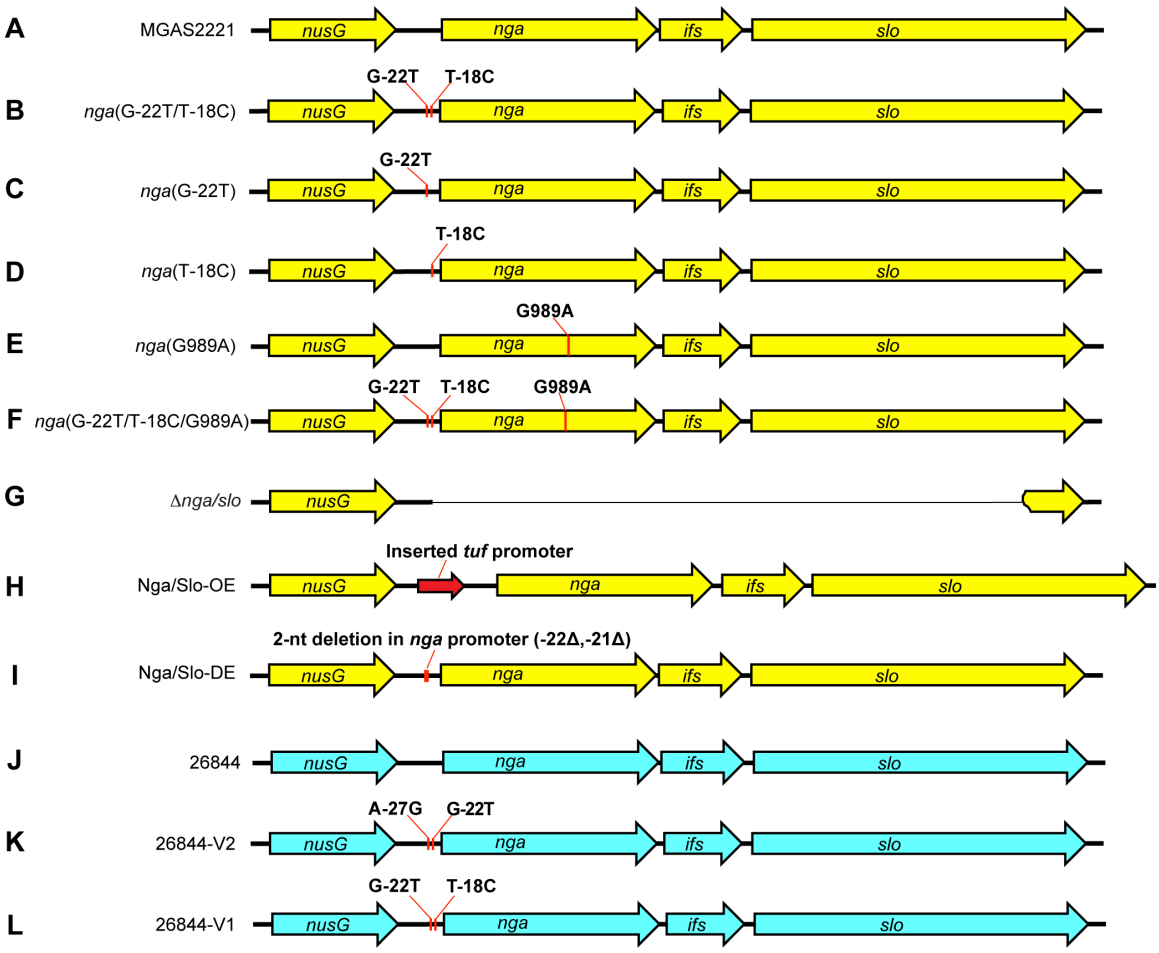
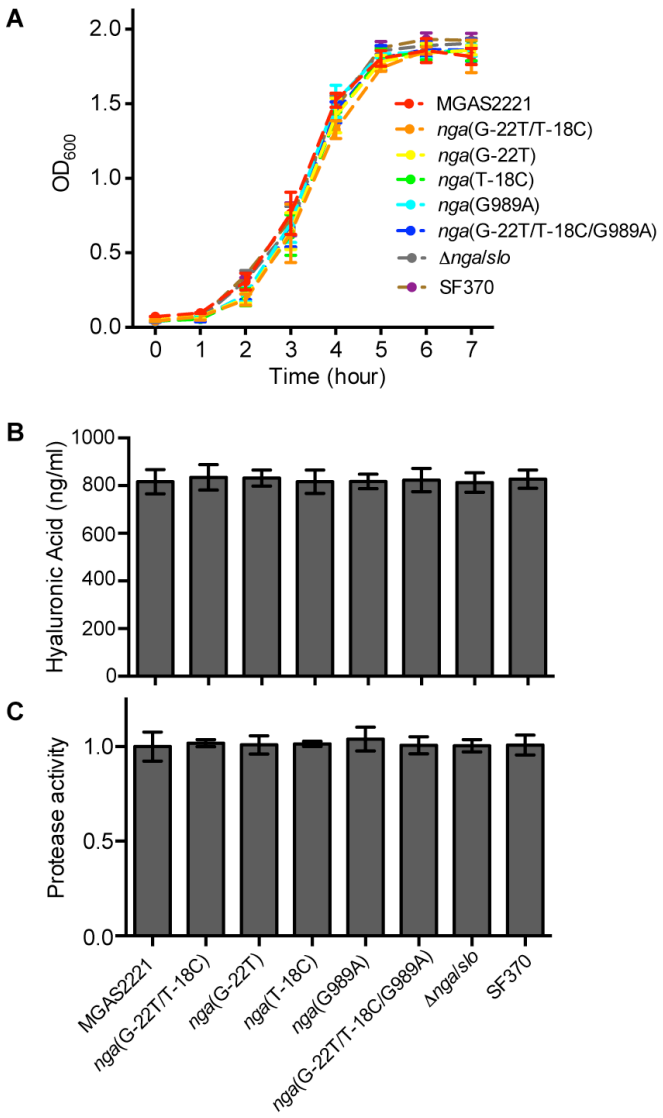
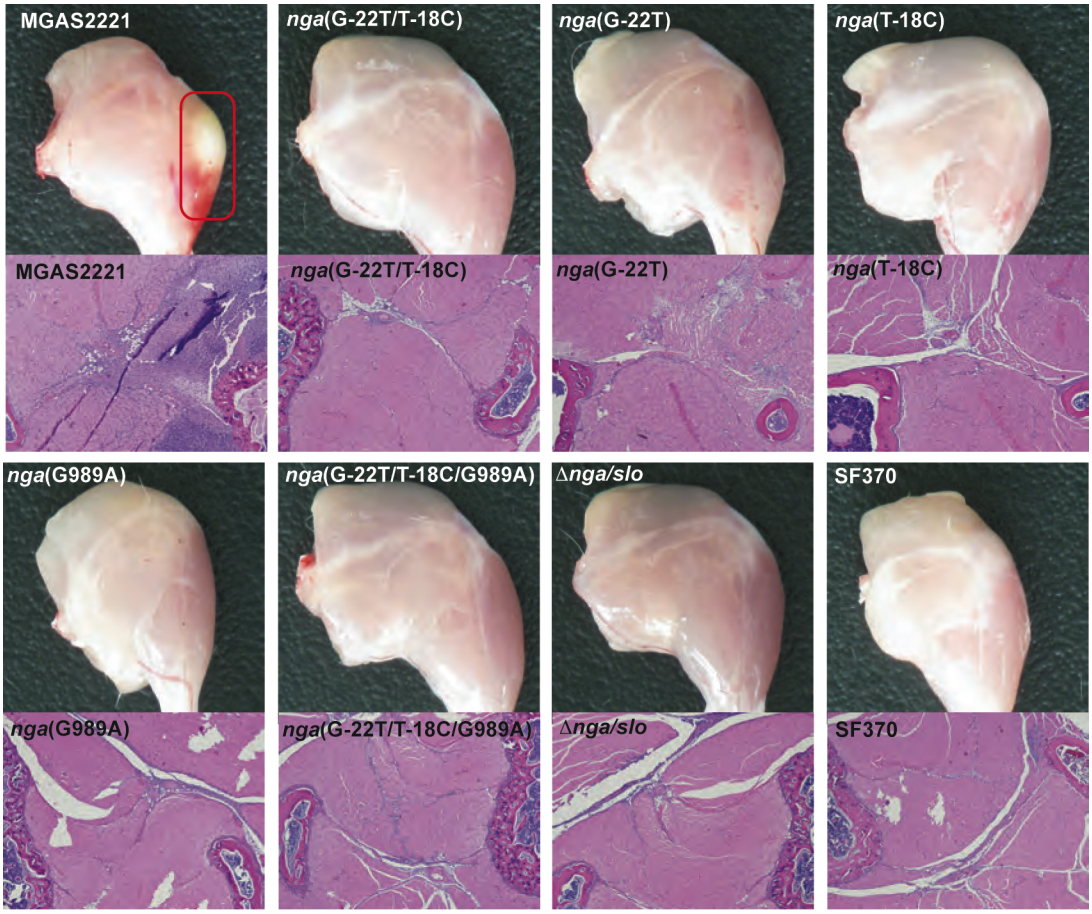


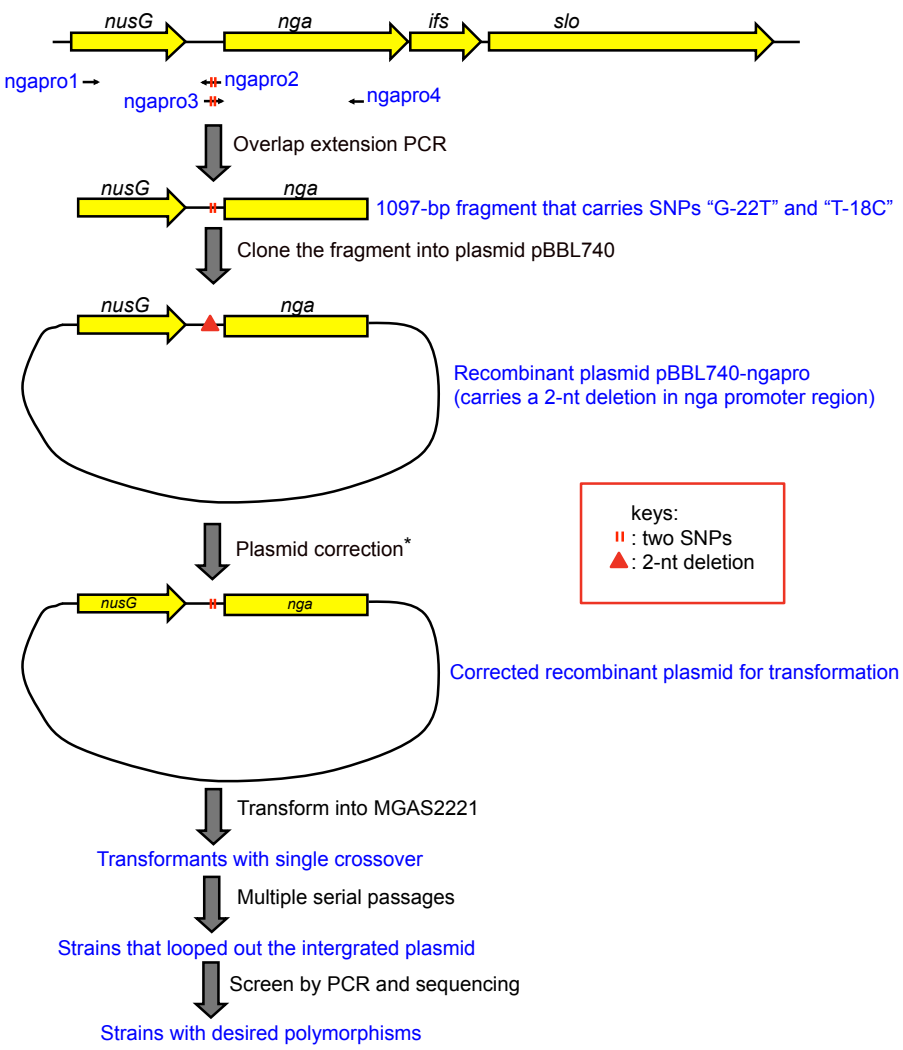
Supplementary Figure 1. Schematic showing *nga-ifs-slo* region of isoallelic mutant strains used in the study. (**A**) Region in wild-type epidemic reference strain MGAS2221 (serotype M1). (**B**) Isoallelic mutant strain *nga*(G-22T/T-18C), with SNPs "G-22T" and "T-18C" in *nga* promoter spacer region. (**C**) Isoallelic mutant strain *nga*(G-22T), with SNP "G-22T" in *nga* promoter spacer region. (**D**) Isoallelic mutant strain *nga*(T-18C), with SNP "T-18C" in *nga* promoter spacer region. (**E**) Isoallelic mutant strain *nga*(G989A), with SNP "G989A" in *nga* coding region. The polymorphism results in either a glycine (nucleotide G) or aspartic acid (nucleotide A) residue in SPN. (**F**) Isoallelic mutant strain *nga*(G-22T/T-18C/G989A), containing all three of the target SNPs. (**G**) *nga-ifs-slo* deletion mutant. (**H**) *tuf* promoter upregulation mutant. (**I**) Two-nucleotide deletion mutant strain in nga promoter spacer region. (**J**) Region in wild-type epidemic serotype M89 reference strain MGAS26844 (variant 3). (**K**) Isoallelic serotype M89 mutant strain 26844-V2 (variant 2), with SNPs "A-27G" and "G-22T" in *nga* promoter spacer region. (**L**) Isoallelic serotype M89 mutant strain 26844-V1 (variant 1), with SNPs "G-22T" and "T-18C" in *nga* promoter spacer region.



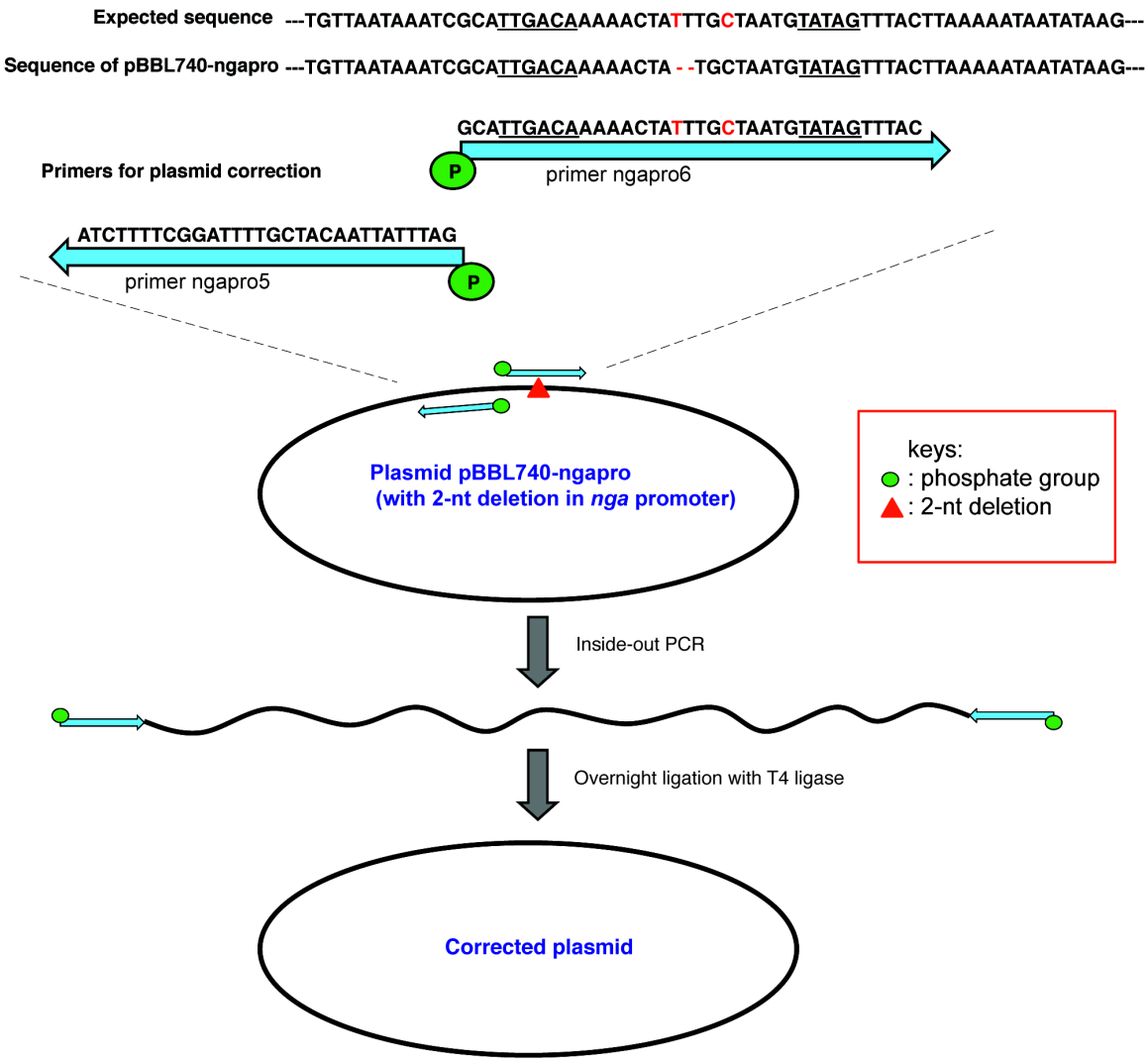
Supplementary Figure 2. In vitro characteristics of M1 reference strains and isoallelic mutants. (**A**) Growth curves of MGAS2221, isoallelic strains and SF370 in THY. (**B**) Production of hyaluronic acid capsule relative to MGAS2221 level. (**C**) Relative protease activity of M1 strains using a standard milk agar assay.



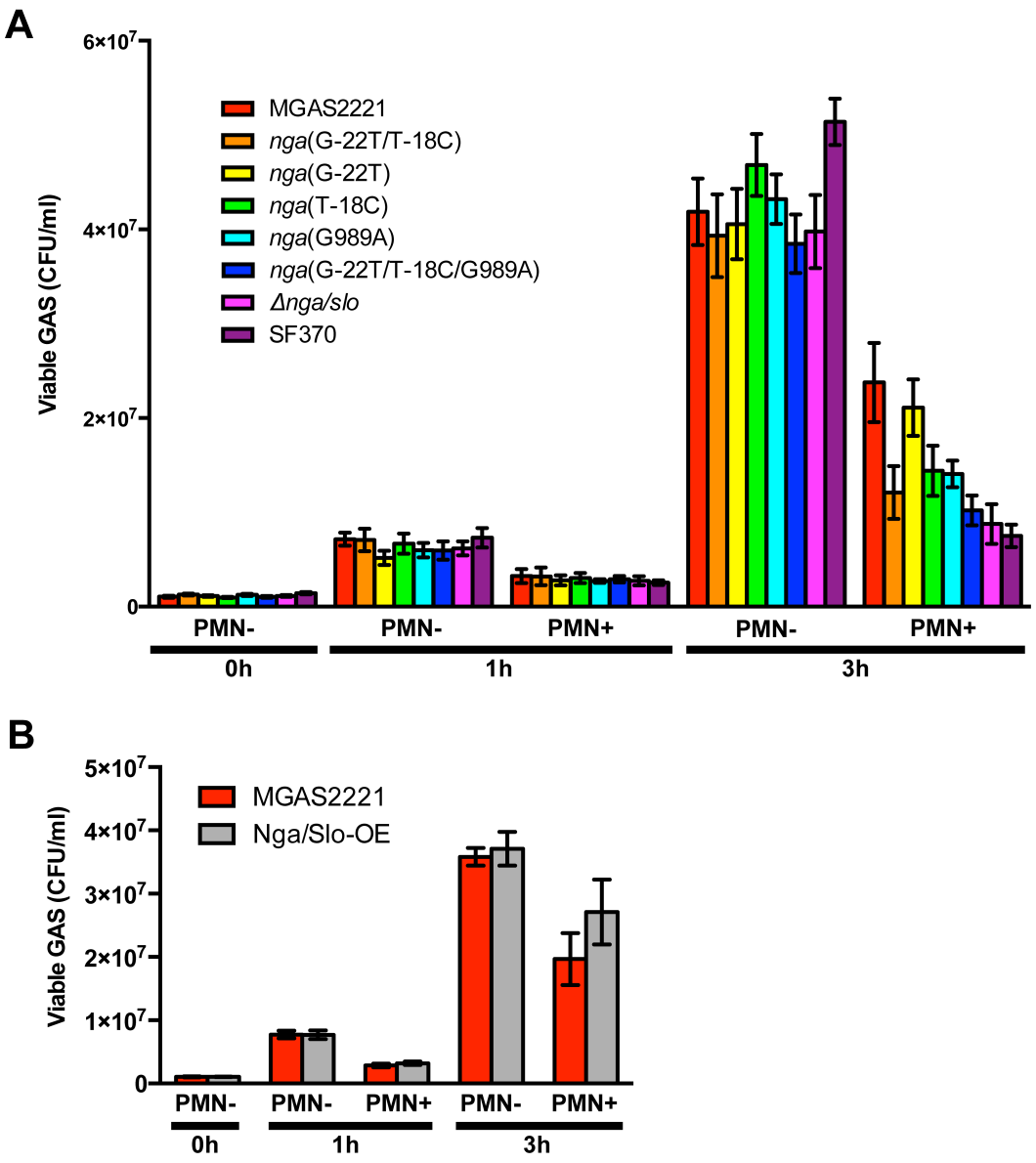
Supplementary Figure 3. Gross and microscopic pathology analysis of virulence in a mouse model of necrotizing fasciitis. Mice were inoculated intramuscularly in the hindlimb with wild-type epidemic strain MGAS2221, isoallelic mutant strains, or pre-epidemic strain SF370. Representative gross pathology and microscopic histopathology of mouse hindlimb lesions at 96 h post-infection. For gross histopathology, mice given wild-type strain MGAS2221 had obvious lesions, whereas mice infected with isoallelic mutant strains or strain SF370 had little or no observable changes. For microscopic histopathology, mice given strain MGAS2221 had severe muscle damage and neutrophil infiltration. All isoallelic mutant strains caused markedly less severe tissue damage. Isoallelic mutant strain *nga*(G-22T) produced an intermediate tissue pathology phenotype.



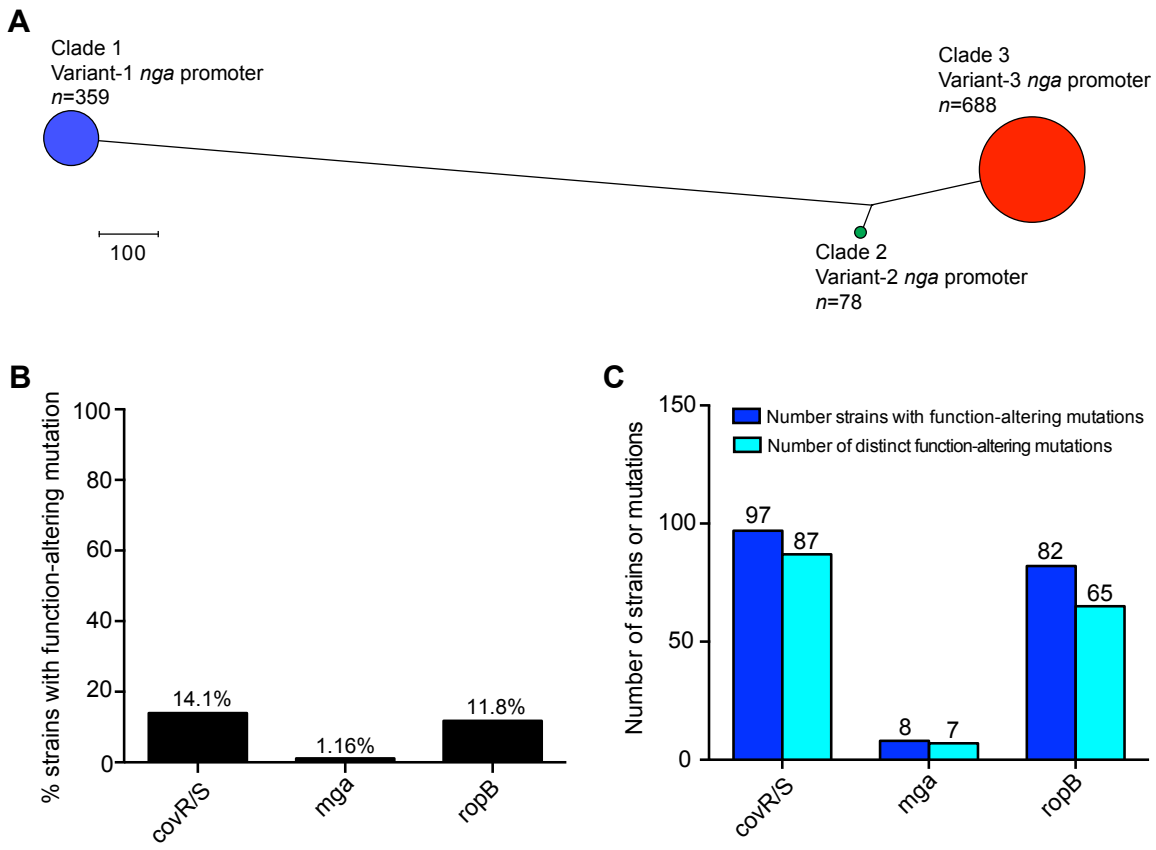
Supplementary Figure 4. Schematic depiction of steps used to generate *nga* promoter mutant strain *nga*(G-22T/T-18C). Overlap extension PCR was used to generate a 1097-bp fragment that spans the spacer region of *nga* promoter using primer sets ngapro1/2 and ngapro3/4. The two SNPs were introduced into the PCR products using primers ngapro2 and ngapro3. The PCR product was then digested with BamHI and cloned into suicide vector pBBL740. After multiple attempts to transform the ligation product into *E. coli* strain JM109, we realized that JM109 was unable to maintain the recombinant plasmid without introducing inactivating mutations into the insert. One of these "flawed" recombinant plasmids, pBBL740-ngapro, which has a 2-nucleotide deletion in the *nga* promoter region, was selected for correction. (Plasmid correction strategy is detailed in Supplementary Figure 5). The corrected plasmid was transformed into parental strain MGAS2221. A verified transformant with a single crossover was passed on nonselective THY agar plates to loop out the integrated plasmid. Chloramphenicol sensitive strains were selected by patching colonies on THY agar plates with and without 10 µg/ml chloramphenicol. PCR and sequencing were used to select for strains that replaced the chromosomally derived sequence "GTTGT" with plasmid derived sequence "TTTGC" in the spacer region of the *nga* promoter.



Supplementary Figure 5. Schematic depiction of strategy used to modify plasmid pBBL740-ngapro for the construction of isoallelic mutant strain *nga*(G-22T/T-18C). Plasmid correction was achieved by inside-out PCR using phosphorylated primers ngapro5 and ngapro6. Primer ngapro6, which covers the spacer region of the *nga* promoter repairs the deletion. Together with primer ngapro5, which is oriented in the opposite direction, the two primers amplify a linear DNA fragment with phosphate groups on the two ends. T4 ligase was used to ligate the two phosphorylated ends and circularize the DNA fragment into a plasmid. After overnight incubation with T4 ligase at 6°C, the ligation product was column purified and electroporated into parental strain MGAS2221.



Supplementary Figure 6. Absolute CFU values of viable GAS at 0h, 1h and 3h post incubation with and without PMN exposure. (**A**) CFU data of Figure 4D. (**B**) CFU data of Figure 5F. CFU data are expressed as the mean ± SEM.



Supplementary Figure 7. Genomic relationships among the *emm89* strains and transcriptional regulator mutations in clade 3 strains that may influence *nga* and *slo* expression. (**A**) Schematic phylogenetic tree representing estimates of overall genomic relationships among the 1,125 *emm*89 strains sequenced. *emm89* strains cluster into three major clades, designated clade 1, clade 2 and clade 3. Strains in each of the three clades have a unique *nga* promoter region pattern. (**B**) Percentage of clade 3 strains with function-altering mutations in major transcriptional regulators that may influence *nga*/*slo* expression. (**C**) Absolute number of clade 3 strains with function-altering mutations in major transcriptional regulators, and number of distinct mutations present in each transcriptional regulator. The data show that only a small minority of the clade 3 *emm89* strains studied have a mutation in these key regulatory genes that may alter the expression of *nga* and *slo*.

Supplementary Table 1. Primers used for constructing isoallelic mutant strains.

Primer name	Sequences	Remarks
ngapro1	GTTCCGCGTGGATCCCCGTGCTATCTTGTTGTCTATGGGT	underlined: BamH1 site
ngapro2	TAAGTAAACTATACATTAGCAAATAGTTTTTGTCAATGCGA	
ngapro3	TCGCATTGACAAAAACTATTTGCTAATGTATAGTTTACTTA	
ngapro4	GTTCCGCGTGGATCCTTTATCAATCTCAATGTGATGCGGT	underlined: BamH1 site
ngapro5	p-GATTTATTAACATCGTTTTAGGCTTTTCTA	phosphorylated on the 5' end
ngapro6	p-GCATTGACAAAAACTATTTGCTAATGTATAGTTTACTT	phosphorylated on the 5' end
ngapro7	CGTGTTTACAAACCAATGGATGACT	
ngaproGT	p-GCATTGACAAAAACTATTTGTTAATGTATAGTTTACTT	phosphorylated on the 5' end
ngaproTC	p-GCATTGACAAAAACTAGTTGCTAATGTATAGTTTACTT	phosphorylated on the 5' end
ngaGA1	GTTCCGCGTGGATCCAATGGAGATACATCTAGCAAGGAGA	underlined: BamH1 site
ngaGA2	TCGCTATATTTTCCGCTATCGACATCTTTTATATTTTCAATTTGGTCAG	
ngaGA3	CTGACCAAATTGAAAATATAAAAGATGTCGATAGCGGAAAATATAGCGA	
ngaGA4	GTTCCGCGTGGATCCTTAGGGTTTGAACCGCTTGGTAAAT	underlined: BamH1 site
ngasloΔ1	GTTCCGCGTGGATCCGCTGGTTCGTCCTCCAAACTTACTC	underlined: BamH1 site
ngasloΔ2	ACCGTTGCTTTGTCTCCCATACCTTAGCCAACTGTTACAAGTTTCAAG	
ngasloΔ3	CTTGAAACTTGTAACAGTTGGCTAAGGTATGGGAGACAAAGCAACGGT	
ngasloΔ4	GTTCCGCGTGGATCCGAGACAGGTTAATTTTTCCACTCGT	underlined: BamH1 site
tuf-nga-1	GTTCCGCGTGGATCCGTGGTGTTAGCCTTACAACGGATGC	underlined: BamH1 site
tuf-nga-2	GTGGTGCTTATAGCAGCGACTTCCTCATCCATTGGTTTGTAAACACGCTT	
tuf-nga-3	AAGCGTGTTTACAAACCAATGGATGAGGAAGTCGCTGCTATAAGCACCAC	
tuf-nga-4	TCTGATTACAATGATGTTAACAATAGTTTTAGGAAAAGCCTCCAATAAAATATA	
tuf-nga-5	TATATTTTATTGGAGGCTTTTCCTAAAACTATTGTTAACATCATTGTAATCAGA	
tuf-nga-6	GTTCCGCGTGGATCCTGCTTGTTTATCTGTTACCCATTTA	underlined: BamH1 site
ngapro-AG	p-GCATTGACAAAGACTATTTGTTAATGTATAGTTTACTT	phosphorylated on the 5' end

Supplementary Table 2. Primers used for TaqMan qRT-PCR analysis.

Primer name	Sequence
rpsl-forward:	CGTGTTGGAACAATGACACCTAA
rpsl-reverse:	CTTCGATAAGGTTGCTCAAACGT
rpsl-probe:	6FAM-CCTAACTCAGCCCTTCGTAAATTCGCTCGT-TAMRA
M1nga-forward:	TCCGAACAGCTAGCGGAGAT
M1nga-reverse:	CACTAATCGATGGGAAAATTAACTCA
M1nga-probe:	6FAM-CAAATTCGCTCAACATTCCTGGTTGCC-TAMRA
M1slo-forward:	GACCTTTAAAGAGTTGCAACGAAAA
M1slo-reverse:	GACCATAAGCTACGTTACTCACAAAGA
M1slo-probe:	6FAM-TGTCAGCAATGAAGCCCCGCC-TAMRA
M89nga-forward	GAATTAGGCGACACCTACACTAA
M89nga-reverse	GTGACCTCTGACAAGGCTAAA
M89nga-probe	6FAM-TGAGGTAACAGAGGTCCATCAGGGA-TAMRA
M89slo-forward	TGGGACAACAACTGGTATAGTAAG
M89slo-reverse	GTGCACTCTCTAGCCATGATAC
M89slo-probe	6FAM-AGCACAGTTATCCCACTAGGAGCT-TAMRA