Supplementary Data for

RNA thermoswitches modulate *Staphylococcus aureus* adaptation to ambient temperatures

Arancha Catalan-Moreno[1], Marta Cela[1], Pilar Menendez-Gil[1], Naiara Irurzun[1], Carlos J. Caballero[1], Isabelle Caldelari[2] and Alejandro Toledo-Arana[1]*

[1]Instituto de Agrobiotecnología. IDAB, CSIC-UPNA-Gobierno de Navarra. 31192-Mutilva, Navarra, Spain.
[2]Université de Strasbourg, CNRS, Architecture et Réactivité de l'ARN, UPR9002, F-67000-Strasbourg, France

*Corresponding author: Laboratory of Bacterial Gene Regulation, Instituto de Agrobiotecnología, CSIC-UPNA-Gobierno de Navarra, Avda. de Pamplona 123, 31192-Mutilva, Navarra, Spain
E-mail: a.toledo.arana@csic.es
Phone: +34 948 16 9752

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Supplementary References

SUPPLEMENTARY TABLES

Table S1. Strains used in this study

Strains	Relevant characteristic(s)	BGR ID [a]	Source or reference
Staphylococcus aureus			
15981	Wild type (WT) strain. MSSA clinical isolate; biofilm positive; PNAG-dependent biofilm matrix	8	(3)
cspA^{3xF}	15981 strain expressing the chromosomic 3xFLAG-tagged CspA protein	239	(4)
cspB^{3xF}	15981 strain expressing the chromosomic 3xFLAG-tagged CspB protein	346	This study
cspC^{3xF}	15981 strain expressing the chromosomic 3xFLAG-tagged CspC protein	240	This study
WT p5'UTR^{cspB}-*gfp*	15981 carrying the p5'UTR^{cspB}-*gfp* plasmid	1570	This study
WT p5'UTR^{cspC}-*gfp*	15981 carrying the p5'UTR^{cspC}-*gfp* plasmid	1398	This study
WT p5'UTR^{cspB}Δ24-*gfp*	15981 carrying the p5'UTR^{cspB}Δ24-*gfp* plasmid	1555	This study
WT p5'UTR^{cspC}Δ24-*gfp*	15981 carrying the p5'UTR^{cspC}Δ24-*gfp* plasmid	1429	This study
WT p5'UTR^{cspB}UAU47AA-*gfp*	15981 carrying the p5'UTR^{cspB}UAU47AA-*gfp* plasmid	1623	This study
WT p5'UTR^{cspB}C50G-*gfp*	15981 carrying the p5'UTR^{cspB}C50G-*gfp* plasmid	1579	This study
WT p5'UTR^{cspB}UU55AA-*gfp*	15981 carrying the p5'UTR^{cspB}UU55AA-*gfp* plasmid	1517	This study
WT p5'UTR^{cspC}UU48A-*gfp*	15981 carrying the p5'UTR^{cspC}UU48A-*gfp* plasmid	1415	This study
WT p5'UTR^{cspB} UU55AA+UU26AA-*gfp*	15981 carrying the p5'UTR^{cspB}UU55AA+UU26AA-*gfp* plasmid	1902	This study
WT p5'UTR^{cspB}U38C+U41C-*gfp*	15981 carrying the p5'UTR^{cspB}p5'UTR^{cspB}U38C+U41C-gfp -*gfp* plasmid	2092	This study
Δ*cspA* p5'UTR^{cspB}-*gfp*	15981 Δ*cspA* strain carrying the p5'UTR^{cspB}-*gfp* plasmid	1571	This study
Δ*cspA* p5'UTR^{cspC}-*gfp*	15981 Δ*cspA* strain carrying the p5'UTR^{cspC}-*gfp* plasmid	1408	This study
Δ*cspB* p5'UTR^{cspB}-*gfp*	15981 Δ*cspB* strain carrying the p5'UTR^{cspB}-*gfp* plasmid	1572	This study
Δ*cspC* p5'UTR^{cspC}-*gfp*	15981 Δ*cspC* strain carrying the p5'UTR^{cspC}-gfp plasmid	1561	This study
cspB^{3xF} Δ*cspA*	15981 Δ*cspA* strain expressing the chromosomic 3xFLAG-tagged CspB protein	1324	This study
cspC^{3xF} Δ*cspA*	15981 Δ*cspA* strain expressing the chromosomic 3xFLAG-tagged CspC protein	725	This study
Δ*cspA* p5'UTR^{cspB}Δ24-*gfp*	15981 Δ*cspA* strain carrying the p5'UTR^{cspB}Δ24-*gfp* plasmid	1566	This study
Δ*cspA* p5'UTR^{cspB}UAU47AA-*gfp*	15981 Δ*cspA* strain carrying thep5'UTR^{cspB}UAU47AA-*gfp* plasmid	2028	This study
Δ*cspA* p5'UTR^{cspB}C50G-*gfp*	15981 Δ*cspA* strain carrying the p5'UTR^{cspB}C50G-*gfp* plasmid	1928	This study
Δ*cspA* p5'UTR^{cspB}UU55AA-*gfp*	15981 Δ*cspA* strain carrying the p5'UTR^{cspB}C50G-*gfp* plasmid	1928	This study
Δ*cspA* p5'UTR^{cspC}Δ24-*gfp*	15981 Δ*cspA* strain carrying the p5'UTR^{cspC}Δ24-*gfp* plasmid	1516	This study
Δ*cspA* p5'UTR^{cspC}UU48A-*gfp*	15981 Δ*cspA* strain carrying the p5'UTR^{cspC}UU48A-*gfp* plasmid	1416	This study
Δ*cspA* p5'UTR^{cspB} UU55AA+UU26AA-*gfp*	15981 Δ*cspA* strain carrying the p5'UTR^{cspB}UU55AA+UU26AA-*gfp* plasmid	1749	This study
Δ*cspA* p5'UTR^{cspB}U38C+U41C-*gfp*	15981 Δ*cspA* strain carrying the p5'UTR^{cspB}U38C+U41C-*gfp* plasmid	2093	This study
Δ*cspB*	15981 strain with deletion of the *cspB* gene	1150	(5)
Δ*cspC*	15981 strain with deletion of the *cspC* gene	195	(5)
Δ*cspBC*	15981 strain with deletion of the *cspB* and *cspC* genes	1251	This study

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Table S1. *Continued*

Strains	Relevant characteristic(s)	BGR ID [a]	Source or reference [b]
Δ24cspB	15981 strain harbouring a chromosomic deletion of the first 24 nucleotides of the *cspB* mRNA	1975	This study
Δ24cspC	15981 strain harbouring a chromosomic deletion of the first 24 nucleotides of the *cspC* mRNA	1987	This study
Δ24cspB^{3xF}	15981 *cspB*^{3xF} strain harbouring a deletion of the first 24 nucleotides of the *cspB* mRNA	1991	This study
Δ24cspC^{3xF}	15981 *cspC*^{3xF} strain harbouring a deletion of the first 24 nucleotides of the *cspC* mRNA	1992	This study
Δ24cspBC	15981 strain harbouring a deletion of the first 24 nucleotides of the *cspB* and *cspC* mRNAs	1917	This study
Escherichia coli			
XL1-Blue	Strain used for cloning experiments	1	Stratagene
IMO1B	Strain used for cloning experiments	1837	(6)

[a] Identification number of the strains stored at the Laboratory of Bacterial Gene Regulation.

Table S2. Plasmids used in this study

Plasmids	Relevant characteristic(s)	Source or reference
pMAD_*cspB*^{3xF}	pMAD plasmid containing the allele for insertion of the 3xFLAG at the C-terminus of the CspB protein	This study
pMAD_*cspC*^{3xF}	pMAD plasmid containing the allele for insertion of the 3xFLAG at the C-terminus of the CspB protein	This study
pCN57	*E. coli-S. aureus* shuttle vector carrying the promoter-less *gfp*mut2 reporter gene. Amp^{R}-Erm^{R}	(7)
pCN47	*E. coli-S. aureus* shuttle vector for cloning. Amp^{R}-Erm^{R}	(7)
pHRG	pCN47 plasmid containing the P*hyper* constitutive promoter, *icaR* RBS and *gfp* reporter gene	This study
p5'UTR^{cspB}-*gfp*	pHRG translation-reporter plasmid carrying the *cspB* 5'UTR fused to the *gfp* reporter gene	This study
p5'UTR^{cspC}-*gfp*	pHRG translation-reporter plasmid carrying the *cspC* 5'UTR fused to the *gfp* reporter gene	This study
p5'UTR^{cspB}Δ24-*gfp*	pHRG translation reporter plasmid carrying the *cspB* 5'UTR that lacks the first 24 nt	This study
p5'UTR^{cspC}Δ24-*gfp*	pHRG translation reporter plasmid carrying the *cspC* 5'UTR that lacks the first 24 nt	This study
p5'UTR^{cspB}UAU47AA-*gfp*	pHRG translation reporter plasmid carrying the *cspB* 5'UTR in which nucleotides 47-UAU-49 were replaced by AA	This study
p5'UTR^{cspB}C50G-*gfp*	pHRG translation reporter plasmid carrying the *cspB* 5'UTR in which nucleotide 50C was substituted by G	This study
p5'UTR^{cspB}UU55AA-*gfp*	pHRG translation reporter plasmid carrying the *cspB* 5'UTR in which nucleotides 55-UU-56 were substituted by AA	This study
p5'UTR^{cspC}UU48A-*gfp*	pHRG translation reporter plasmid carrying the *cspC* 5'UTR in which nucleotides 48-UU-49 were substituted by A	This study
p5'UTR^{cspB}UU55AA+UU26 AA-*gfp*	p5'UTR^{cspB}UU55AA-*gfp* plasmid carrying an additional mutation in which nucleotides 26-UU-27 were substituted by AA	This study
p5'UTR^{cspB}UU26AA-*gfp*	pHRG translation reporter plasmid carrying the *cspB* 5'UTR in which nucleotides 26-UU-27 were substituted by AA	This study
p5'UTR^{cspB}U38C+U41C-*gfp*	pHRG translation reporter plasmid carrying the *cspC* 5'UTR in which 38U and 41U were substituted by C	This study
pMAD	*E. coli-S. aureus* shuttle vector with a thermosensitive origin of replication for Gram-positive bacteria. It contains the *bgaB* gene that encodes β-galactosidase under the control of a constitutive promoter. Amp^{R}-Erm^{R}	(8)
pMAD_Δ*cspA*	pMAD plasmid containing the allele for the deletion of the *cspA* gene	(4)
pMAD_Δ*cspB*	pMAD plasmid containing the allele for the deletion of the *cspB* gene	(5)
pMAD_Δ*cspC*	pMAD plasmid containing the allele for the deletion of the *cspC* gene	(5)
pMAD_Δ*24cspB*	pMAD plasmid containing the allele for the deletion of the first 24 nucleotides of the *cspB* gene	This study
pMAD_Δ*24cspC*	pMAD plasmid containing the allele for the deletion of the first 24 nucleotides of the *cspC* gene	This study
pMAD_Δ*24cspB*^{3xF}	pMAD plasmid containing the allele for the deletion of the first 24 nucleotides of the *cspB*^{3xF} gene	This study
pMAD_Δ*24cspC*^{3xF}	pMAD plasmid containing the allele for the deletion of the first 24 nucleotides of the *cspC*^{3xF} gene	This study

Table S3. Oligonucleotides used in this study

Oligonucleotide name	Sequence [a]
Construction of pMAD plasmid for 24 deletion of *cspB* gene	
CspB_A EcoRI	*GAATTC*AACTTGGTATAACGTCATTG
CspB_D24_Izq	AAGACCAACTATACGCTCAT
CspB_D24_Drcha	ATGAGCGTATAGTTGGTCTTATTGTAGTGTATTTGTTTAGAATATCCT
CspB_D BamHI	GGATCCTTAGTTGTTTATTGGAATTG
Construction of pMAD plasmid for 24 deletion of *cspC* gene	
CspC_A BglII	*AGATCT*TTAGTTCGTCAAGGCTTGG
CspC_D24_Izq	AACTTTCATTATACACTTTT
CspC_D24_Drcha	AAAAGTGTATAATGAAAGTTATGTGAGTTATTTATATAGAATATTCTC
CspC_D BamHI	*GGATCC*CTCAATAATTAATCAGTCTTAA
Construction of pMAD plasmid for chromosomic 3xFLAG-labelling of the *cspB* gene	
cspB_A EcoRI	*GAATTC*AACTTGGTATAACGTCATTG
3xFcspB_B	TTATAATCACCGTCATGGTCTTTGTAGTCAACAGTTTGTACGTTAACTGC
3xFcspB_C	GACTACAAAGACCATGACGGTGATTATAAAGATCATGATATCGACTACA AAGATGACGACGATAAATAATCTTACAACATAAAACGACTCATTA
CspB_D BamHI	*GGATCC*TTAGTTGTTTATTGGAATTG
Construction of pMAD plasmid for chromosomic 3xFLAG-labelling of the *cspC* gene	
CspC_A BglII	*AGAT*CTTTAGTTCGTCAAGGCTTGG
3xFcspC_B	ACCGTCATGGTCTTTGTAGTCCATTTTAACTACGTTTGCAGCTT
3xFcspC_C	GACTACAAAGACCATGACGGTGATTATAAAGATCATGATATCGACTACA AAGATGACGACGATAAATAATTTTAACTTATTCAAACAGT
CspC_D BamHI	*GGATCC*CTCAATAATTAATCAGTCTTAA
Construction of pHRG plasmid	
pHyper-RBSicaR-GFP	*GCATGC*AATTTTGCAAAAAGTTGTTGACTTTATCTACAAGGTGTGGCATA ATGAATTCAGTAGGGGGTTATAAAAATTGACTAGTAAAGGAGAAGAACT TTTCACT
GFPend-AscI	*GGCGCG*CCTTATTTGTATAGTTCATCCATGCCAT
Construction of plasmids expressing 5'UTR *cspB and cspC* mRNA and its mutants	
5UTR_cspB_FW_EcoRI	*GAATTC*ACGTAATAAAAGCTCGTGAAT
5UTR_cspB_RV_SpeI	*ACTAGT*TGTACCGTTATTCATATAGAAAACC
5'UTR_cspC_FW_EcoRI	*GAATT*CAAGTAATAAAGAGCGTGAAGAAA
5'UTR_cspC_RV_SpeI	*ACTAGT*TGTACCGTTATTCATATTGAATACC
M5B_D24_EcoRI	*GAATTC*ATTGTAGTGTATTTGTTTAGAATATCC
M5C_D24_EcoRI	*GAATTC*ATGTGAGTTATTTATATAGAATATTCTCCT
M5B_C50G_EcoRI	*GAATTC*ACGTAATAAAAGCTCGTGAATTAAATTGTAGTGTATTTGTTTAG AATATGCTCTTTTTTAGTTATGAAT
M5B_UU55AA_EcoRI	*GAATTC*ACGTAATAAAAGCTCGTGAATTAAATTGTAGTGTATTTGTTTAG AATATCCTCTAATTTAGTTATGAAT
M5B_UAU47AA_EcoRI	*GAATTCACGTAATAAAAGCTCGTGAAT*TAAATTGTAGTGTATTTGTTTAG AAAACCTCTTTTTT
M5C_UU48A_EcoRI	*GAATTC*AAGTAATAAAGAGCGTGAAGAAAAATGTGAGTTATTTATATAGA ATAACTCCTTTTCATT
M5B_2U_26_2_EcoRI	*GAATTC*ACGTAATAAAAGCTCGTGAATTAAAAAGTAGTGTATTTG
M5B_U38C-U41C_EcoRI	*GAATTC*ACGTAATAAAAGCTCGTGAATTAAATTGTAGTGTATTCGTCTAG AATATCCT
Probe for Northen blot assays	
anti-GFP probe	TTATTTGTATAGTTCATCCATGCCATGTGTAATCCCAGCAGCTGTTACAA ACTCAAGAAGGACCATGTGG
anti_3xFLAG_probe	TTTATCGTCGTCATCTTTGTAGTCGATATCATGATCTTTATAATCACCGT CATGGTCTTTGTAGTC

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Table S3. *Continued*

Oligonucleotide name	Sequence [a]
Molecular beacons	
5UTR_B_FAM_IQ	FAM_CGTAATAAAAGCTCGTGAATTAAATTGTAGTGTATTTGTTTAGAAT ATCCTCTTTTTTAGTTATGAATTTGTTACA_IQ
5UTR_C_FAM_IQ	FAM_AGTAATAAAGAGCGTGAAGAAAAATGTGAGTTATTTATATAGAATA TTCTCCTTTTCATTTATGAATTTGTTACA_IQ
Synthesis of *cspB* 5'UTR mRNA	
T7_5UTRcspB_Fw	TAATACGACTCACTATAGGGACGTAATAAAAGCTCGTGAA
5UTR_cspB_RV_SpeI	ACTAGTTGTACCGTTATTCATATAGAAAACC
Synthesis of *cspC* 5'UTR mRNA	
T7_5UTRcspC_fw	TAATACGACTCACTATAGGGAAGTAATAAAGAGCGTGAAG
5'UTR_cspC_RV_SpeI	ACTAGTTGTACCGTTATTCATATTGAATACC

[a] Restriction enzymes sites and 3xFLAG sequences are indicated in italic and underlined font, respectively

Table S4. Blastn comparative analysis showing the conservation of the *S. aureus* CspB thermoswitch among different *Staphylococcus* species.

Staphylococcus species	Strain	Accession ID	Total CSPs	Thermo switch	Locus tag ID	*csp* genes including a thermoswitch / Sequence identity to *cspB* 5'UTR (%)			
S. aureus	NCTC 8325	NC_007795.1	3	2		*cspB*	100	*cspC*	85
S. argenteus	MSHR1132	NC_016941.1	3	2	SAMSHR1132_	*RS13405*	100	*RS03890*	85
S. schweitzeri	NCTC13712	NZ_LR134304.1	3	2	EL116_	*RS13645*	98	*RS04340*	87
S. simiae	NCTC13838	NZ_LT906460.1	3	2	CKV88_	*RS12300*	96	*RS03665*	85
S. succinus	14BME20	NZ_CP018199.1	3	2	BK815	*RS09355*	94	*RS08380*	75
S. devriesei	NCTC13828	UHCZ01000002	3	2	DYD94_	*RS10710*	89	*RS10715*	87
S. pasteuri	SP1	NC_022737.1	3	2	STP1_	*RS07870*	88	*RS09745*	83
S. warneri	NCTC11044	NZ_LR134269.1	3	2	EL082_	*RS11195*	88	*RS09370*	83
S. cornubiensis	NW1	FXUZ01000007	3	2	CCE82_	*RS08550*	88	*RS08575*	87
S. felis	ATCC 49168	NZ_CP027770.1	3	2	C7J90_	RS03590	87	*RS00805*	77
S. massiliensis	CCUG 55927	NZ_JH815593.1	3	2	A33S_	*RS0106390*	93	*RS0107335*	89
S. edaphicus	CCM 8730	MRZN01000034	3	1	BTJ66_	*RS13275*	94		
S. saprophyticus	ATCC 15305	NC_007352.1	3	1	SSP_	*RS12515*	93		
S. stepanovicii	NCTC13839	NZ_LT906462.1	3	1	CKV64_	*RS00450*	93		
S. petrasii	NCTC13835	UHDU01000001	2	1	DYD86_	*RS11030*	91		
S. haemolyticus	JCSC1435	NC_007168.1	2	1	SH_	*RS11570*	91		
S. lutrae	ATCC 700373	NZ_CP020773.1	2	1	B5P37_	*RS08760*	89		
S. pseudintermedius	HKU10-03	NC_014925.1	2	1	SPSINT_	*RS03305*	88		
S. delphini	NCTC12225	NZ_LR134263.1	2	1	EL101_	*RS10200*	88		
S. intermedius	NCTC 11048	UHDP01000003	2	1	DYA52_	*RS11680*	88		
S. lugdunensis	HKU09-01	NC_013893.1	2	1	SLGD_	*RS00875*	87		
S. hominis	C80	NZ_GL545254.1	2	1	HMPREF0798_	*RS00715*	87		
S. schleiferi	2317-03	NZ_CP010309.1	2	1	RN70_	*RS09475*	88		
S. fleurettii	FDAARGOS_682	NZ_CP046351.1	2	1	FOB90_	*RS10505*	88		
S. caprae	26D	NZ_CP031271.1	2	1	DWB96_	*RS10220*	84		
S. saccharolyticus	NCTC11807	UHDZ01000001	2	1	DYE57_	*RS09170*	83		
S. auricularis	NCTC12101	NZ_LS483491.1	2	1	DQL57_	*RS02425*	83		
S. epidermidis	ATCC 14990	NZ_CP035288.1	2	1	EQW00_	*RS09740*	83		
S. capitis	AYP1020	NZ_CP007601.1	2	1	ayp1020_	*RS00755*	83		
S. chromogenes	20B	NZ_CP031471.1	2	1	DWB92_	*RS09990*	81		
S. hyicus	ATCC 11249	NZ_CP008747.1	2	1	SHYC_	*RS09620*	80		
S. agnetis	908	NZ_CP009623.1	2	1	EP23_	*RS00105*	80		
S. rostri	DSM 21968	PPRF01000144	2	1	CD122_	*RS11505*	80		
S. muscae	ATCC 49910	NZ_CP027848.1	2	1	C7J88_	*RS09350*	80		
S. microti	DSM 22147	JXWY01000057	2	1	TP70_	*RS08325*	80		
S. equorum	KS1039	NZ_CP013114.1	2	1	SE1039_	*RS00970*	77		
S. nepalensis	JS11	NZ_CP017466.1	2	1	BJG89_	*RS13175*	78		
S. carnosus	TM300	NC_012121.1	2	1	SCA_	*RS02225*	76		
S. piscifermentans	NCTC13836	NZ_LT906447.1	2	1	CKV71_	*RS10125*	76		
S. condimenti	DSM 11674	NZ_CP015114.1	2	1	A4G25_	*RS05475*	76		
S. gallinarum	DSM 20610	JXCF01000001	2	1	SH09_	*RS00140*	75		
S. cohnii	532	LATV01000001	2	1	XA21_	*RS00240*	76		
S. xylosus	SMQ-121	NZ_CP008724.1	2	1	SXYLSMQ121_	*RS11945*	74		
S. pettenkoferi	VCU012	AGUA01000053	1	0					
S. kloosii	NCTC12415	UHDQ01000002	1	0					

Table S5. Prediction of putative mutually exclusive alternative structures in the 5'UTRs of *csp* genes.

Bacterial species	*csp* gene	5'UTR length (nt)[a]	Alternative structures
***Enterococcus faecalis* V583**	**(NC_004668)**		
	EF0781	116	
	EF1367	134	√
	EF1726	32	
	EF1991	117	√
	EF2925	109	
	EF2939	ND	
***Bacillus subtilis* 168**	**(NC_000964)**		
	cspC	115	
	cspB	119	√
	cspD	85	
***Clostridium perfringens* str. 13**	**(NC_003366)**		
	cspL		√
***Pseudomonas aeruginosa* PA14**	**(NC_008463)**		
	PA14_05960 (cspB)	151	√
	PA14_21760 (capB)	151	√
	PA14_30200 (cspD)	109	
	PA14_39180	ND	
	PA14_49410	ND	
	PA14_51840	ND	
***Salmonella* Typhimurium str. SL1344**	**(NC_016810)**		
	cspA	164	√
	cspB	145	√
	cspC	ND	
	cspD	87	
	cspE	43	
	cspH	23	

[a] 5'UTR length annotated using previous transcriptomic data (1, 2). ND: not determined.

SUPPLEMENTARY FIGURES

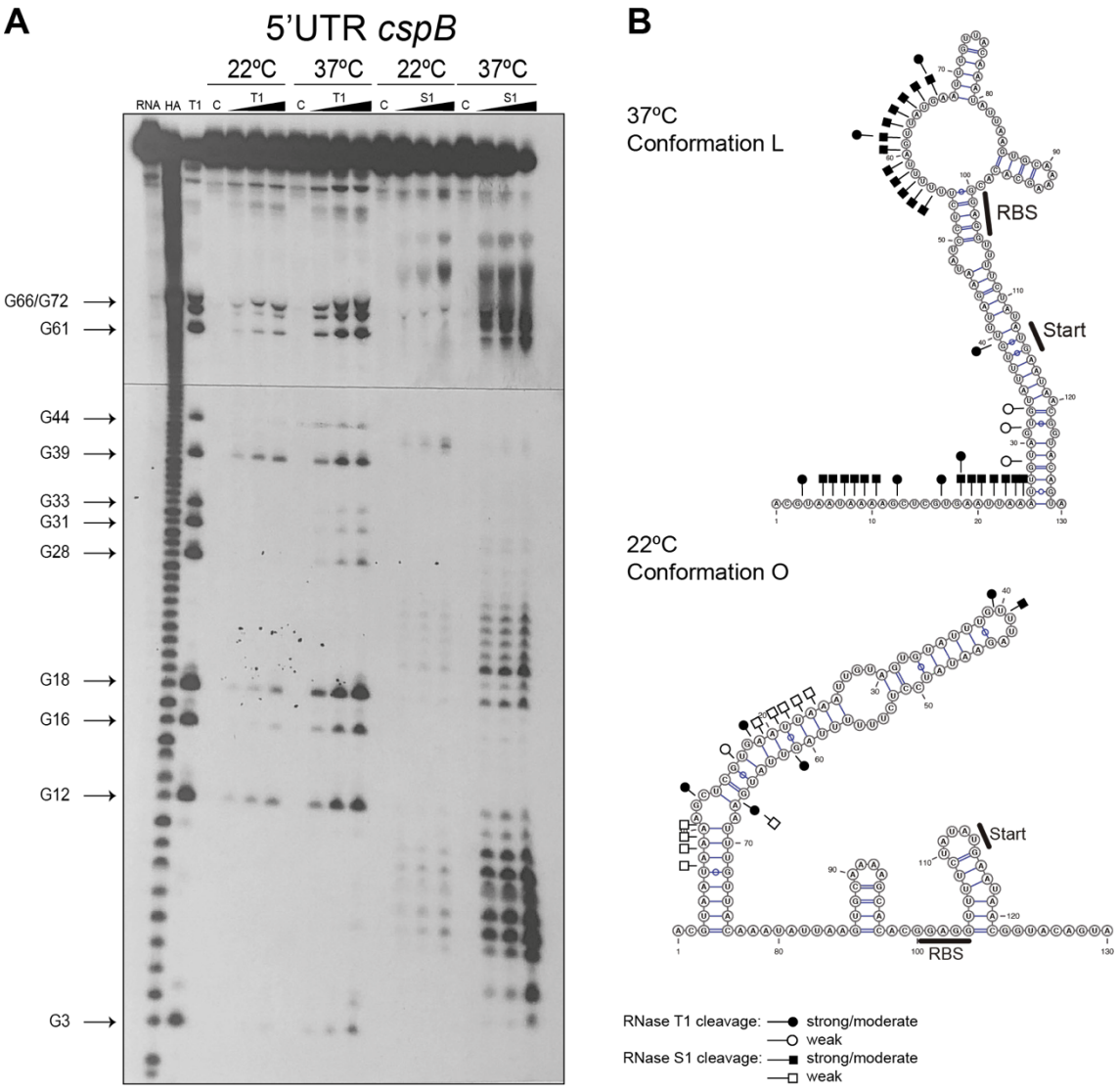

Figure S1. Enzymatic probing at different temperatures of the alternative structures found in the *cspB*. (**A**) Electrophoretic migration of the radiolabelled *cspB* 5'UTR after RNase T1 (T1) and RNAse S1 (S1) cleavage at 22 and 37ºC. Samples were subjected to denaturation at 90ºC and renaturation at 22 or 37ºC for 15 min. Lane C: incubation control in the absence of RNase T1/S1. Cleavage reactions were performed in the presence of increasing concentrations of T1: $1x10^{-3}$ U/µl, $2x10^{-2}$ U/µl and $5x10^{-2}$ U/µl or S1:$5x10^{-5}$ U/µl, $2x10^{-5}$ U/µl and $1x10^{-5}$ U/µl. Lanes RNA: RNA control; HA: alkaline hydrolysis ladder and T1: RNAse T1 control. (**B**) Model of *cspB* 5'UTR secondary structure at 37ºC (conformation L) and 22ºC (conformation O) derived from the probing data shown in A. The RBS sequence and start codon are indicated.

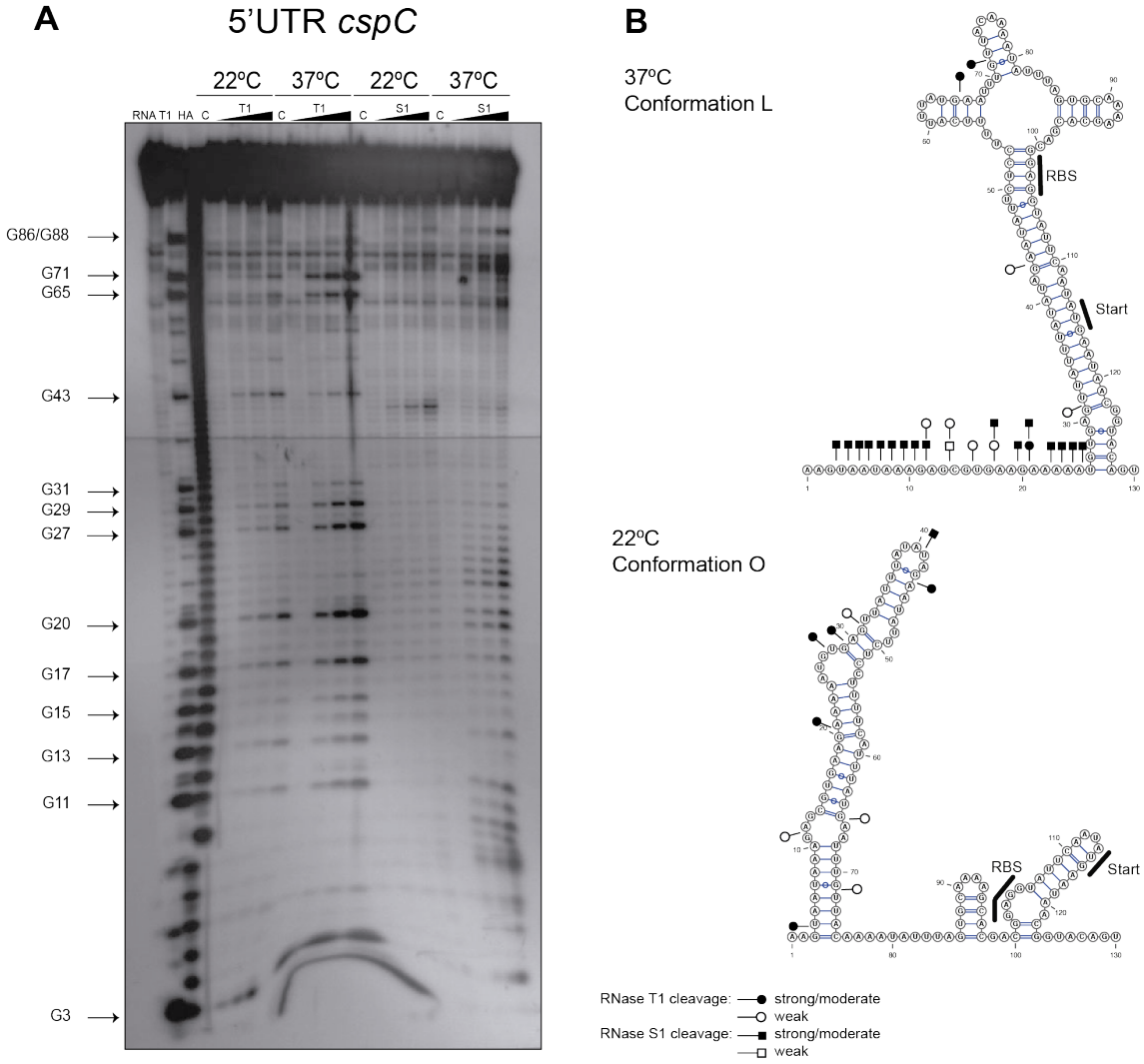

Figure S2. Enzymatic probing at different temperatures of the alternative structures found in the *cspC* 5'UTRs. (**A**) Electrophoretic migration of the radiolabelled *cspC* 5'UTR after RNase T1 (T1) and RNAse S1 (S1) cleavage at 22 and 37ºC. Samples were treated as previously described in Figure S1. (**B**) Model of *cspC* 5'UTR secondary structure at 37ºC (conformation L) and 22ºC (conformation O) derived from the probing data shown in A. The RBS sequence and start codon are indicated.

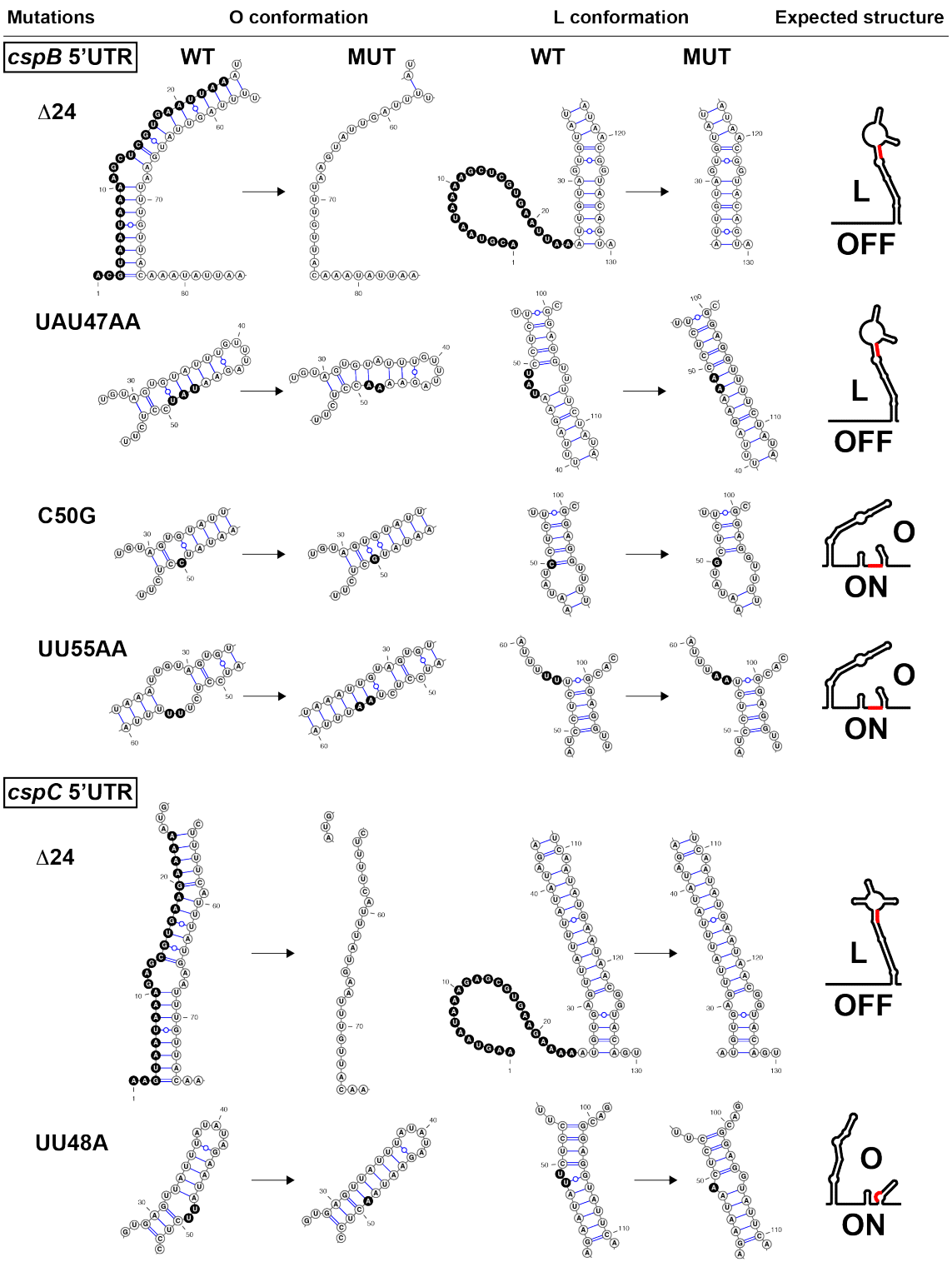

Figure S3. Schematic representation of the *cspB* and *cspC* 5'UTR mutations. The wild type (WT) and mutated (MUT) structures are represented for the O and L conformations . Mutated nucleotides are depicted in black. The expected mutated 5'UTR structure O or L (translation ON/OFF) is also showed.

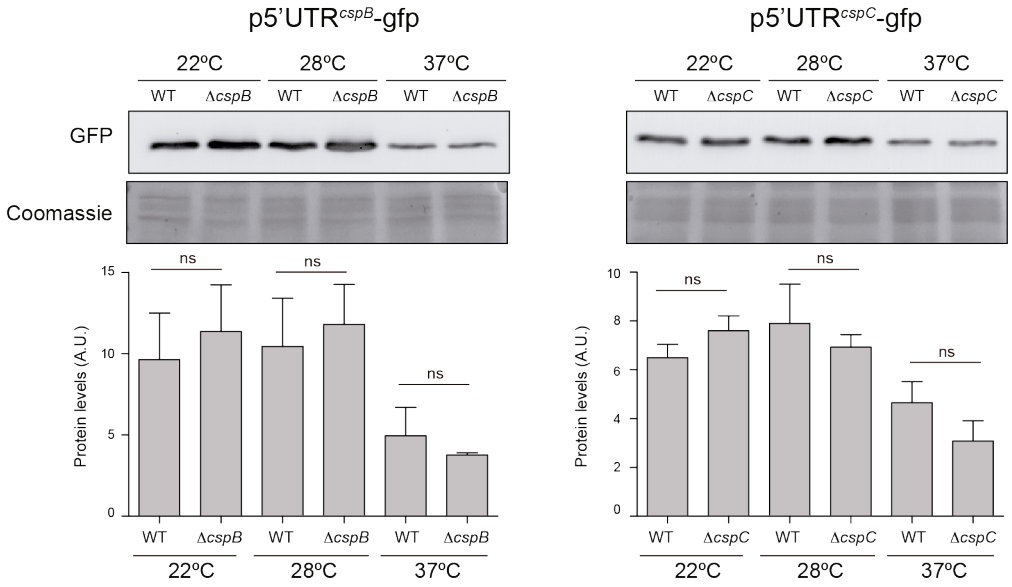

Figure S4. The CspB and CspC protein expression is not auto-regulated in *S. aureus*. Western blot analyses of GFP levels expressed from the *S. aureus* WT, Δ*cspB and* Δ*cspC* strains carrying the *cspB* and *cspC* 5'UTR-GFP reporters, which were grown in MH at 22, 28 and 37ºC. The GFP production was developed as described in Figure 2. Bar plots show the mean and standard deviation of the GFP levels from three independent biological replicates, which were determined by densitometry of protein bands using ImageJ (https://imagej.nih.gov/ij/). Asterisk represent statistical significance (p<0.05, Mann-Whitney U test); ns, not significant. Representative images from the triplicates are shown.

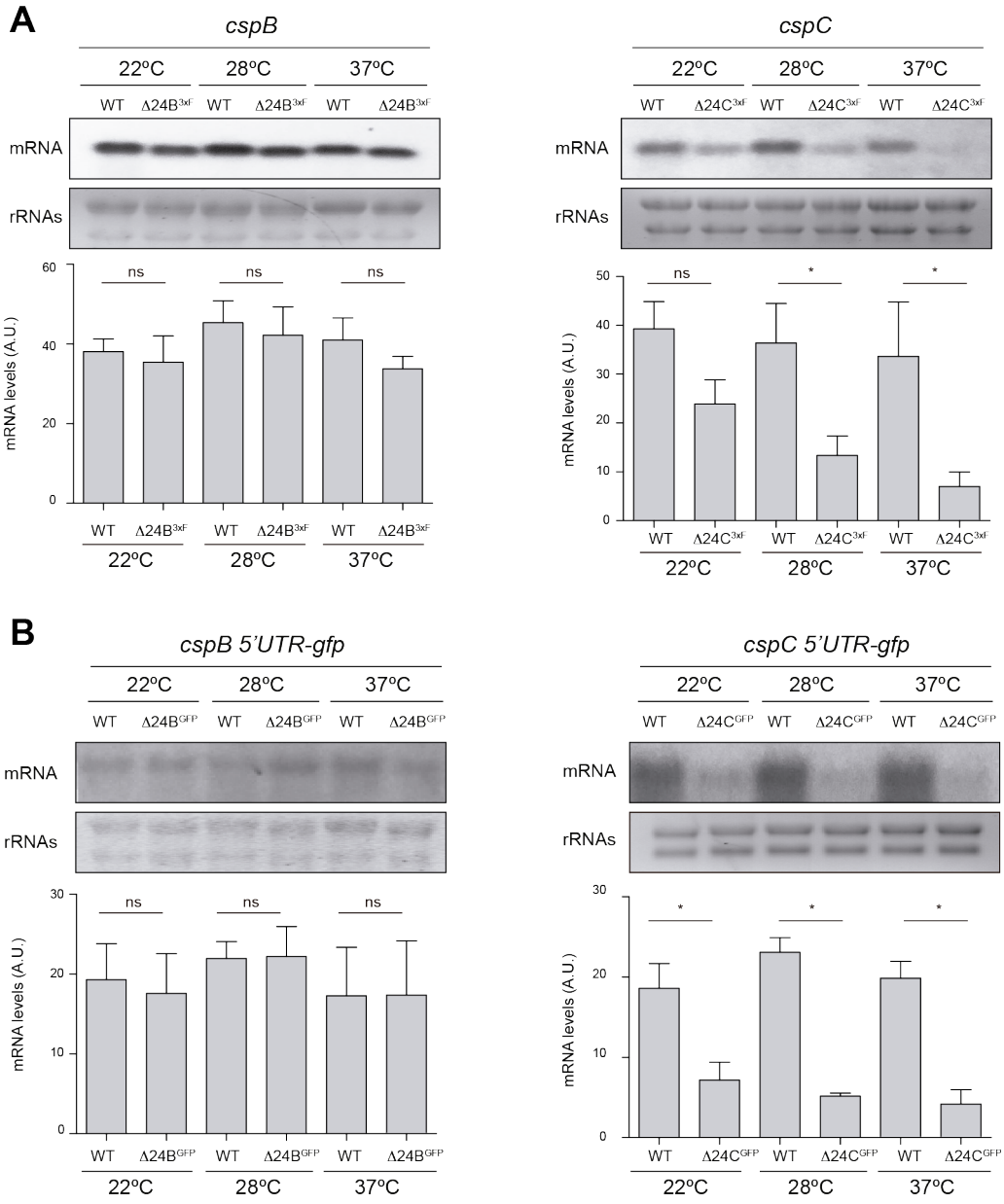

Figure S5. Different effects on the mRNAs levels when deleting the first 24 nucleotides in *cspB* and *cspC* 5'UTRs. (**A**) Northern blot showing the chromosomal expression of *cspB*^{3xF} and *cspC*^{3xF} mRNAs from the WT and Δ24 strains grown in MH at 22, 28 and 37ºC. (**B**) Northern blot results of the chimeric *5'UTR-gfp* mRNA levels expressed from the *S. aureus* carrying the *cspB* and *cspC* WT and Δ24 5'UTR-GFP translational-reporter plasmids after growth in MH at 22, 28 and 37ºC. rRNAs stain gel portions are included as loading controls. Bar plots represent the mean and standard deviation of mRNAs levels from three independent biological replicates, which were determined by densitometry of protein bands using ImageJ (https://imagej.nih.gov/ij/). Asterisk represents statistical significance (p<0.05, Mann-Whitney U test); ns, not significant. Representative images from the triplicates are shown.

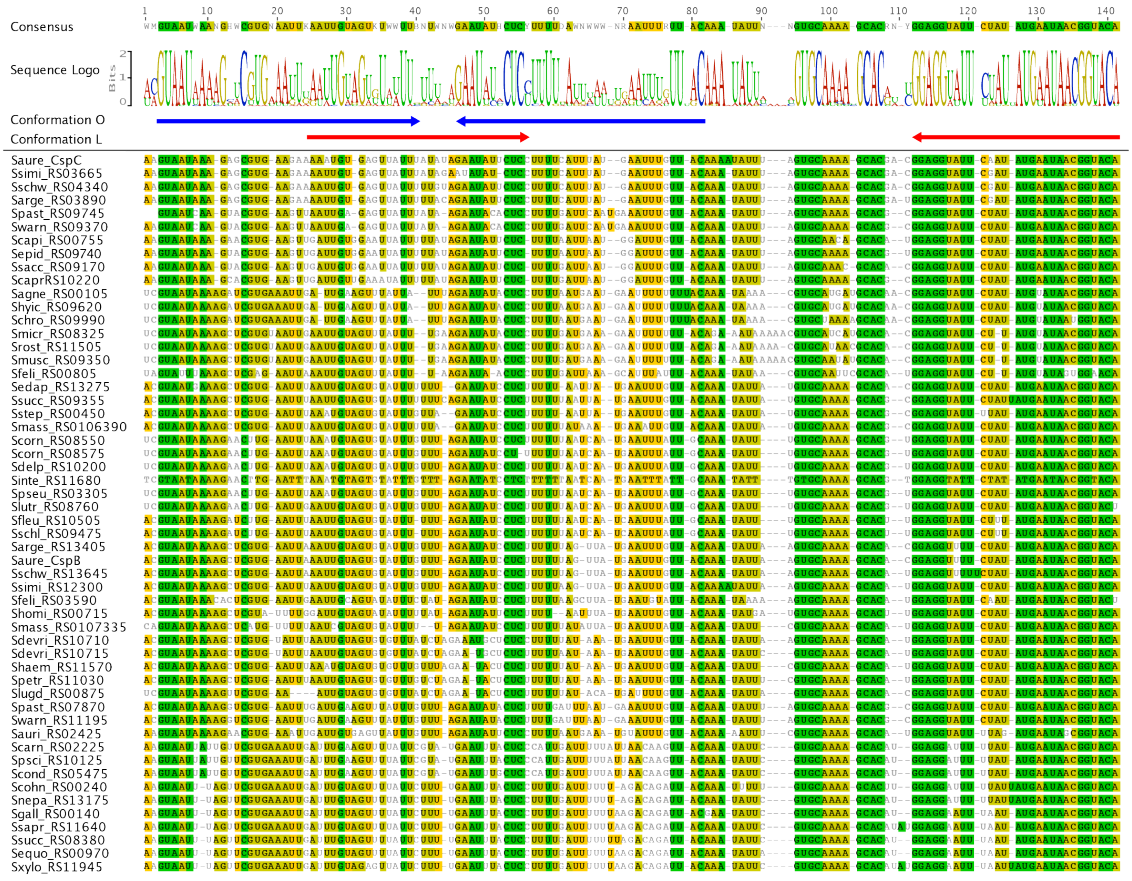

Figure S6. Multiple sequence alignments of *csp* 5'UTRs from different *Staphylococcus* species. The consensus sequence and sequence logo are shown. Blue and red arrows represent the nucleotides that form conformation O or L, respectively. Nucleotides were coloured in function of their degree of identity as follows: dark green 100%, light green 80-99%, yellow 60-89% and white less that 60%. *Saure, S. aureus; Ssimi, S. simiae; Sschw, S. schweitzeri; Sarge, S. argenteus; Spast, S. pasteuri; Swarn, S. warneri; Scapi, S. capitis; Sepid, S. epidermidis; Ssacc, S. saccharolyticus; Scapra, S. caprae; Sagne, S. agnetis; Shyic, S. hycus; Schro, S. chromogenes; Smusc, S. muscae; Sfeli, S. felis; Sedap, S. edaphicus; Ssucc, S. succinus ; Sstep, S. stepanovicii; Smass, S. massiliensis; Scorn, S. cornubiensis; Sdelp, S. delphini; Sinte, S. intermedius; Spseu, S. pseudointermedius; Slutr, S. lutrae; Sfleu, S. fleurettii; Shomi, S. hominis; Smass, S. massiliensis; Sdevr, S. devriesei; Shaem, S. haemolyticus; Spetr, S. petrasii, Slugd, S. lugdunensis; Spast, S. pasteuri; Sauri, S. auricularis; Scarn, S. carnosus; Spsci, S. pscifermentans; Scond, S. condimenti; Scohn, S. cohnii; Snepa, S. nepalensis; Sgall, S. gallinarum; Ssapr, S. saprophyticus; Ssucc, S. succinus; Sequo, S. equorum; Sxylo, S. xylosus.*

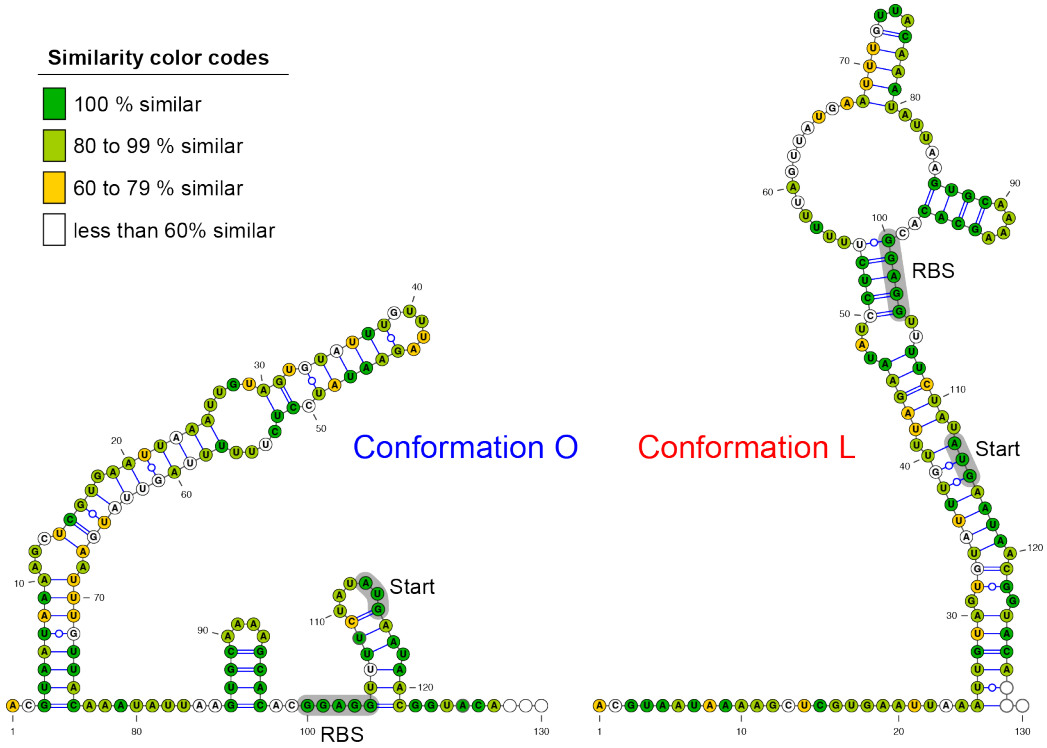

Figure S7. The nucleotides required for adopting the alternative conformations are highly conserved. RNA structures were predicted by the mfold web server **(9)** and visualized and drawn with the VARNA software **(10)**. Nucleotides were coloured according to the identity percentage. The RBS sequence, the start codon and the anti-RBS region are grey shaded.

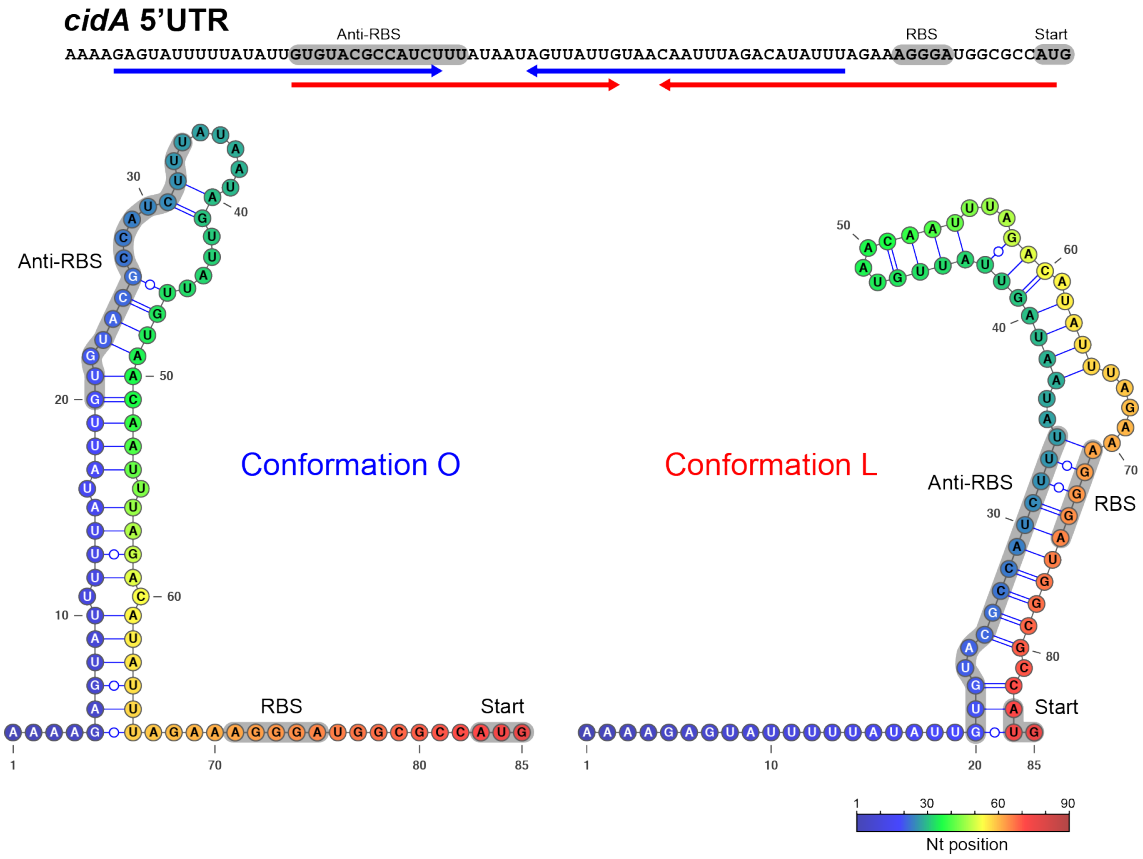

Figure S8. Putative alternative RNA structures in the longer *cidA* 5'UTR. RNA structures were predicted by the mfold web server **(9)** and visualized and drawn with the VARNA software **(10)**. Structures were coloured according to the nucleotide positions as indicated in the colour scale. The arrows below the 5'UTR sequences indicate the interacting nucleotide regions. Blue arrows, conformation O; Red arrows, conformation L. The ribosome binding site (RBS), the start codon and the anti-RBS region are grey shaded.

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