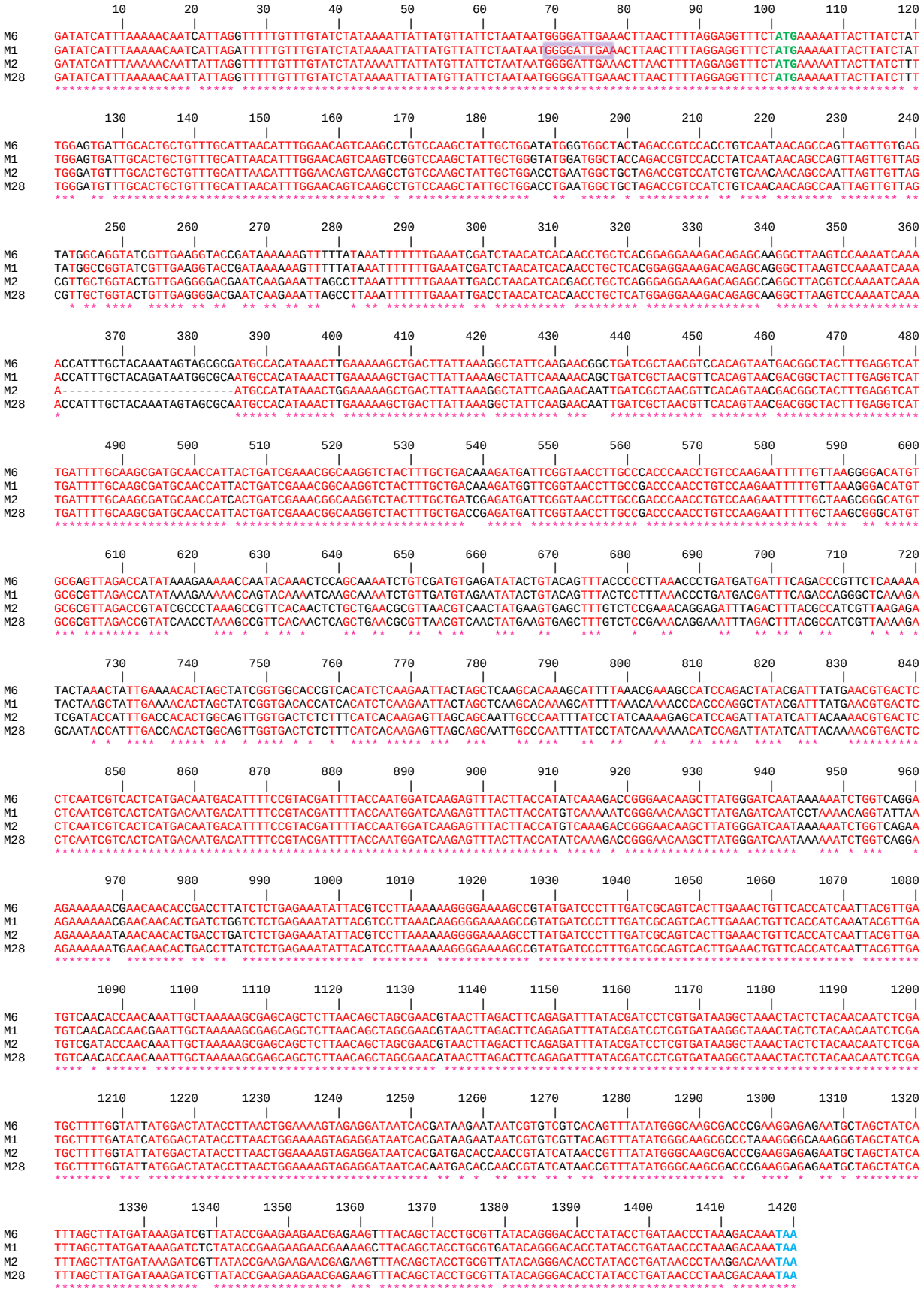

Figure S1

Alignment of the *ska* genes from the serotype M1, M2, M6, and M28 strains used in this study.

The *ska* genes, and 100 nucleotides upstream of the ATG start codon, were aligned. Nucleotides that are identical across all four *ska* alleles are colored red. The start codon is colored green. The stop codon is colored blue. The nine nucleotides that base-pair to the FasX sRNA in serotype M1 GAS are highlighted in a purple box.

Strain Name	Information	Reference
MGAS2221	Serotype M1 clinical GAS isolate that has been genome sequenced	Sumby *et al*., 2006
2221ΔFasX	MGAS2221 derivative in which the *fasX* gene has been replaced with a spectinomycin resistance cassette	Ramirez-Pena *et al*., 2010
2221ΔFasX + vector	2221ΔFasX containing the empty shuttle vector pDCBB	Ramirez-Pena *et al*., 2010
2221ΔFasX + pFasX	2221ΔFasX containing the pDCBB-derivative pFasX which contains a wild-type *fasX* allele downstream of the natural promoter	Ramirez-Pena *et al*., 2010
MGAS10270	Serotype M2 clinical GAS isolate that has been genome sequenced	Beres *et al*., 2007
M2 + vector	MGAS10270 containing the empty shuttle vector pDCBB	This work
M2 + pFasX	MGAS10270 containing the pDCBB-derivative pFasX which contains a wild-type *fasX* allele downstream of the natural promoter	This work
M2ΔFasX	MGAS10270 derivative in which the *fasX* gene has been replaced with a spectinomycin resistance cassette	This work
M2ΔFasX + vector	M2ΔFasX containing the empty shuttle vector pDCBB	This work
M2ΔFasX + pFasX	M2ΔFasX containing the pDCBB-derivative pFasX which contains a wild-type *fasX* allele downstream of the natural promoter	This work
M2ΔPIL	MGAS10270 derivative in which the pilus operon genes *10270_spy0109* to *0111* have been partially or fully deleted and a spectinomycin resistance cassette inserted	This work
MGAS10394	Serotype M6 clinical GAS isolate that has been genome sequenced	Banks *et al*., 2004
M6ΔFasX	MGAS10394 derivative in which the *fasX* gene has been replaced with a spectinomycin resistance cassette	This work
M6ΔFasX + vector	M6ΔFasX containing the empty shuttle vector pDCBB	This work
M6ΔFasX + pFasX	M6ΔFasX containing the pDCBB-derivative pFasX which contains a wild-type *fasX* allele downstream of the natural promoter	This work
MGAS6180	Serotype M28 clinical GAS isolate that has been genome sequenced	Green *et al*., 2005
M28ΔFasX	MGAS6180 derivative in which the *fasX* gene has been replaced with a spectinomycin resistance cassette	This work
M28ΔFasX + vector	M28ΔFasX containing the empty shuttle vector pDCBB	This work
M28ΔFasX + pFasX	M28ΔFasX containing the pDCBB-derivative pFasX which contains a wild-type *fasX* allele downstream of the natural promoter	This work

Table S1
Table of GAS strains used in this study.

Primer name	Sequence (5' - 3')	Role
M2PILA	gtcaggatccAAATTGATGCCGCTTCCACGATAAAGCC	Used in construction of pilus operon mutant in M2 GAS
M2PILB	CTAAGAAAATGAGACGGAAAATCCAGCTGAATACAACAACTTCTTTG	Used in construction of pilus operon mutant in M2 GAS
M2PILC	CAAAGAAGTTGTTGTATTCAGCTGGATTTTCCGTCTCATTTTCTTAG	Used in construction of pilus operon mutant in M2 GAS
M2PILD	gcatggatccAAGGTAATAACTTACCTGGCTTAC	Used in construction of pilus operon mutant in M2 GAS
M2PILE	GGTGATATAAATTAAATAGAGGTAG	Used in construction of pilus operon mutant in M2 GAS
M2PILF	TACTCTCTCAATCCTTCTTTTTGCTTAC	Used in construction of pilus operon mutant in M2 GAS
FASXA	TCGTGTGGATATAGCCAAACG	Used in construction of *fasX* mutant in M2 GAS
M2FASXB	gttatagttattataacatgtattCATTAATTATTATAGCGAAAAATACTTC	Used in construction of *fasX* mutant in M2 GAS
FASXC	ctatttaaataacagattaaaaaaattataagGGGTTTTTTGATAGGTAAATAC	Used in construction of *fasX* mutant in M2 GAS
M2FASXD	ATCTGAATTAAGAGGGGTAAGAG	Used in construction of *fasX* mutant in M2 GAS
M2FASXSF	GAAGTATTTTTCGCTATAATAATTAATGaatacatgttataataactataac	Used in construction of *fasX* mutant in M2 GAS
FASXSR	gtatttacctatcaaaaaacccCTTATAATTTTTTTAATCTGTTATTTAAATAG	Used in construction of *fasX* mutant in M2 GAS
FASXE	GAGACAGCCAAAGGCAGTAGACG	Used in construction of *fasX* mutant in M2 GAS
M2FASXF	AAGTGGACAAGAAAGTGAAGAC	Used in construction of *fasX* mutant in M2 GAS
M6fasXD	GCAATCATAACTGGCATTTGAATTAAG	Used to replace primer M2FASXD in construction of the M6 *fasX* mutant
M28fasXM	TTAAACAAAGGCATGAGCATGAG	Used with FASXA to amplify the SPEC cassette from strain 2221ΔfasX for creation of M28 *fasX* mutant
107TMF	AAAAGGGAGTCAGAAAGTAACTTGGT	Taqman primer for *M1.cpa*
107TMR	CCGGAACTTGTTTTGGCATT	Taqman primer for *M1.cpa*
107TMP	CGTCAAGCTTTGAAGCAACTGATTGATCC	Taqman probe for *M1.cpa*
109TMF	ACTACTGTCAACGAAGACGGAAATAAG	Taqman primer for *M1.tee1*
109TMR	CGTATTTGATCCACCTTTATCTGAATT	Taqman primer for *M1.tee1*
109TMP	TGACTTTAGTCATCGGTGTGTTCAAAGCTACACC	Taqman probe for *M1.tee1*
111TMF	AAACCGAAAAATCGGAGCTTATT	Taqman primer for *M1.111*
111TMR	CGTAACTCACCATTTCACCAGTTG	Taqman primer for *M1.111*
111TMP	AAACACCGGAACCTCATCAACCAGATACAA	Taqman probe for *M1.111*
M2.109 TMF	CGGTAGCGGGACCAACTG	Taqman primer for *M2.fctA*
M2.109 TMR	TCTGGTGTCCTCAGCTCT-TACAAC	Taqman primer for *M2.fctA*
M2.109 TMP	TAGCCCAGTTGCACAGTTTGCGACAA	Taqman probe for *M2.fctA*
M2.110 TMF	GAAGGGACTCCAACTGAAGG	Taqman primer for *M2.110*
M2.110 TMR	AATTCCAATGTGCCAACAAA	Taqman primer for *M2.110*
M2.110 TMP	CGCCATCTTCAGTTGATTTCAATTGG	Taqman probe for *M2.110*
M2.113 TMF	TAGGCAAAGCTACTTTTG-TGTTGAA	Taqman primer for *M2.113*
M2.113 TMR	TCCCGGTTTTATGTTTTC-AAAAG	Taqman primer for *M2.113*
M2.113 TMP	TCTACTGTCTCGTGACTTATTTCTGACTTATCATGGTCA	Taqman probe for *M2.113*
M6.159 TMF	GGAGCAGCCCCAGCACTAA	Taqman primer for *M6.fctX*
M6.159 TMR	CCGCTTATGGTGCTCCAA-TT	Taqman primer for *M6.fctX*
M6.159 TMP	CCACGTTCTTTTCGCTCCTTAACTCCCA	Taqman probe for *M6.fctX*
M6.160 TMF	CGAATGAAGGTTCAGGTA-CAGTATTATTAG	Taqman primer for *M6.tee6*
M6.160 TMR	GCTACCTGTCGAAGGTAA-TTCACCTA	Taqman primer for *M6.tee6*
M6.160 TMP	ACTGACATCCCTAACACCAA	Taqman probe for *M6.tee6*
M6.srtB TMF	AAGAAGCCCTCAGAAATGGA	Taqman primer for *M6.srtB*
M6.srtB TMR	TGAATGCCATCCCTAAATGA	Taqman primer for *M6.srtB*
M6.srtB TMP	TTGGGATTCAGCCCGTTCCA	Taqman probe for *M6.srtB*
M28.cpa TMF	TTGGGACAGAATACCATCCA	Taqman primer for *M28.cpa*
M28.cpa TMR	TCCCAACTCACCGACTACTG	Taqman primer for *M28.cpa*
M28.cpa TMP	TCCATACGAATCACGTCAACCAAATCA	Taqman probe for *M28.cpa*
M28.lepA TMF	TTGAAAGTTGGTCGAATTGC	Taqman primer for *M28.lepA*
M28.lepA TMR	AACAGTCCTCCTTCTTGCGT	Taqman primer for *M28.lepA*
M28.lepA TMP	CCTCATCGCCAGCTTGAGCG	Taqman probe for *M28.lepA*
M28.109 TMF	AATAACCGTGACACTCAA-GTTCCA	Taqman primer for *M28.fctA*
M28.109 TMR	AGCCACAATGCTAAGAAC-TGCAA	Taqman primer for *M28.fctA*
M28.109 TMP	CTGGTGTTGTAGGCACCCTTGCTCCA	Taqman probe for *M28.fctA*
TUFA TMF	AACTACTTTAACAGCTGCAATCACAACT	Taqman primer for *tufA*
TUFA TMR	AGAAGCGTAATCTTTTGGTTGGTT	Taqman primer for *tufA*
TUFA TMP	TATTGGCACGTCGCTTGCCTTCATC	Taqman probe for *tufA*
SKATM2 TMF	CGGCTACTTTGAGGTCATTGATT	Taqman primer for *M1.ska / M2.ska*
SKATM2 TMR	CCGAACCATCTTTGTCAGCAA	Taqman primer for *M1.ska / M2.ska*
SKATM2 TMP	CAAGCGATGCAACCATTACTGATCGAAAC	Taqman probe for *M1.ska / M2.ska*
SKATM2 TMF	CGGCTACTTTGAGGTCATTGATT	Taqman primer for *M6.ska*
M6SKATMR	CCGAATCATCTTTGTCAGCAA	Taqman primer for *M6.ska*
SKATM2 TMP	CAAGCGATGCAACCATTACTGATCGAAAC	Taqman probe for *M6.ska*
SKATM2 TMF	CGGCTACTTTGAGGTCATTGATT	Taqman primer for *M28.ska*
M28SKATMR	CCGAATCATCTCGGTCAGCAA	Taqman primer for *M28.ska*
SKATM2 TMP	CAAGCGATGCAACCATTACTGATCGAAAC	Taqman probe for *M28.ska*
UNR218	CTGActtaatacgactcactataggAGAAGGGAGATAAATGAAAAAGAAAC	Used with UNR219 to amplify the 5' end of gene 113 from M2 GAS and place it downstream of a T7 promoter for use in in vitro transcription
UNR219	ATTTGGGATAGGATTAGTAAAG	Used with UNR218 to amplify the 5' end of gene 113 from M2 GAS and place it downstream of a T7 promoter for use in in vitro transcription
UNR123	cttaatacgactcactataggTAAATAAAGATTTACGAAGTC	Used with FASEND9 to amplify *fasX* or the *fasX* derivative *fasXA72-77* and allow their use in in vitro transcription assays by placing a T7 promoter upstream
FASEND9	aaaaaacccggcaagccgggct	Used with UNR123 to amplify *fasX* or the *fasX* derivative *fasXA72-77* and allow their use in in vitro transcription assays by placing a T7 promoter upstream
UNR291	agcttaatacgactcactatagGAGAGAAACAGTGAGAGAGAAGATATTAATAAC	Used in association with UNR292 to amplify the 5' UTR and initial coding region of *M6.tee6* and allow its use in *in vitro* transcription assays by placing a T7 promoter upstream
UNR292	GTGCAGTATCATCTTTTGATAAAGCTG	Used in association with UNR291 to amplify the 5' UTR and initial coding region of *M6.tee6* and allow its use in *in vitro* transcription assays by placing a T7 promoter upstream
UNR212	CTGActtaatacgactcactataggTGGAGAGAGAGAAAATGAAAAAAATAAATTATTACTTGC	Used in association with UNR214 to amplify the 5' UTR and initial coding region of *M28.fctA* and allow its use in *in vitro* transcription assays by placing a T7 promoter upstream
UNR214	GATTTTACGTTTTGATTTAAAGAAG	Used in association with UNR212 to amplify the 5' UTR and initial coding region of *M28.fctA* and allow its use in *in vitro* transcription assays by placing a T7 promoter upstream
UNR217	ATATGAGTAAACTTGGTCTGACAGtcatttatcatcgtcatctttataatcTTCTACCTTAACTAATGGATAACTTTC	Used in association with UNR218 to amplify the 5'-UTR and first 158 codons of *M2.113* for use in *in vitro* transcription and translation reactions
UNR218	CTGActtaatacgactcactataggAGAAGGGAGATAAATGAAAAAGAAAC	Used in association with UNR217 to amplify the 5'-UTR and first 158 codons of *M2.113* for use in *in vitro* transcription and translation reactions
UNR291	agcttaatacgactcactatagGAGAGAAACAGTGAGAGAGAAGATATTAATAAC	Used in association with UNR293 to amplify the 5'-UTR and first 158 codons of *M6.tee6* for use in *in vitro* transcription and translation reactions
UNR293	ATATGAGTAAACTTGGTCTGACAGtcatttatcatcgtcatctttataatcTAATGATGATTTTGCAACACTTGTTTGTCC	Used in association with UNR291 to amplify the 5'-UTR and first 158 codons of *M6.tee6* for use in *in vitro* transcription and translation reactions
UNR220	ATATGAGTAAACTTGGTCTGACAGtcatttatcatcgtcatctttataatcTCCTCCATTACCATCATTAACAAC	Used in association with UNR356 to amplify the 5'-UTR and first 158 codons of *M28.fctA* for use in *in vitro* transcription and translation reactions
UNR356	CTGActtaatacgactcactatagGGAAAATCTCAACTCTATTAAGAGTG	Used in association with UNR220 to amplify the 5'-UTR and first 158 codons of *M28.fctA* for use in *in vitro* transcription and translation reactions
SPDTRN	ATATGAGTAAACTTGGTCTGACAGcatttatcatcgtcatctttataatcTCCGACATAAGATAGACCATTTAACC	Used in association with T7SPD to amplify the 5'-UTR and first 158 codons of *spd* for use in *in vitro* transcription and translation reactions
T7SPD	cttaatacgactcactatagGGTTAGTGAGCGAAATTAGAAAAGAGG	Used in association with SPDTRN to amplify the 5'-UTR and first 158 codons of *spd* for use in *in vitro* transcription and translation reactions

Table S2
Table of primers and probes used in this study.