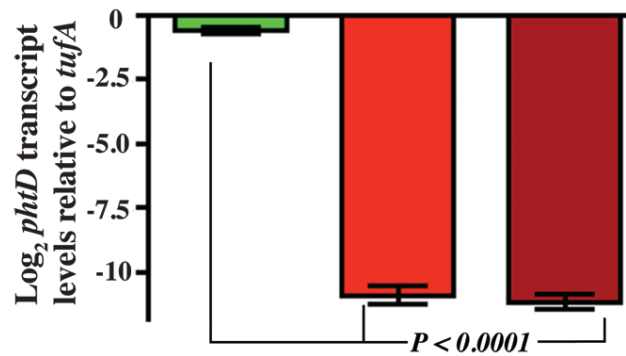
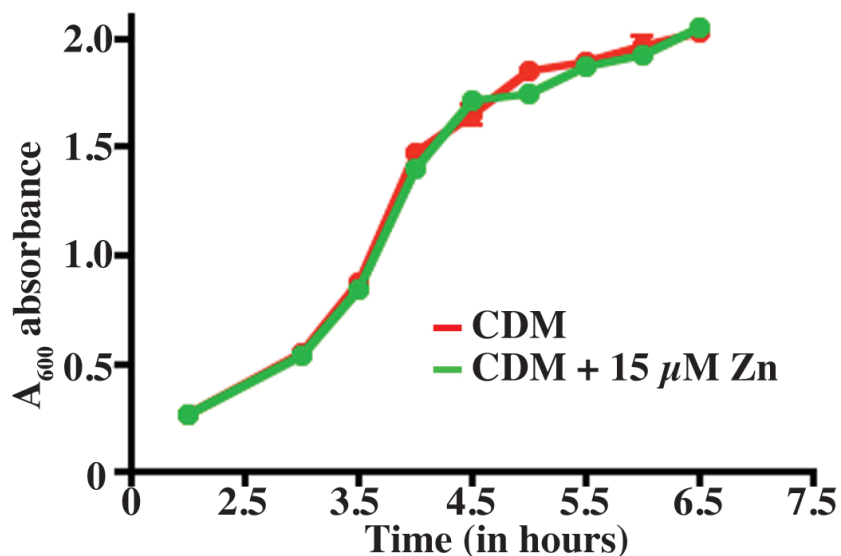


Supplementary figure S1

A)

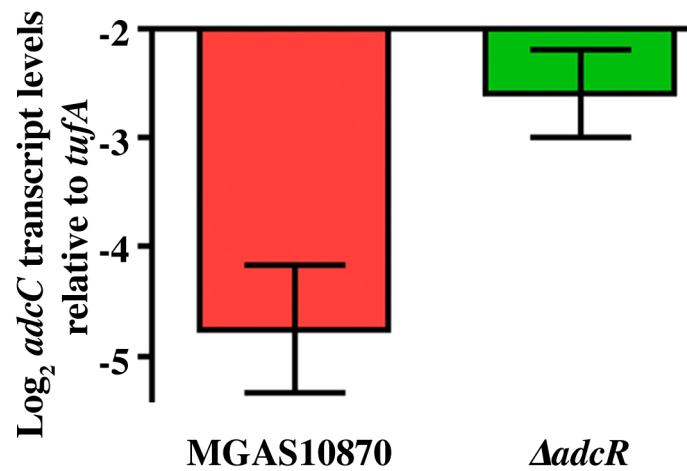


B)



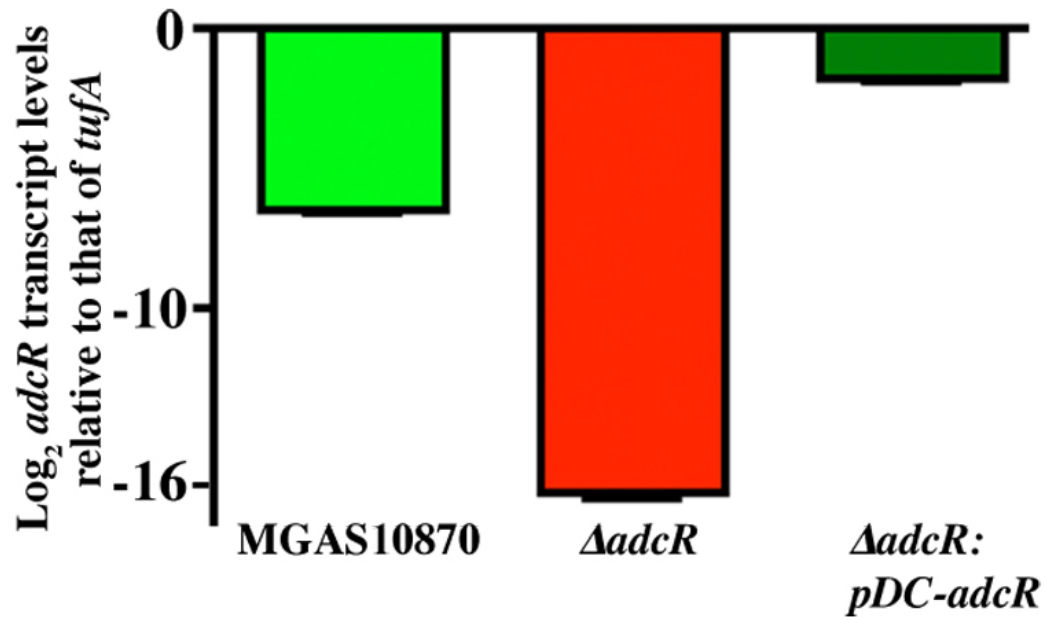
Supplementary figure S1. A) Transcript levels of *phtD* in strain MGAS10870 grown in chemically defined medium containing no (colored green), 10 μM (colored red), and 15 μM (colored dark red) zinc, as measured by qRT-PCR. Samples were collected at late-exponential ($A_{600} \sim 1.0$) phase of growth for transcript level analysis. Triplicate biological replicates were grown and analyzed in 3 replicates. Data graphed are mean \pm standard deviation. B) Growth pattern of GAS grown in CDM or CDM supplemented with 15 μM zinc.

Supplementary figure S2



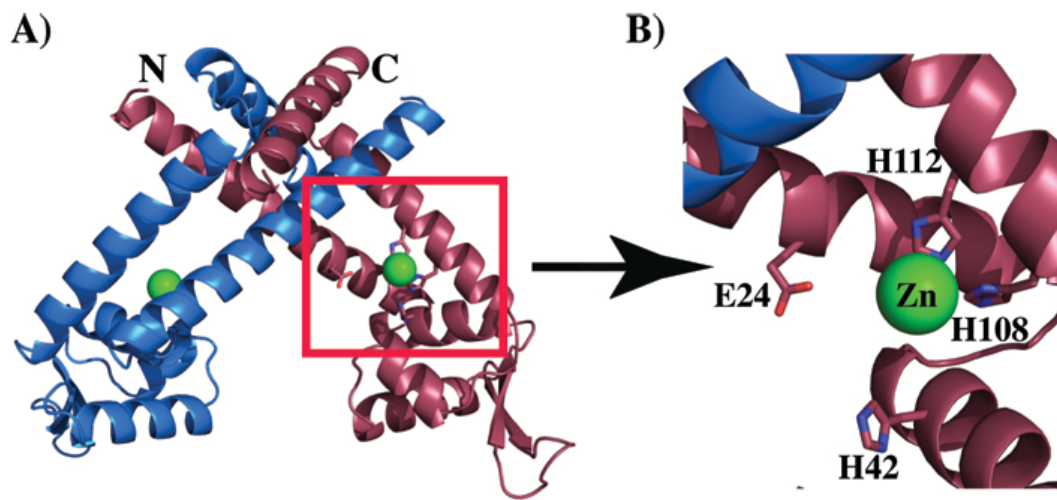
Supplementary figure S2. In-frame deletion of *adcR* gene in parental serotype MGAS10870 does not cause polar effect in the expression of downstream genes. Transcript levels of *adcC* in strain MGAS10870 and isogenic mutant Δ *adcR* strain as measured by qRT-PCR. Samples were collected at late-exponential ($A_{600} \sim 1.0$) phase of growth for transcript level analysis. Triplicate biological replicates were grown and analyzed in 3 replicates. Data graphed are mean \pm standard deviation.

Supplementary figure S3



Supplementary figure S3. Transcript levels of *adcR* in indicated strains as measured by qRT-PCR.

Supplementary figure S4



Supplementary Figure S4. A) Model of GAS AdcR dimer using the structure of AdcR from *S. pneumoniae* (PDB code – 3TGN). Individual subunits of AdcR are color coded in blue and pink. Zinc bound to the primary metal sensing site in the structure of pneumococcal AdcR is shown as a green sphere and the side chains of metal-coordinating amino acids are depicted in ball and stick representation. The N- and C-termini are labeled. B) A magnified view of the metal-binding site (boxed in red in panel A) with the metal binding ligands labeled.

Supplementary Table S1. Bacterial strains and plasmids used in this study.

Strain or plasmid	Description	Reference
Strains		
MGAS10870	Invasive isolate, serotype M3	(26)
		(Shelburne et
MGAS10870: <i>pDC</i>	MGAS10870 with empty vector, Cm ⁺	al., 2011)
Δ <i>adcR</i>	MGAS10870 Δ <i>adcR</i>	This study
Δ <i>adcR</i> : <i>pDC</i>	MGAS10870 Δ <i>adcR</i> , empty vector, Cm ⁺	This study
Δ <i>adcR</i> - <i>pDC</i> : <i>adcR</i>	MGAS10870 Δ <i>adcR</i> , <i>adcR</i> ⁺ , Cm ⁺	This study
Plasmids		
<i>pDC123</i>	Low-copy number plasmid capable of replication in GAS and <i>Escherichia coli</i> , Cm ⁺	(31)
<i>pDC</i> : <i>adcR</i>	<i>pDC123</i> with entire <i>adcR</i> gene plus promoter, Cm ⁺	This study
	Temperature-sensitive <i>E. coli</i> -Gram positive	
<i>pJL1055</i>	shuttle vector used for allelic replacement	(27,28)

Supplementary Table S2. Primers and probes used in this study

Primer	Sequence (5'-3')	Target
adcR-KO 5'	GAGATTTATGGGGACTTTAGAAGAATTAGAAGGG TAATCC	5' primer for 5' region of <i>adcR</i>
adcR-KO 3'	GGATTACCCTTCTAATTCTTCTAAAGTCCCCATAA ATCTC	3' primer for 5' region of <i>adcR</i>
adcR-For	TGCTTTGAGATGGGATCCCAATATAAAG	5' primer for 5' region of <i>adcR</i> to generate upstream flanking region
adcR-Rev	AACTTGTTCCACTCGAGTCGTGATGATC	3' primer for 3' region of <i>adcR</i> to generate downstream flanking region
pDCadcR-F	GGAAGATCTCTATTGTGATGAATCGCTGTC	5' <i>adcR</i> primer for complementation
pDCadcR-R	GGAATCCATATGCTAAAAATCAGGTTGGTC	3' <i>adcR</i> primer for full length <i>adcR</i> complementation
adcR qRT 5'	ATATTAGTCAGGCGGCAGTAAC	5' primer for <i>adcR</i> qRT-PCR
adcR qRT 3'	CACCCTAGCATCAACCGTATC	3' primer for <i>adcR</i> qRT-PCR
adcR probe	TGGTCAAACAAGACATGCTAGCAGGA	Probe for <i>adcR</i> qRT-PCR
tufA qRT 5'	CAACTCGTCACTATGCGCACAT	5' primer for <i>tufA</i> qRT-PCR
tufA qRT 3'	GAGCGGCACCAGTGATCAT	3' primer for <i>tufA</i> qRT-PCR
tufA probe	CTCCAGGACACGCGGACTACGTAAAAA	Probe for <i>tufA</i> qRT-PCR
phtD probe	CCAATTCCTGATGTGACGCCTAACCCCT	Probe for <i>phtD</i> qRT-PCR
phtD qRT 5'	GCCCCAGGTCGTAGGAAAG	5' primer for <i>phtD</i> qRT-PCR
phtD qRT 3'	CCGTTATCTGGCTGATGACCTT	3' primer for <i>phtD</i> qRT-PCR
adcC probe	TGCCCGTATGTTTGCTTCTGACCCTGATAT	Probe for <i>adcC</i> qRT-PCR
adcC qRT 5'	TGGTCAAAAACAACGTGTGGTTA	5' primer for <i>adcC</i> qRT-PCR
adcC qRT 3'	TTGTTGGCTCGTCTAGCACAA	3' primer for <i>adcC</i> qRT-PCR
hasA probe	CGCCATGCTCAAGCGTGGGC	Probe for <i>hasA</i> qRT-PCR

hasA qRT 5'	ACCGTTCCTTGTCAATAAAGG	5' primer for <i>hasA</i> qRT-PCR
hasA qRT 3'	CGTCAGCGTCAGATCTTTCAA	3' primer for <i>hasA</i> qRT-PCR
pET21bdc R-5'	GGGAATTCCATATGGACATTCATTCACATCAG	5' <i>perR</i> primer for the construction of overexpression plasmid
pET21bdc R-3'	CCGCTCGAGAAAATCAGGTTGGTCTTTTGCT	3' <i>perR</i> primer for the construction of overexpression plasmid

Supplementary Table S3. Genes that are differentially regulated in GAS during zinc starvation

Locus tag	Spy Number^a	Fold-change^b	Annotation^c
<u>Genes</u>			
<u>Downregulated</u>			
<u>Zinc homeostasis</u>			
<i>adcA</i>	SpyM3_0466	61.5	Extracellular zinc-binding subunit
<i>adcB</i>	SpyM3_0071	3.8	ABC transporter - permease
<i>adcC</i>	SpyM3_0070	3.3	ABC transporter – ATP-binding protein
<i>adcAII</i>	SpyM3_1725	364.0	Extracellular zinc-binding subunit
<i>phtD</i>	SpyM3_1724	341.2	Polyhistidine triad protein D
<i>phtY</i>	SpyM3_1035	198.5	Polyhistidine triad protein Y
<i>rpsN</i>	SpyM3_1615	156.1	30S ribosomal protein subunit S14
<u>Transporters</u>			
<i>dppA</i>	SpyM3_1718	7.3	Dipeptide surface lipoprotein
<i>dppB</i>	SpyM3_1719	7.3	Dipeptide ABC transporter permease
<i>dppC</i>	SpyM3_1720	6.0	Dipeptide ABC transporter permease
<i>dppD</i>	SpyM3_1721	6.2	Dipeptide ABC transporter ATP-binding subunit
<i>dppE</i>	SpyM3_1722	6.5	Dipeptide ABC transporter ATP-binding subunit
<i>mtsA</i>	SpyM3_0381	3.4	ABC transporter - Extracellular metal-binding subunit
<i>mtsB</i>	SpyM3_0391	4.1	ABC transporter - ATP-binding protein
<i>mtsC</i>	SpyM3_0320	3.5	ABC transporter – permease
<u>Genes upregulated</u>			
<u>Hypothetical proteins</u>			
<i>M3_0025</i>	SpyM3_0025	2.0	Choline binding protein
<i>M3_1666</i>	SpyM3_1666	2.2	Hypothetical protein

Supplementary Table S4. Genes that are upregulated in Δ *adcR*.

Locus tag	Spy Number^a	Fold-change^b	Annotation^c
<u>Zinc homeostasis</u>			
<i>adcA</i>	SpyM3_0466	15.6	Extracellular zinc-binding subunit
<i>adcB</i>	SpyM3_0071	4.3	ABC transporter - permease
<i>adcC</i>	SpyM3_0070	4.5	ABC transporter – ATP-binding protein
<i>adcAII</i>	SpyM3_1725	119.1	Extracellular zinc-binding subunit
<i>phtD</i>	SpyM3_1724	92.7	Polyhistidine triad protein D
<i>phtY</i>	SpyM3_1035	67.3	Polyhistidine triad protein Y
<i>rpsN</i>	SpyM3_1615	74.9	30S ribosomal protein subunit S14
<i>adh1</i>	SpyM3_0037	2.2	alcohol dehydrogenase
<i>adh2</i>	SpyM3_0036	3.5	alcohol dehydrogenase
<u>Transporters</u>			
<i>dppA</i>	SpyM3_1718	3.4	Dipeptide surface lipoprotein
<i>dppB</i>	SpyM3_1719	2.2	Dipeptide ABC transporter permease
<i>dppC</i>	SpyM3_1720	2.1	Dipeptide ABC transporter permease
<i>dppD</i>	SpyM3_1721	2.1	Dipeptide ABC transporter ATP-binding subunit
<i>dppE</i>	SpyM3_1722	2.2	Dipeptide ABC transporter ATP-binding subunit
<i>M3_0790</i>	SpyM3_0790	2.3	Na ⁺ driven multidrug efflux pump
<u>Virulence factors</u>			
<i>sagB</i>	SpyM3_0481	2.1	Streptolysin-S biosynthesis protein
<i>MF</i>	SpyM3_1745	3.9	Mitogenic factor 25K precursor
<i>sdn</i>	SpyM3_1409	3.9	Streptodornase – phage associated
<i>prsA</i>	SpyM3_1740	2.2	Foldase/prolyl-peptidyl isomerase
<u>Enzymes</u>			
<i>arcA</i>	SpyM3_1196	3.9	Arginine deaminase
<i>arcB</i>	SpyM3_1194	4.4	Ornithine carbamoyltransferase
<i>arcC</i>	SpyM3_1191	4.2	Carbamate kinase
<i>nagB</i>	SpyM3_1065	2.6	N-acetylglucosamine-6-phosphate isomerase
<i>M3_0788</i>	SpyM3_0788	4.1	Short chain

<i>M3_0788</i>	SpyM3_0788	4.1	Short chain dehydrogenase/oxidoreductase
<i>M3_0789</i>	SpyM3_0789	3.1	Short chain dehydrogenase/oxidoreductase
<u>Hypothetical proteins</u>			
<i>M3_1195</i>	SpyM3_1195	4.2	Hypothetical protein
<i>M3_1192</i>	SpyM3_1192	3.8	Hypothetical protein
<i>M3_1723</i>	SpyM3_1723	2.1	Hypothetical protein
<u>Others</u>			
<i>M3_0404</i>	SpyM3_0404	4.2	PTS system transporter subunit IIABC
thrs	SpyM3_0365	2.4	Threonyl-tRNA synthetase

Supplementary Table S5. Genes that are downregulated in *ΔadcR*

Locus tag	Spy Number^a	Fold-change^b	Annotation^c
<u>Zinc</u>			
<u>homeostasis</u>			
<i>adcR</i>	SpyM3_0069	717.5	Adhesin-competence repressor
<u>Transporters</u>			
<i>cadX</i>	SpyM3_1819	2.8	Cadmium efflux pump accessory protein
<i>cadD</i>	SpyM3_1818	2.2	Cadmium resistance protein
<i>fhuC.2</i>	SpyM3_1558	2.5	Ferrichrome ABC transporter–ATP-binding protein
<i>fhuG.2</i>	SpyM3_1559	2.6	Ferrichrome ABC transporter– permease
<i>M3_0655</i>	SpyM3_0655	2.3	ABC transporter–permease
<i>M3_0656</i>	SpyM3_0656	2.2	ABC transporter–ATP-binding protein
<u>Virulence</u>			
<u>Factors</u>			
<i>speA3</i>	SpyM3_1301	3.3	Exotoxin A precursor – phage associated
<i>scpA</i>	SpyM3_1726	3.3	C5A peptidase precursor
<i>hasA</i>	SpyM3_1851	14.9	Hyaluronate synthase
<i>hasB</i>	SpyM3_1852	15.5	UDP-glucose-6-dehydrogenase
<i>hasC</i>	SpyM3_1853	14.0	UDP-glucose-pyrophosphorylase
<i>prtS</i>	SpyM3_0298	28.2	Cell envelope proteinase
	SpyM3_1698	4.8	Streptokinase A precursor
<i>ska</i>			
<u>Enzymes</u>			
<i>Nga</i>	SpyM3_0128	2.1	NAD glycohydrolase
<i>adk</i>	SpyM3_0061	2.4	Adenylate kinase
<i>purF</i>	SpyM3_0021	3.1	Amidophosphoribosyltransferase
<u>Ribosomal</u>			
<u>proteins</u>			
<i>rplN</i>	SpyM3_0050	2.2	50S ribosomal protein subunit L14
<i>rplX</i>	SpyM3_0051	2.1	50S ribosomal protein subunit L24
<i>rplF</i>	SpyM3_0055	2.0	50S ribosomal protein subunit L6
<i>rpmC</i>	SpyM3_0048	2.0	50S ribosomal protein subunit L29
<u>Hypothetical</u>			
<u>proteins</u>			
<i>M3_0105</i>	SpyM3_0105	3.1	Hypothetical protein
<i>M3_0129</i>	SpyM3_0129	2.0	Hypothetical protein
<i>M3_0305</i>	SpyM3_0305	3.4	Hypothetical protein
<i>M3_0307</i>	SpyM3_0307	2.5	Hypothetical protein
<i>M3_0583</i>	SpyM3_0583	6.6	Hypothetical protein
<i>M3_1414</i>	SpyM3_1414	3.2	Hypothetical protein
<i>M3_1415</i>	SpyM3-1415	2.5	Hypothetical protein
<i>M3_1417</i>	SpyM3_1417	2.4	Hypothetical protein
<i>M3_1424</i>	SpyM3_1424	3.1	Hypothetical protein

<i>M3_1424</i>	SpyM3_1424	3.1	Hypothetical protein
<i>M3_1425</i>	SpyM3_1425	3.2	Hypothetical protein
<i>M3_1428</i>	SpyM3_1428	2.1	Hypothetical protein
<i>M3_1429</i>	SpyM3_1429	2.6	Hypothetical protein
<i>M3_1430</i>	SpyM3_1430	2.9	Hypothetical protein
<i>M3_1434</i>	SpyM3_1434	2.1	Hypothetical protein
<i>M3_1683</i>	SpyM3_1683	4.6	Hypothetical protein
<i>M3_1820</i>	SpyM3_1820	2.5	Hypothetical protein
<i>M3_1821</i>	SpyM3_1821	2.7	Hypothetical protein
<i>M3_1822</i>	SpyM3_1822	2.6	Hypothetical protein
Other			
<i>murM.2</i>	SpyM3_0846	2.5	Anti resistance factor
<i>M3_0157</i>	SpyM3_0157	2.1	Regulatory protein RofA related
<i>M3_1208</i>	SpyM3_1208	2.4	Cell wall hydrolase-phage associated
<i>M3_1421</i>	SpyM3_1421	2.3	Tail protein-phage associated
<i>M3_1436</i>	SpyM3_1436	2.7	Terminase small subunit-phage associated