCACCGATCT	Taqman primer for *proS*
PROSTMP	CACGCATGATGGTCTTGAATTTCTCA	Taqman probe for *proS*
SCPCTMF	AAGGAGCTTGGGACAAGGGATA	Taqman primer for *scpC*
SCPCTMR	TGATGGGCCGGATCGA	Taqman primer for *scpC*
SCPCTMP	CAATAACTGCGACAACCTTGCCTTGTCCT	Taqman probe for *scpC*
SLOTMF	GACCTTTAAAGAGTTGCAACGAAAA	Taqman primer for *slo*
SLOTMR	GACCATAAGCTACGTTACTCACAAAGA	Taqman primer for *slo*
SLOTMP	TGTCAGCAATGAAGCCCCGCC	Taqman probe for *slo*
SPD3TMF	GGCGATCTTGACAATCTGCAA	Taqman primer for *spd3*
SPD3TMR	CAGCCAGGAGGATTGAATTTAAGT	Taqman primer for *spd3*
SPD3TMP	CCAACCTTCGCACATCCAGCTAAAAG	Taqman probe for *spd3*
SKATMF	CGGCTACTTTGAGGTCATTGATT	Taqman primer for *ska*
SKATMR	CCGAACCATCTTTGTCAGCAA	Taqman primer for *ska*
SKATMP	CAAGCGATGCAACCATTACTGATCGAAAC	Taqman probe for *ska*
ROCATMF	AGGGCTATAAGCGCAAAGAA	Taqman primer for *rocA*
ROCATMR	GGCTTTCTTTCCAGACCATT	Taqman primer for *rocA*
ROCATMP	TGAGCCAACATCACAACATCAAGAATG	Taqman probe for *rocA*
GRABTMF	GCATCAGTATTAGTCGGTTCAACAGT	Taqman primer for *grab*
GRABTMR	GGTTCCGCCATTTGGAATAA	Taqman primer for *grab*
GRABTMP	TGTTGACTCACCTATCGAACAGCCTCGA	Taqman probe for *grab*
SICTMF	CTGAGGACACCCCTCGTTTC	Taqman primer for *sic*
SICTMR	TCTTGTGGATTTTTTTGAGGAGTATG	Taqman primer for *sic*
SICTMP	TGAACCTCGTGTGACAGAAAAACCGCA	Taqman probe for *sic*
SDAD2TMF	AAGAAAATGAAATCGAAACTACTGATGAC	Taqman primer for *sdaD2*
SDAD2TMR	GCCCATTACTTGCCACATATACAG	Taqman primer for *sdaD2*
SDAD2TMP	AAAACGAGCCTGACACGGATGCACTAAA	Taqman probe for *sdaD2*
SCLATMF	TGCAGAAAAACCTGCTGACAA	Taqman primer for *sclA*
SCLATMR	GGTGTCTTAGCCATATTGCCATT	Taqman primer for *sclA*
SCLATMP	AGCTAACCAAACGCCAGAACGCCG	Taqman probe for *sclA*
ENDOSTMF	CGCAAGGACGAGGACAAAGC	Taqman primer for *endoS*
ENDOSTMR	CCCACCTGTCTTAGGTTGCCA	Taqman primer for *endoS*
ENDOSTMP	TGCATACCGTTCGGCACGCGTTCCAGT	Taqman probe for *endoS*
SAGCTMF	CTGCTGAGTTGCTAGCTGAACAAC	Taqman primer for *sagC*
SAGCTMR	CTTTGGTAATCGCACAAGGCATCTG	Taqman primer for *sagC*
SAGCTMP	CGGCGATGCTCCAAAGCATCTAAACGCGAAC	Taqman probe for *sagC*
UNR24	AGTCATGCATAAAAAGAGTCTGGGACTATTGTCTCAG	To be used with UNR25 to amplify rocA from M1 GAS genome in order to make plasmid pRocA-M1 (or from M3/M18 genomes to make pRocA-M3/M18)
UNR25	AGTCAGATCTGAGATCAACAACTGATAAATCTGAG	To be used with UNR24 to amplify rocA from M1 GAS genome in order to make plasmid pRocA-M1 (or from M3/M18 genomes to make pRocA-M3/M18)
UNR544	TGATGTTAAAGGTATGAATAATCACTCAAAATCAACTTAAGAGTCTGAGACAATAG	To be used with UNR547, UNR548, UNR608 and UNR610 for amplifying truncated rocA alleles for creation of plasmids pRocA-M1$^{1-328}$, pRocA-M1$^{1-267}$, pRocA-M1$^{1-221}$, and pRocA-M1$^{1-89}$
UNR547	GATTTTGAGTGATTATTCATACCTTTAACATCACTTCTTATTTTTTGCCTCAATTAATTTAGCTAAAACAATATG	To be used with UNR544 for amplifying a truncated rocA allele for the creation of plasmid pRocA-M1$^{1-328}$
UNR548	GATTTTGAGTGATTATTCATACCTTTAACATCAATCATTTTGTTCGATTCCAATCTTAAGACGACTAGACACGGTAGTTAATAGC	To be used with UNR544 for amplifying a truncated rocA allele to create plasmid pRocA-M1$^{1-276}$
UNR608	GATTTTGAGTGATTATTCATACCTTTAACATCAACGCTCTAAATGAATTAATAAAGTAAGAAAC	To be used with UNR544 for amplifying a truncated rocA allele to create plasmid pRocA-M1$^{1-221}$
UNR610	GATTTTGAGTGATTATTCATACCTTTAACATCAAAACTTAGCTTTTATTGATTTACCATAG	To be used with UNR544 for amplifying a truncated rocA allele to create plasmid pRocA-M1$^{1-89}$

Table S7
Table of primers and probes used in this study.