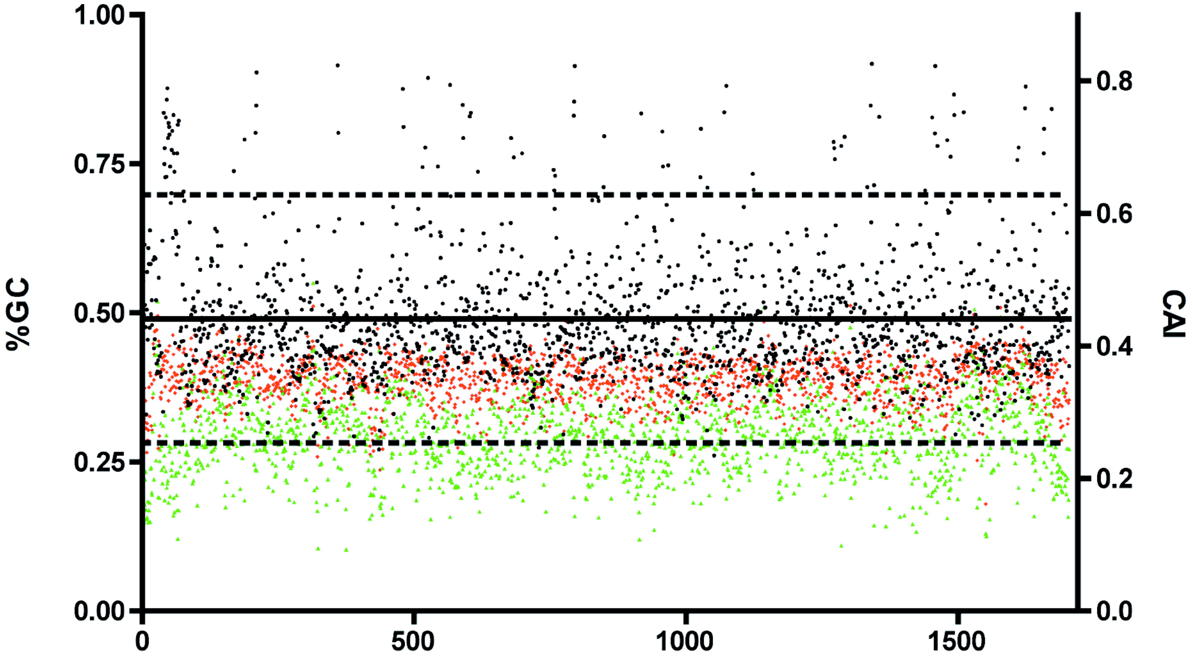
NZ131 – Supplemental Materials

Supplemental Figure 1. Codon adaptation index (CAI) and %G+C analysis of the NZ131 genome. The CAI of each predicted ORF is indicated (black points) with the mean CAI indicated by the solid line. The dotted lines indicated the limits defined by two standard deviations above and below the mean. The percentage %G+C for each predicted ORF is shown for the total coding region (red) and the third base position for each codon (green).



Supplemental Table 1. GAS strains used in these studies. All strains are from the University of Oklahoma collection or were recent clinical isolates.

Strain	M serotype	Origin	Case
		Non invasive GAS M49	
NZ131	49	New Zealand	APSAGN
2	49	New Zealand	APSAGN
3	49	New Zealand	Scarlet fever
4	49	New Zealand	Scarlet fever
5	49	England	Scarlet fever
6	49	England	Scarlet fever
7	49	England	Scarlet fever
8	49	New Zealand	Scarlet fever
9	49	New Zealand	Scarlet fever
10	49	New Zealand	Scarlet fever
11	49	New Zealand	Pharyngitis
12	49	New Zealand	Scarlet fever
13	49	Denmark	Pharyngitis
14	49	Japan	Pharyngitis
15	49	Japan	Pharyngitis
16	49	USSR	Scarlet fever
17	49	Thailand	Scarlet fever
		Invasive GAS M49*	
1	49	CA	Sepsis
2	49	CA	Sepsis
3	49	CA	Sepsis
4	49	CA	Sepsis
5	49	CA	Sepsis
6	49	Minnesota	Sepsis
7	49	CA	Sepsis
8	49	CA	Sepsis
9	49	CA	Sepsis
10	49	CA	Sepsis
11	49	CA	Sepsis
12	49	CA	Sepsis
13	49	Minnesota	Sepsis
14	49	CA	Sepsis
15	49	Oregon	Sepsis
16	49	Minnesota	Sepsis
17	49	CA	Surgical material
18	49	CA	Sepsis
19	49	CA	Sepsis

20	49	CA	Sepsis
21	49	CA	Sepsis
22	49	CA	Sepsis
		Other GAS Strains**	
SF370	1	USA	Wound (41)
287	1	New Zealand	Tonsillitis
	2	USA	
587	3	USA	Skin lesion
	3	USA	
	4	USA	
	4	USA	
	6	USA	
	9	USA	
	9	USA	
	11	USA	
	11	USA	
365	12	USA	Pharyngitis
	12	USA	
	22	USA	
	22	USA	
	22	USA	
	25	USA	
	25	USA	
785	28	Trinidad	
	28	USA	
	29	USA	
120	49	England	
D914	53	Germany	
291	58	USA	
	58	USA	
260	60	New Zealand	APSGN
261	60	New Zealand	Tonsillitis
	80	USA	
	80	USA	
5082-01	82	USA	
5082-02	82	USA	
5082-03	82	USA	
5082-04	82	USA	
5082-05	82	USA	
5082-06	82	USA	
5082-07	82	USA	
5082-08	82	USA	
5082-09	82	USA	
5082-10	82	USA	

	87	USA
	87	USA
	103	USA
	103	USA
	107	USA
	111	USA
3602-00	118	USA
	118	USA

*Provided by Bernard Beale from the CDC collection of invasive strains collected in the USA during a six-year period from 2000 to 2006

**Strains with no associated strain number were recent clinical isolates. The *emm* type was confirmed by DNA sequencing.

Supplemental Table 2. Oligonucleotide primers used for PCR analysis.

Sequence (5' – 3')	Gene Target	Product Size (bp)
ACTTGCTACTGGTTGCCGATT	Prophage NZ131.1 int	354
CGGAAAGCGTGAAAGGTAAAT		
GCCACGAATACCAAAAGAAGC	Prophage NZ131.2 int	323
AAGCGATAGCGTAGTCGAAGG		
TCTGTTAAGAACGACGGCTCA	Prophage NZ131.3 int	303
ATTGACATCAGTGTGCCCAAC		
GGGCTTGCTGTTCCTGACTAT	nudA	303
CACACGAGATGGTGCTAAGTCC		
CGTCCTTCCAAACTCCCATAGA	nudB	355
CCCTTTGAATGTGTGCAACGTG		
ACGCTATTAAATTCAGAACCTTC	nudC	626
ATTTACGATTTTTCCAGCTATCT		
GCGAAGTGACCCAAGATGAT	Spy1989-1988 junction	480
GATAGCAACCCCTTGACTGC		
AAGCGGATGTGATTGTAGCC	Spy1988-1987 junction	461
CAGGCAATTCCACCTGATCT		

Supplemental Table 3. Transcript mapping of the nudABC region. The PCR primers shown were used to map the presence of genes on a cDNA created from a polycistronic mRNA from strain SF370 (*nud*ABC-) or NZ131 (*nud*ABC+). The results show that nudABC is an addition to an operon common to all GAS genomes. The specificity of all amplified products was confirmed by DNA sequencing (not shown).

		cDNA Region Amplified	
Primer	**Sequence**	**SF370**	**NZ131**
89b88.L	GCGAAGTGACCCAAGATGAT	+	-
89b88.R	GATAGCAACCCCTTGACTGC		
88b87.L	AAGCGGATGTGATTGTAGCC	+	+
88b87.R	CAGGCAATTCCACCTGATCT		
89bO1.L	ACGATAGTGGCGAAATGA	-	+
89bO1.R	GGCATCATCAACTTGGACCT		
O1bNDX.L	TGCGTCTGAATGACGAGAAG	-	+
O1bNDX.R	CCAGGTGTCAGGAAAAGGAA		
NDXbO3.L	GCGATTTCGCAAGATATGGT	-	+
NDXbO3.R	CCCACTGAGATATAGGAAGCTTTA		

Supplemental Table 4. Survey of NZ131 prophage integrases in M49 strains.

Non-Invasive M49 GAS			
	NZ131.1 *int*	NZ131.2 *int*	NZ131.3 *int*
1	+	+	+
2	+	+	+
3	-	+	-
4	-	+	-
5	+	-	-
6	+	-	-
7	-	-	-
8	-	+	-
9	-	+	-
10	-	+	-
11	-	-	-
12	-	-	-
13	-	+	-
14	-	+	-
15	-	-	-
16	-	-	-
Invasive M49 GAS			
1	-	+	-
2	-	+	-
3	-	+	-
4	-	+	-
5	-	+	-
6	-	-	-
7	-	+	-
8	-	+	-
9	-	+	-
10	-	-	-
11	-	+	-
12	+	+	-
13	-	-	+
14	-	-	-
15	-	-	-
16	+	-	-
17	-	+	-
18	-	+	-
19	-	+	-
20	-	+	-
21	-	+	-
22	-	-	-