Supplementary Data

The regulon of the staphylococcal RNA chaperone CspA and its auto-regulation

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This file includes the Supplementary Tables S1, S2 and S3, the Supplementary Figures S1, S2, S3 and S4 and their references. Additionally, supplementary Tables S4 S5 and S6, including proteome and targetome results are provided as Excel files. The map of CspA binding signals can be browsed at http://rnamaps.unavarra.es/.

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	(1)
Δ*cspA*	15981 strain with deletion of the *cspA* gene	83	This study
15981 *cspA*^{3xFLAG}	15981 strain expressing the 3xFLAG-tagged CspA protein from the chromosome	239	This study
15981 *gdpP*^{3xFLAG}	15981 strain expressing the 3xFLAG-tagged GdpP protein from the chromosome	305	This study
WT pCN51	15981 strain carrying the pCN51 plasmid	105	This study
Δ*cspA* pCN51	15981 Δ*cspA* strain carrying the pCN51 plasmid	107	This study
Δ*cspA* pCspA^{3xFLAG}	15981 Δ*cspA* strain carrying the pCspA^{3xFLAG} plasmid	268	This study
WT pSigB$^{3x\ FLAG}$	15981 strain carrying the pSigB^{3xFLAG} plasmid	656	This study
WT pCspC^{3xFLAG}	15981 strain carrying the pCspC^{3xFLAG} plasmid.	651	This study
WT pCspA^{3xFLAG}	15981 strain carrying the pCspA^{FLAG} plasmid	505	This study
WT pCspA^{3xFLAG}Δ5'UTR	15981 strain carrying the pΔ5'CspA^{3xFLAG} plasmid	649	This study
WT pM5U^{3xFLAG}	15981 strain carrying the pM5U^{3xFLAG} plasmid	746	This study
WT pM5UC^{3xFLAG}	15981 strain carrying the pM5UC^{3xFLAG} plasmid	748	This study
Δ*cspA* pSigB$^{3x\ FLAG}$	15981 Δ*cspA* strain carrying the pSigB$^{3x\ FLAG}$ plasmid	657	This study
Δ*cspA* pCspC^{3xFLAG}	15981 Δ*cspA* strain carrying the pCspC^{3xFLAG} plasmid	652	This study
Δ*cspA* pCspA^{3xFLAG}Δ5'UTR	15981 Δ*cspA* strain carrying the pΔ5'CspA^{3xFLAG} plasmid	650	This study
Δ*cspA* pM5U^{3xFLAG}	15981 Δ*cspA* strain carrying the pM5U^{3xFLAG} plasmid	747	This study
Δ*cspA* pM5UC^{3xFLAG}	15981 Δ*cspA* strain carrying the pM5UC^{3xFLAG} plasmid	749	This study
Δ*rnc*	15981 strain with deletion of the *rnc* gene	409	(2)
Δ*rnc* pCspA^{3xFLAG}	15981 Δ*rnc* strain carrying the pCspA^{3xFLAG} plasmid	1036	This study
Δ*rnc* Δ*cspA*	15981 strain with deletion of the *rnc* and *cspA* genes	989	This study
Δ*rnc* Δ*cspA* pCspA^{3xFLAG}	15981 Δ*cspA* Δ*rnc* strain carrying the pCspA^{FLAG} plasmid	968	This study
Escherichia coli			
XL1-Blue	Strain used for cloning experiments	1	Stratagene
XL1-Blue pCN40	XL1Blue strain carrying the pCN40 plasmid	18	(3)
XL1-Blue pCN51	XL1Blue strain carrying the pCN51 plasmid	20	(3)
BL21pGEX-6P-2::cspA	BL21 (DE3) expressing CspA with a GST tail from the pGEX-6P plasmid	631	This study

[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	*E. coli-S. aureus* shuttle vector with a thermosensitive origin of replication for Gram-positive bacteria. The vector contains the *bgaB* gene encoding a β-galactosidase under the control of a constitutive promoter as reporter of plasmid presence. Amp^R, Erm^R	(4)
pMAD *cspA*	pMAD plasmid containing the allele for deletion of the *cspA* coding region	This study
pMAD *cspA*^{3xFLAG}	pMAD plasmid containing the allele for insertion of the 3xFLAG at the C-terminus of the CspA protein	This study
pMAD *gdpP*^{3xFLAG}	pMAD plasmid containing the allele for insertion of the 3xFLAG at the C-terminus of the GdpP protein	This study
pCN40	*E. coli-S. aureus* shuttle vector to express genes under the control of the P*blaZ* promoter. Low copy number. Amp^R-Em^R	(3)
pCN51	*E. coli-S. aureus* shuttle vector to express genes under the control of the P*cad* inducible promoter. Low copy number. Amp^R-Em^R	(3)
pSigB^{3xFLAG}	pCN40 plasmid expressing the 3xFLAG-tagged SigB protein	This study
pCspC^{3xFLAG}	pCN51 plasmid expressing the 3xFLAG-tagged CspC protein	This study
pCspA^{3xFLAG}	pCN51 plasmid expressing the 3xFLAG-tagged CspA protein from the entire *cspA* mRNA molecule	This study
pΔ5'CspA^{3xFLAG}	pCN51 plasmid expressing the 3xFLAG-tagged CspA protein from a *cspA* mRNA lacking the 5'UTR	This study
pMA-T_CspA_M5U	pMA-T plasmid carrying the synthetic *cspA_M5U* gene	This study
pMA-T_CspA_M5UC	pMA-T plasmid carrying the synthetic *cspA_M5UC* gene	This study
pCspA^{3xFLAG}-M5U	pCN51 plasmid expressing the 3xFLAG-tagged CspA protein from a *cspA* mRNA carrying a mutation that substituted the 5U motif within the 5'UTR	This study
pCspA^{3xFLAG}-M5UC	pCN51 plasmid expressing the 3xFLAG-tagged CspA protein from a *cspA* mRNA carrying a mutation that substituted the 5U motif and a compensatory one to restore the stem-loop structure within the 5'UTR	This study

Table S3. Oligonucleotides used in this study

Oligonucleotide name	Sequence [a]
Probe for Northern blots assays	
anti_3xFLAG_probe	TTTATCGTCGTCATCTTTGTAGTCGATATCATGATCTTTATAATCACCGTCATGGTCTTTGTAGTC
Construction of pMAD plasmid for deletion of *cspA* gene	
CspA_A_BamHI	*GGATCC*TCAAACTTGATTTTCAGAGG
CspA_B_SalI	*GTCGAC*AATCTGAAACCTCCAAGACT
CspA_C_SalI	*GTCGAC*ATTCTTAGATTTGAATCATTG
CspA_D_EcoRI	*GAATTC*GATTTTCTAATTCCATCATCTCTG
CspA_E	TGTCTCATTTTTACCACCTC
CspA_F	CCACACTTTAAACAAGCATC
Construction of pMAD plasmid for chromosomic 3xFLAG-labeling of the *cspA* gene	
CspA_3xF_A_EcoRI	*GAATTC*ACTACCAATAATTGATTAAATGAATATT
CspA_3xF_B_NcoI	*CCATGG*TT<u>ACTATTTATCGTCGTCATCTTTGTAGTCGATATCATGATCTTTATAATCACCGTCATGGTCTTTGTAGTC</u>TAGTTTAACAACGTTTGCAGC
CspA_3xF_C_NcoI	*CCATGG*TTCTTAGATTTGAATCATTGATTTTAAC
CspA_3xF_D_BamHI	*GGATCC*GAATTAACAACAAATATAATGTATAAAAC
Construction of pMAD plasmid for chromosomic 3xFLAG-labeling of the *gdpP* gene	
GdpP_3xF_A_EcoRI	*GAATTC*TGAATCAACAGTGATGTATGCAG
GdpP_3xF_B_BamHI	*GGATCC*TT<u>ACTATTTATCGTCGTCATCTTTGTAGTCGATATCATGATCTTTATAATCACCGTCATGGTCTTTGTAGTC</u>TAATTGTTCTGTAATTGCTTGTTGT
GdpP_3xF_C_BamHI	*GGATCC*AGTAGGAGTGAAGATGCATGAA
GdpP_3xF_D_NcoI	*CCATGG*GTACCTTCAACTTCTTTATCTAATTTAACA
GdpP_3xF_E	CTAATCCATTGTTGATATATATGGAACC
GdpP_3xF_F	GCGGCATTTGATTTTGCT
Construction of plasmid expressing the SigB³ˣᶠᴸᴬᴳ protein	
EcoRI_sigB_fw	*GAATTC*AAAGAGCAGGTGCGAAATA
SpeI_sigB_rev	*ACTAGT*TTGATGTGCTGCTTCTTGTA
SpeI_3xF_TT_pCN51	*ACTAGT*<u>GACTACAAAGACCATGACGGTGATTATAAAGATCATGATATCGACTACAAAGATGACGACGATAAATAA</u>GGCGCGCCTATTCTAAATG
NarI_3xF_TT_pCN51	AAA*GGCGCC*TGTCACTTTGC
Construction of plasmid expressing the CspC³ˣᶠᴸᴬᴳ protein	
CspC +1 BamHI	*GGATCC*AATAAAGAGCGTGAAGAAAATGTG
3xFcspC_B	ACCGTCATGGTCTTTGTAGTCCATTTTAACTACGTTTGCAGCTT
3xFcspC_C	<u>GACTACAAAGACCATGACGGTGATTATAAAGATCATGATATCGACTACAAAGATGACGACGATAAATAA</u>TTTTAACTTATTCAAACAGT
CspC ter KpnI	*GGTACC*CCATTATATATTGTTAAATCTCCAAC
Construction of plasmid expressing CspA³ˣᶠᴸᴬᴳ and its variants	
CspA_+1_BamHI	*GGATCC*AAGCAGATGATTATTCCATATTG
CspA_ter_EcoRI	*GAATTC*CTATTGCAAACAATGTTGGTGATATAA
CspA_D5UTR_BamHI	*GGATCC*TCTTGGAGGTTTCAGATTATGAAACAAGGTACAGTTAA
ssDNA oligonucleotide gel shift assays	
f-A1	AAGCAGATGATTATTCCATATTGCAAAGAATATTTGAGTGAAATTTGCATGTATTTTTTGTA
f-A2	TTTTTTGTAATATGCGAATAAGCATATTGAATGAATTTTAGTCTTGGAGGTTTCAGATTATG
f-A3	CTTGGAGGTTTCAGATTATGAAACAAGGTACAGTTAAATGGTTTAACGCTGAAAAAGGAT
f1-ΔT	AAGCAGATGATTATTCCATATTGCAAAGAATATTTGAGTGAAATTTGCATGTA
f2-ΔT	GTAATATGCGAATAAGCATATTGAATGAATTTTAGTCTTGGAGGTTTCAGATTATG
f-CDS	GAAGTAGTTGAAGGCGACCGCGGTCCACAAGCTGCAAACGTTGTTAAACTATAA

[a] Restriction enzymes sites, T7 promoter and 3xFLAG sequences included into the oligonucleotides are indicated in italic, bold and underlined format, respectively.

Tables S4, S5 and S6 can be found as Supplementary Excel files.

Table S4. Results of comparative label-free LC-MS-based proteomics. Up- and down-regulated proteins with a fold change ratio ($\Delta cspA$ vs WT) higher than 2 and a P-value lower than 0.05 are included. Coloured rows show proteins encoded by CspA-targeted transcripts. Specifically, yellow rows highlight proteins in which the CspA-binding peak was nearby or included in the CDS. Orange rows indicate proteins encoded by polycistronic transcripts, where the CspA-binding peak was not contained in the CDS. The red row highlights CspA protein, which is deleted in the $\Delta cspA$ strain. Each CspA-regulated protein is classified in category, subcategory, subsystem and role according to the SEED database (http://pseed.theseed.org/) (5).

Table S5. CspA[3xFLAG] RIP-chip results. The genomic coordinates (Summit_pos) and the tiling intensity signals (Summit_value) for the CspA-binding peaks are indicated. Boundaries, length and features of the CspA-targeted transcripts as well as description of the closest feature to the corresponding peak is provided.

Table S6. Table summarizing the list of putative mRNAs targeted by CspA for which the protein levels were found significantly affected in the $\Delta cspA$ mutant strain.
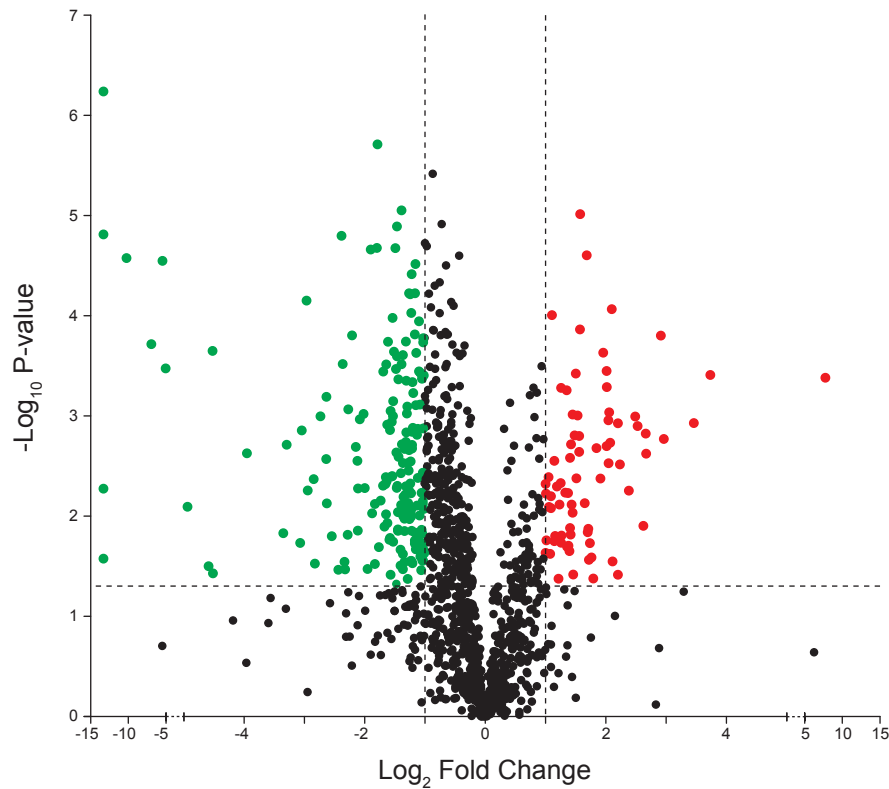
Figure S1. Volcano Plot for differential gene expression determined by label-free proteomics in the Δ*cspA* mutant compared to WT strain. Scattered points represent genes: the x-axis is the log ratios (log$_2$ fold change) of Δ*cspA* mutant vs WT strain, whereas the y-axis is the negative log of the P-value. Red and green dots are the proteins that were significantly up- and down-regulated (considering a fold change > 2 and a P-value <0.05), respectively. Detailed information about these proteins is available in Table S4.
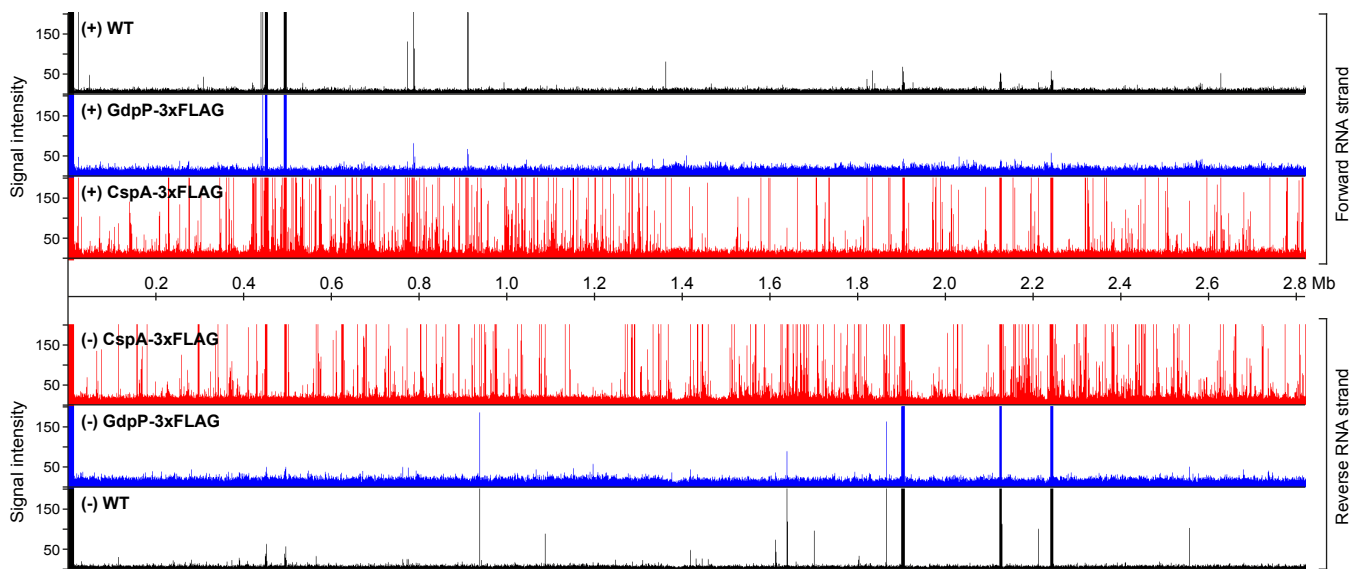
Figure S2. RIP-on-chip genomic maps. IGB plot showing the tiling array signals of the RIP-on-chip assays from strains CspA[3xFLAG] in red, GdpP[3xFLAG] in blue and the untagged WT in black along the forward and reverse genomic strands. *S. aureus* NCTC 8325 strain sequence was used as reference genome. These maps can be browsed at http://rnamaps.unavarra.es/.
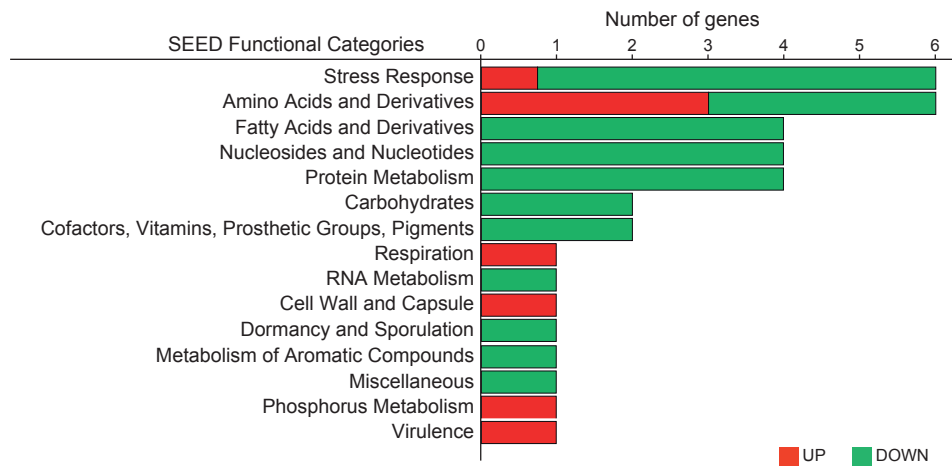
Figure S3. Functional classification of differentially expressed CspA targets. The plot represents the number of differentially expressed CspA targets that could be classified into different functional SEED categories (http://pseed.theseed.org/) (5). The number of up-regulated and down regulated targets are represented as red and green bars, respectively. Details of those genes are included in Table S4 and S6.
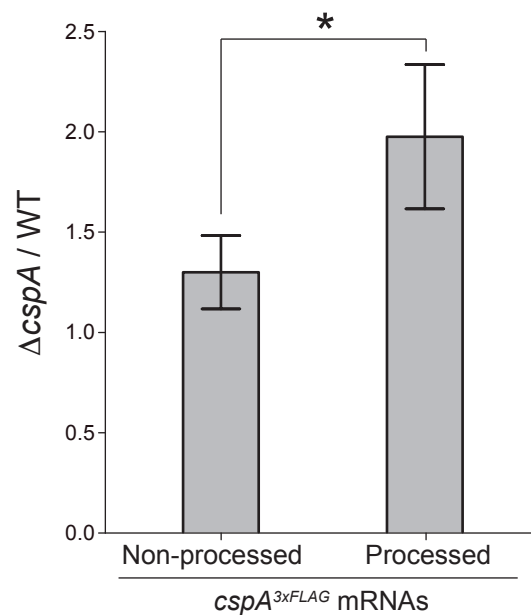

Figure S4. Comparison of non-processed and processed $cspA^{3xFLAG}$ mRNAs levels in the $cspA$ mutant vs the WT strain. Bands were quantified by densitometry of Northern blot autoradiographies using Fiji (https://fiji.sc/). The mean ratios and standard deviations from four independent experiments are shown. The asterisk indicates statistical significance of the ratio differences ($P = 0.0154$).
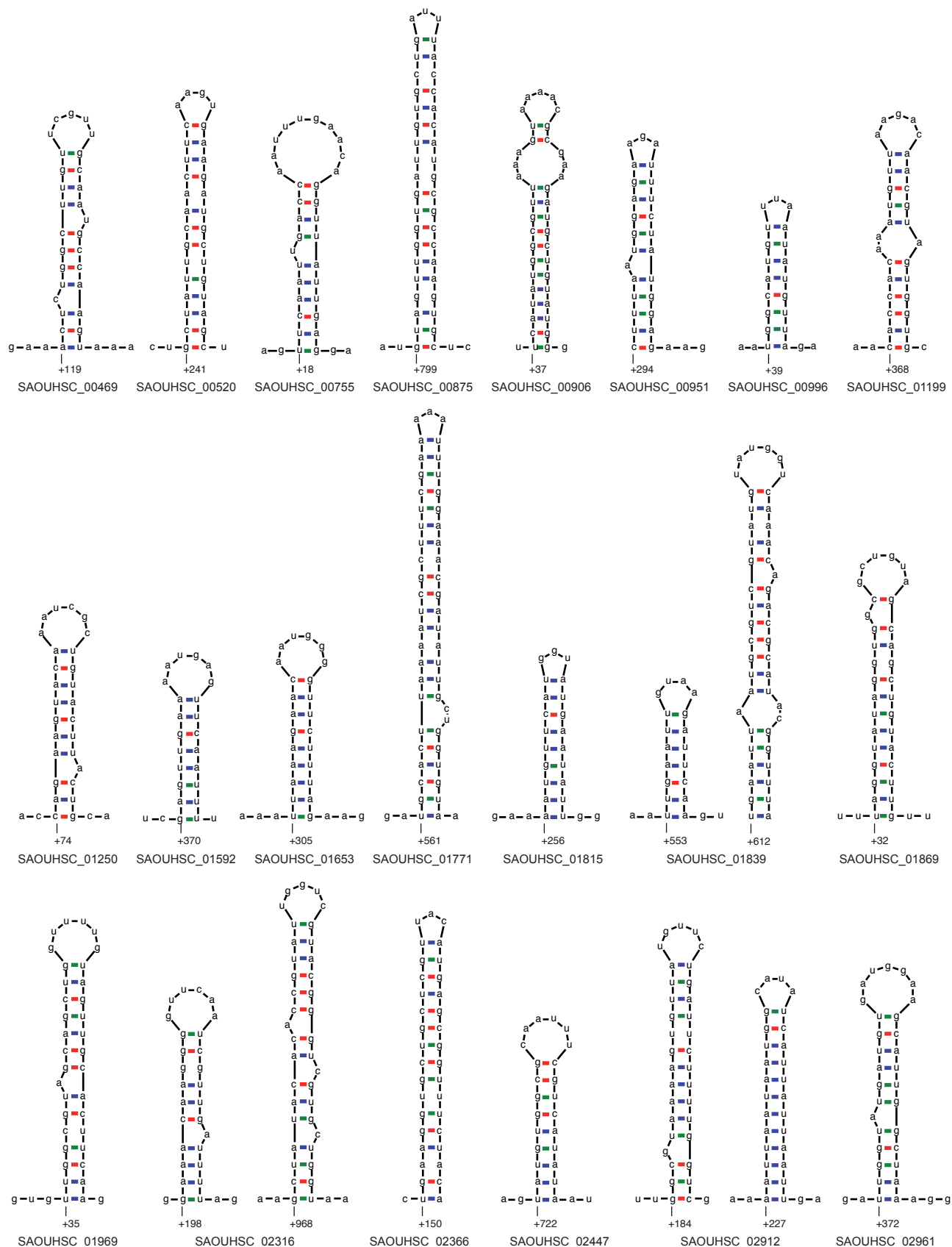
Figure S5. Putative ribosome stalling structures. M-fold predictions of RNA secondary structures located at mRNAs that are directly regulated by CspA and presented RFPs as previously described (6). Numbers indicate the position of the hairpins from the start codon of the corresponding CDS. Gene ID from *S. aureus* NCTC 8325 are included.

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References

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