Supporting Information

Gryllos *et al.* 10.1073/pnas.0803815105

SI References

1. Alberti S, Ashbaugh CD, Wessels MR (1998) Structure of the *has* operon promoter and regulation of hyaluronic acid capsule expression in group A *Streptococcus*. *Mol Microbiol* 28:343–353.
2. Gryllos I, Levin JC, Wessels MR (2003) The CsrR/CsrS two-component system of group A *Streptococcus* responds to environmental Mg^{2+}. *Proc Natl Acad Sci USA* 100:4227–4232.
3. Gryllos I, *et al.* (2007) Mg^{2+} signalling defines the group A streptococcal CsrRS (CovRS) regulon. *Mol Microbiol* 65:671–683.
4. Beres SB, *et al.* (2002) Genome sequence of a serotype M3 strain of group A *Streptococcus*: Phage-encoded toxins, the high-virulence phenotype, and clone emergence. *Proc Natl Acad Sci USA* 99:10078–10083.
5. Ferretti JJ, *et al.* (2001) Complete genome sequence of an M1 strain of *Streptococcus pyogenes*. *Proc Natl Acad Sci USA* 98:4658–4663.

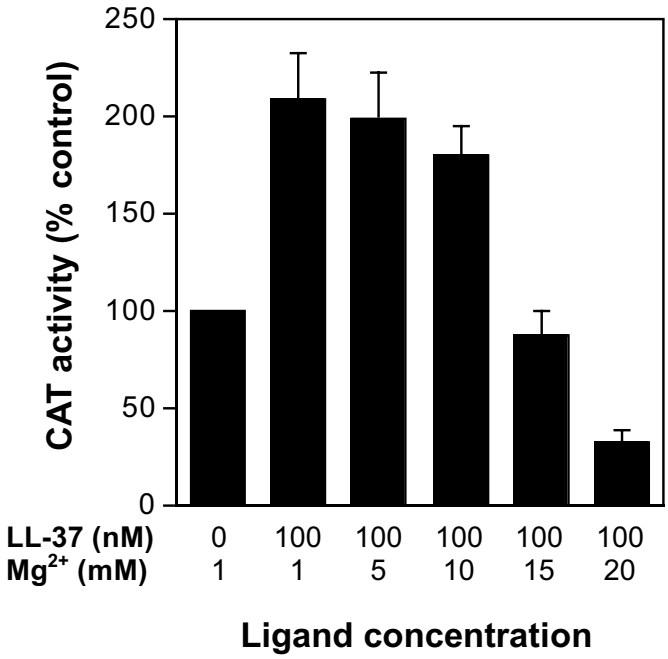

Fig. S1. Opposing effects of LL-37 and Mg^{2+} on GAS capsule gene expression. Exposure of the GAS reporter strain 003CAT to increasing concentrations of Mg^{2+} inhibited the stimulatory effect of LL-37 on *has* promoter activity. CAT activities from bacteria grown in the presence of supplemental $MgCl_2$ and/or LL-37 are shown as percentage of activity of that from control 003CAT cultures in unsupplemented medium (1 mM $MgCl_2$). Results shown are means ± SD from at least 3 independent experiments.

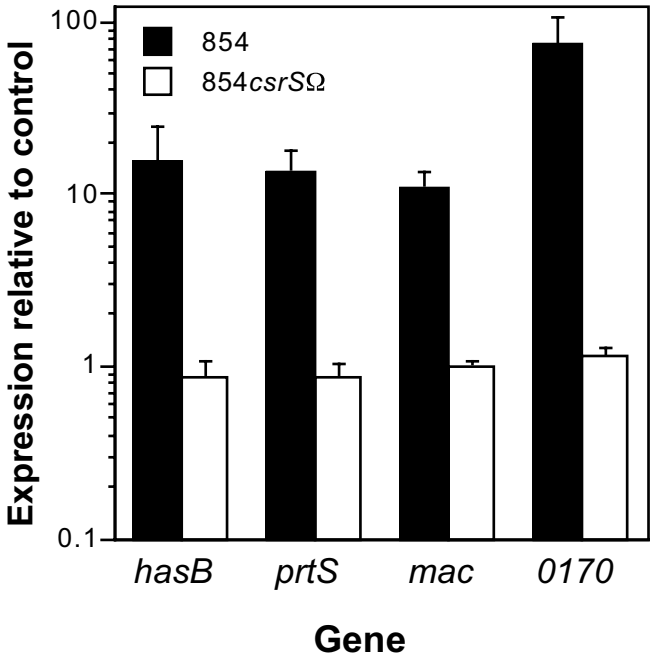

Fig. S2. LL-37-induced changes in CsrRS-regulated gene expression in wild-type GAS strain 854 and its isogenic *csrS* mutant 854*csrS*Ω. Data represent expression of *hasB*, *prtS*, *mac*, and *SPy0170* during growth in the presence of 100 nM LL-37 relative to that in control cultures of the same strain in unsupplemented medium. Data for strain 854 are the same as those in Fig. 3 and are shown here for comparison with those for strain 854*csrS*Ω. Results shown are means ± SD from at least 3 independent experiments.

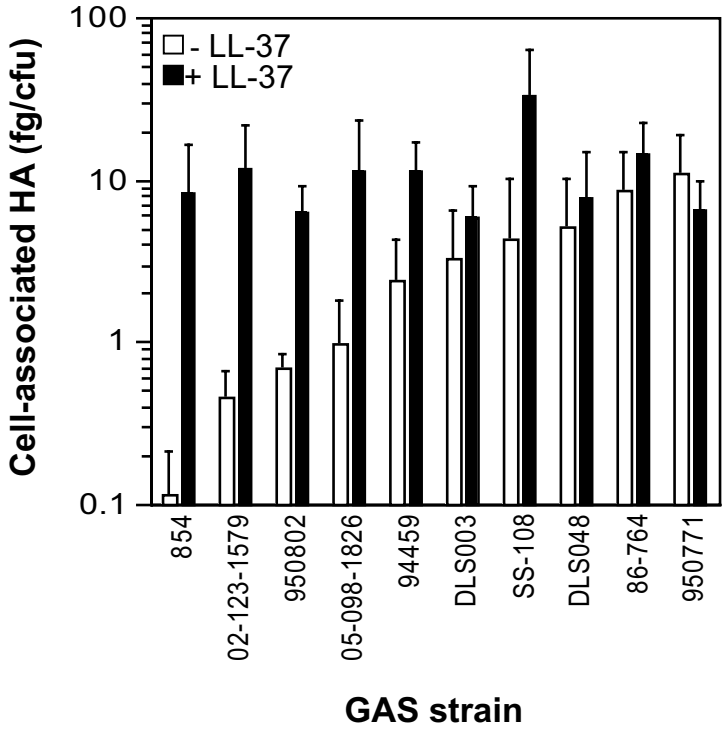

Fig. S3. Effect of LL-37 on HA capsule production in 10 wild-type GAS strains. Cell-associated HA was extracted from bacteria grown in the presence or absence of 100 nM LL-37. Note the marked increase in HA production in the presence of LL-37 for strains that express low amounts of capsule under standard growth conditions. Results shown are means ± SD from at least 3 independent experiments.

Table S1. AMPs tested for stimulation of *has* operon expression in GAS

Peptide	Description	Amino acid sequence	pI
LL-37	Human cathelicidin	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	10.6
RL-37	Rhesus cathelicidin	RLGNFFRKVKEKIGGGLKKVGQKIKDFLGNLVPRTAS	11.2
CRAMP	Mouse cathelicidin	GLLRKGGEKIGEKLKKIGQKIKNFFQKLVPQPEQ	10.2
SMAP29	Sheep cathelicidin	RGLRRLGRKIAHGVKKYGPTVLRIIRIAG	12.3
HNP1	Human α-defensin	ACYCRIPACIAGERRYGTCIYQGRLWAFCC	8.7
HNP3	Human α-defensin	DCYCRIPACIAGERRYGTCIYQGRLWAFCC	8.3
HNP4	Human α-defensin	VCSCRLVFCRRTELRVGNCLIGGVSFTYCCTRV	9.0
HD5	Human α-defensin	ATCYCRHGRCATRESLSGVCEISGRLYRLCCR	9.0
HD6	Human α-defensin	AFTCHCRRSCYSTEYSYGTCTVMGINHRFCCL	8.4
HBD1	Human β-defensin	GLGHRSDHYNCVSSGGQCLYSACPIFTKIQGTCYRGKAKCCK	9.1
HBD2	Human β-defensin	GIGDPVTCLKSGAICHPVFCPRRYKQIGTCGLPGTKCCKKP	9.3
HBD3	Human β-defensin	GIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK	10.1
RC-101	θ-defensin analog	Cyclic [GICRC ICGKG ICRCI CGR]	9.0
PG-1	Porcine protegrin	RGGRLCYCRRRFCVCVGR-NH_2	10.7

Table S2. GAS strains used in this study

Strain	M type	Clinical syndrome	Source
854	1	Retroperitoneal abscess	Brigham and Women's Hospital
854*csrS*Ω	1	N/A	This study
DLS048	1	Necrotizing fasciitis	D. Stevens, University of Washington
DLS003	3	Necrotizing fasciitis	D. Stevens, University of Washington
003CAT	3	N/A	1
003*csrS*Ω	3	N/A	2
003*csrS*Ω(pORI23)	3	N/A	3
003*csrS*Ω(pORI-*csrS*)	3	N/A	3
86-764	3	Rheumatic fever	E. Kaplan, University of Minnesota
950771	3	Necrotizing fasciitis	E. Kaplan, University of Minnesota
950802	3	Necrotizing fasciitis	E. Kaplan, University of Minnesota
94459	4	Necrotizing fasciitis	E. Kaplan, University of Minnesota
02-123-1579	5	Bacteremia	Children's Hospital Boston
SS-108	29	Unknown	R. Facklam, Centers for Disease Control and Prevention
05-098-1826	Unknown	Pharyngitis	Children's Hospital Boston

Table S3. Oligonucleotide primers used in this study

Primer*	Sequence (5′–3′)	Position†	Source
qRT-PCR			
rt0132-F (*SPy0170*)	ttatgatcgcaactgctgctg	23–43	3
rt0132-R	tcaggagcattttgtccgtag	128–108	3
rt0132-R4‡	ggaccagtgtgtccgtagaaa	137–117	This study
rt0298-F (*prtS*)	cgcgttagccttaaaacagc	4447–4466	3
rt0298-R	aggcaggctgacaacaacttc	4566–4546	3
rt0583-F (*mac*)	ccacagcagggaatatgcttca	290–311	3
rt0583-R	caaacatctgttcgccattg	406–387	3
rt0583-F3 (mac)§	gctaacgtacgcatcaacca	756–785	This study
rt0583-R3	ccagcggaattaacaccaac	911–892	This study
rt1800-F (*recA*)	tgattctggtgcggttgatc	282–301	3
rt1800-R	atttacgcatggcctgactc	415–396	3
rt1852-F (*hasB*)	tccccaaacgctaattgaag	825–844	3
rt1852-R	ttaaacggtaaaccccgact	952–933	3
rt-emm3-F (*emm*)	gctttagaagaagcaaacagca	1327–1348	This study
rt-emm3-R	tcagggatttgtgagtctgat	1535–1515	This study
emm typing			
emm1	tattcgcttagaaaattaa	28–46	Centers for Disease Control and Prevention
emm2	gcaagttcttcagcttgttt	1493–1474	Centers for Disease Control and Prevention

*Numbers for qRT-PCR primers indicate M-type 3 strain MGAS315 (4) or M-type 1 strain SF370 ORFs (5). F, forward; R, reverse.
†Nucleotide position relative to the start codon of ORFs on M-type 3 strain MGAS315 or M-type 1 strain SF370 chromosome.
‡Reverse primer specific to M-type 1 ORF SPy0170, used with primer rt0132-F for mRNA detection in M-type 1 strain 854.
§Primer pair rt0583-F3/rt0583-R3 used for *mac*-specific mRNA detection in strains 02-123-1579 (M5) and SS-108 (M29).