Supporting Information

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SI Materials and Methods

Bacterial Strains and Culture Conditions. The strains and oligonucleotides used in this study are listed in Table S1. *Staphylococcus aureus* 15981 wild type and its isogenic mutants were grown in trypticase soy broth containing 0.25% glucose (TSB-gluc) (Pronadisa). *Enterococcus faecalis* JH2-2, *Listeria monocytogenes* EGD, and *Bacillus subtilis* MIC1982 were grown in brain heart infusion medium (Pronadisa). *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) was grown in Luria-Bertani broth (Pronadisa). When required for selective growth, medium was supplemented with appropriate antibiotics at the following concentrations: erythromycin (Em), 1.5 $\mu g \cdot mL^{-1}$, and ampicillin, 100 $\mu g \cdot mL^{-1}$.

Allelic Exchange of Chromosomal Genes. To generate a *pnp* deletion mutant, we amplified by PCR two fragments of 500 bp that flanked the left (primers A and B) and right sequences (primers C and D) of the genes targeted for deletion (Table S1). The PCR products were purified and cloned separately in the pGEM-T Easy vector (Promega). Fragments were then fused by ligation into the shuttle plasmid pMAD, and the resulting plasmid was transformed into *S. aureus* 15981 by electroporation (1). For inactivation of *rnc* (RNase III encoding gene), codons 56–200 encompassing the RNA binding and the catalytic site were deleted and replaced by the cat gene using pLUG519 plasmid, a pMAD derivative kindly provided by Pascale Romby, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France (2). Homologous recombination experiments were performed as described (3). Em-sensitive white colonies, which no longer contained the pMAD plasmid, were tested by PCR using primers E and F (Table S1).

RNA Extractions. Bacteria were grown in 20 mL of TSB-gluc at 37 °C under shaking conditions (200 rpm) until the culture reached an OD_{600} of 0.8. Cultures were centrifuged, and the pellets were frozen in liquid nitrogen and stored at −80 °C until needed. Total RNA from bacterial pellets was extracted by using TRIzol reagent method as described (4). Briefly, bacterial pellets were resuspended into 400 μL of solution A (glucose 10%, Tris 12.5 mM, pH 7.6, EDTA 10 mM) and mixed to 60 μL of 0.5M EDTA. Resuspended cells were transferred into Lysing Matrix B tubes (MP Biomedicals) containing 500 μL of acid phenol (Ambion) and mixed. Bacteria were mechanically lysed by using the Fastprep apparatus (BIO101) at speed 6.0 during 45 s at 4 °C. After lysis, tubes were centrifuged 10 min at 17,900 × *g* at 4 °C. The aqueous phase was transferred to 2-mL tubes containing 1 mL of TRIzol, mixed, and incubated for 5 min at room temperature. 100 μL of chloroform were added, mixed gently, and incubated for 3 min at room temperature. Tubes were centrifuged for 10 min at 17,900 × *g* at 4 °C. The aqueous phase was transferred into a 2-mL tube containing 200 μL of chloroform, mixed, and incubated for 5 min at room temperature. Tubes were centrifuged for 5 min at 17,900 × *g* at 4 °C. RNA contained in the aqueous phase was precipitated by addition of 500 μL of isopropanol and incubated 15 min at room temperature. Tubes were centrifuged for 15 min at 17,900 × *g* at 4 °C. RNA pellets were washed with 75% ethanol. Dried RNA pellets were resuspended in DEPC-treated water. RNA concentrations were quantified, and RNA qualities were determined by using Agilent RNA Nano LabChips (Agilent Technologies). RNAs were stored at −80 °C until needed.

Construction of cDNA Libraries for dRNA-seq. Short RNA libraries for Illumina sequencing were prepared from the RNA fraction that contains RNAs shorter than 50 nt by adapting the described method (5). All of the oligonucleotides used in this protocol are listed in Table S1. This fraction was purified from total RNA (100 μg) with flashPAGE fractionator (Ambion). For that, electrophoresis was performed at 75-V constant voltage during 15 min. The extracted product was incubated with 2 U of tobacco acid pyrophosphatase (TAP) (Epicenter) for 2 h at 37 °C to convert 5′ triphosphate groups to monophosphates. The reaction was terminated by phenol-chloroform treatment and ethanol precipitation. The TAP treated short RNA fraction was ligated to the 3′ ModBan oligonucleotide by using the truncated T4 RNA ligase2 as described (5). This was followed by a second gel purification on 10% TBE-Urea denaturing polyacrylamide gel (Novex; Invitrogen) to remove the nonligated 3′ ModBan oligonucleotide. The extracted product was ligated to 5′ RNA Illumina adapter by using T4 RNA ligase (Invitrogen) in a final volume of 20 μL. For the first strand synthesis of cDNA, SuperScript III (Invitrogen) and the RT oligonucleotide, complementary to the ModBan 3′ linker, was used according to the manufacturer's instructions. The resulting RT product was then amplified by using Sol_5_SBS3 and Sol_3_ModBan adapters for 20 cycles of PCR (for oligonucleotides, see Table S1). The resulting cDNA (120–150 nt) was purified from a 3% low melting agarose gel (MetaPhor; Cambrex) by using MinElute Gel extraction kit (QIAGEN) and sent for sequencing. The libraries were sequenced by using 36 single-read cycles on a Genome Analyzer II (Illumina) at the Cold Spring Harbor Laboratory (CSHL) facilities.

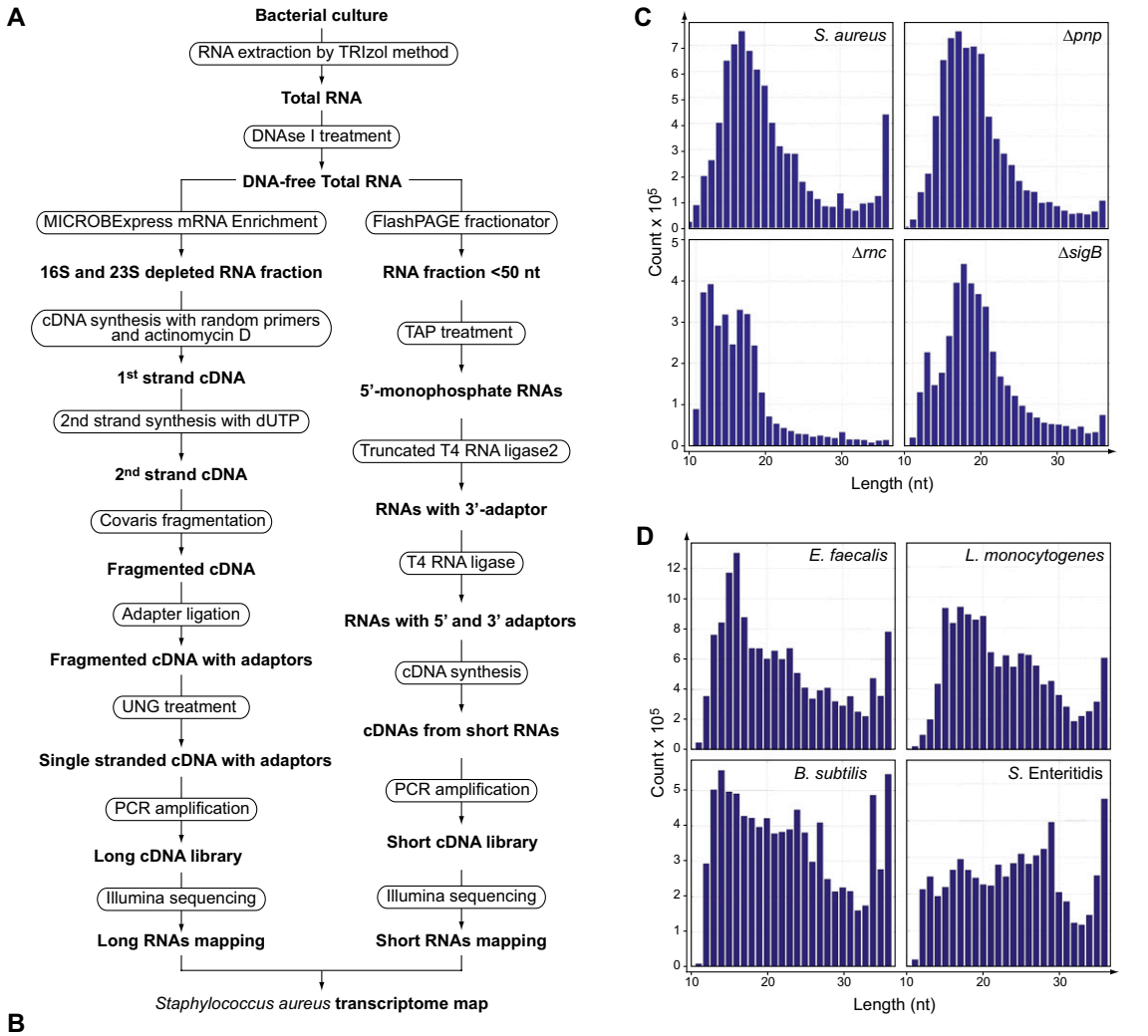
The protocol to prepare long RNA libraries for Illumina sequencing was created by adapting the described dUTP second strand method (6). 10 μg of total RNA was depleted from 16S and 23S RNAs by using the MicroExpress kit (Ambion). Reverse transcription (RT) of the recovered RNA was performed with SuperScript III reverse transcriptase (Invitrogen) in a 25-μL reaction, containing 100 ng of RNA, 2 μL of 50 ng/μL random primers, 2.5 μL of 5× First Strand Buffer, 1.25 μL of 1 M MgCl2, 1.25 μL of 10mM dNTPs, 2.5 μL of 1 M DTT, and 6 ng/μL freshly made actinomycin D (which inhibits DNA-dependent DNA polymerase activity of reverse transcriptase). The resulting cDNA was purified by using MinElute cleanup kit (QIAGEN). Second-strand cDNA was synthesized by adding 30 μL of first strand reaction, 22.5 μL of RNase free water, and 22.5 μL of the following reaction (1 μL of 5× first Strand Buffer, 15 μL of 5× second Strand Buffer, 0.5 μL of MgCl2, 1 μL of DTT, 4 μL of 10 mM dNTPs with dTTP replaced by dUTP, 40 U of *Escherichia coli* DNA polymerase, 10 U of *E. coli* DNA ligase, and 2 U of *E. coli* RNase H). The final mix was incubated at 16 °C for 2 h. cDNA was fragmented by using the Covaris ultrasonicator to produce cDNA fragments around 200–300 nt. After second-strand synthesis, double-stranded cDNAs were end repaired by T4 DNA polymerase, followed by A-tailing with Klenow DNA polymerase (exo-). The resulting DNA fragments were ligated to Illumina linkers and treated with 1 U of Uracil-*N*-Glycosylase (UNG; Applied Biosystems) to degrade the uridine-containing strand. 18-cycle PCR was then performed with Phusion DNA Polymerase (Finnzymes) to generate the final sequencing library. The libraries were sequenced by using 76 single-read cycles on a Genome Analyzer II at the CSHL facilities.

Read Mapping and Statistics Analysis. The small RNA-seq reads were aligned to the *S. aureus* NCTC8325 reference genome by using the following procedure. First, the 3′ adapter was clipped off using a simple local alignment algorithm. Then, the remaining sequences were aligned to the reference genome by using STAR, the suffix arrays based alignment tool (http://gingeraslab.cshl.edu/STAR/). For alignments of 10–19 bases long, up to 1 mismatch was allowed; for alignments >20 bases long, up to 2 mismatches were allowed. Alignments of <10 bases were discarded, and spliced alignments were prohibited. Fig. S1 summarizes the mapping statistics. Only uniquely mapping reads were used in the further analysis. Long RNAs were mapped with STAR. Only uniquely and concordantly mapped pairs were kept in further analysis. The average mismatch number was 0.23 per 76-mer reads. Number of mapped reads per nucleotide were visualized by using the Integrated Genome Browser (IGB) (Version 6.2.2) from http://genoviz.sourceforge.net/ (7).

Riboprobes Synthesis. Strand-specific riboprobes to detect sense and antisense RNA signals of *sarA, lexA, recF, yhcRS, clpP, agr, sae*, SA_00086, and SA_00410 genes were synthesized from a PCR product containing a T7 phage promoter sequence on one of its extremities (see Table S1 for the oligonucleotides). One microgram of these PCR products was used as a matrix for in vitro transcription reaction with phage T7 RNA polymerase, 0.5 mM each ATP, GTP, CTP, and 50 mCi of [α-^{32}P] UTP using the Maxiscript kit (Ambion). The probes were then treated with TURBO DNase I at 37 °C for 30 min, and reactions were stopped by addition of 1 μL of 0.5 M EDTA. The riboprobes were purified on Bio-Spin 30 columns following the manufacturer's recommendations (Bio-Rad), and they were immediately used.

Northern Blots. Northern blots were performed as described (4). Briefly, 12 μg of total RNA was separated in precast agarose gels (Sigma) by using 1× NorthernMax Mops as running buffer (Ambion). After electrophoresis, gels were stained with ethidium bromide and photographed to verify equal loading of RNA samples. Then, RNAs were transferred onto Nytran membranes (0.2-μm pore size) (Sigma) by using NorthernMax One Hour Transfer buffer reagent as described in the manufacturer's protocol (Ambion). RNA was UV cross-linked to the membrane by using the UV Stratalinker 1800 (Stratagene). Membranes were prehybridized for at least 30 min in ULTRAhyb solution (Ambion) at 65 °C, followed by addition of labeled strand-specific riboprobe and overnight hybridization at 65 °C. Membranes were washed twice with 2× SSC-0.1% SDS for 5 min at 65 °C. The size of the transcripts was estimated by comparison with RNA Millenium molecular weight standards (Ambion). Autoradiography images were registered at different exposition times according to each gene.

1. Arnaud M, Chastanet A, Débarbouillé M (2004) New vector for efficient allelic replacement in naturally nontransformable, low-GC-content, gram-positive bacteria. *Appl Environ Microbiol* 70:6887–6891.
2. Huntzinger E, et al. (2005) *Staphylococcus aureus* RNAIII and the endoribonuclease III coordinately regulate *spa* gene expression. *EMBO J* 24:824–835.
3. Valle J, et al. (2003) SarA and not sigmaB is essential for biofilm development by Staphylococcus aureus. *Mol Microbiol* 48:1075–1087.
4. Toledo-Arana A, et al. (2009) The *Listeria* transcriptional landscape from saprophytism to virulence. *Nature* 459:950–956.
5. Czech B, et al. (2008) An endogenous small interfering RNA pathway in Drosophila. *Nature* 453:798–802.
6. Parkhomchuk D, et al. (2009) Transcriptome analysis by strand-specific sequencing of complementary DNA. *Nucleic Acids Res* 37:e123.
7. Nicol JW, Helt GA, Blanchard SG Jr., Raja A, Loraine AE (2009) The Integrated Genome Browser: Free software for distribution and exploration of genome-scale datasets. *Bioinformatics* 25:2730–2731.



B

Short RNA libraries	*S. aureus*	Δ*rnc*	Δ*sigB*	Δ*pnp*	*E. faecalis*	*B. subtilis*	*L. monocytogenes*	*S.* Enteritidis
All sequenced reads	26,412,413	25,475,137	19,543,115	13,217,027	32,652,018	20,583,003	24,247,879	25,528,253
Uniquely mapped reads	9,078,873	4,884,478	5,595,211	7,046,095	15,253,616	9,782,465	14,525,969	7,876,673
Uniquely mapped reads - filtered	7,778,726	3,160,181	4,220,164	6,300,332	14,326,630	9,254,300	13,291,269	6,313,630
Multi-mapped reads	13,510,752	13,978,234	10,798,948	5,240,577	13,730,707	7,953,616	7,594,869	16,311,243
Average mapped length	19.6	14.9	18.0	19.3	21.6	22.7	22.2	22.4

Long RNA libraries	*S. aureus*	Δ*rnc*	Δ*sigB*	Δ*pnp*
All sequenced read pairs	42,244,261	39,108,704	41,856,408	44,000,351
Uniquely mapped reads	9,708,928	11,724,454	11,501,966	15,259,644
Multi-mapped reads	22,953,090	21,091,830	23,659,293	19,647,315

Fig. S1. Experimental design for long and short RNA sequencing and summary of mapping results. (*A*) Summary flowchart illustrating the experimental design to determine the *S. aureus* transcriptome. (*B*) Summary of mapping statistics. (*C*) The plot shows the size distribution of mapped reads of short RNA libraries of *S. aureus* wild type and its corresponding isogenic Δ*rnc,* Δ*pnp*, and Δ*sigB* mutants. (*D*) The plot shows the size distribution of mapped reads of short RNA libraries of *Enterococcus faecalis*, *Listeria monocytogenes*, *Bacillus subtilis*, and *Salmonella enterica* ser. Enteritidis.

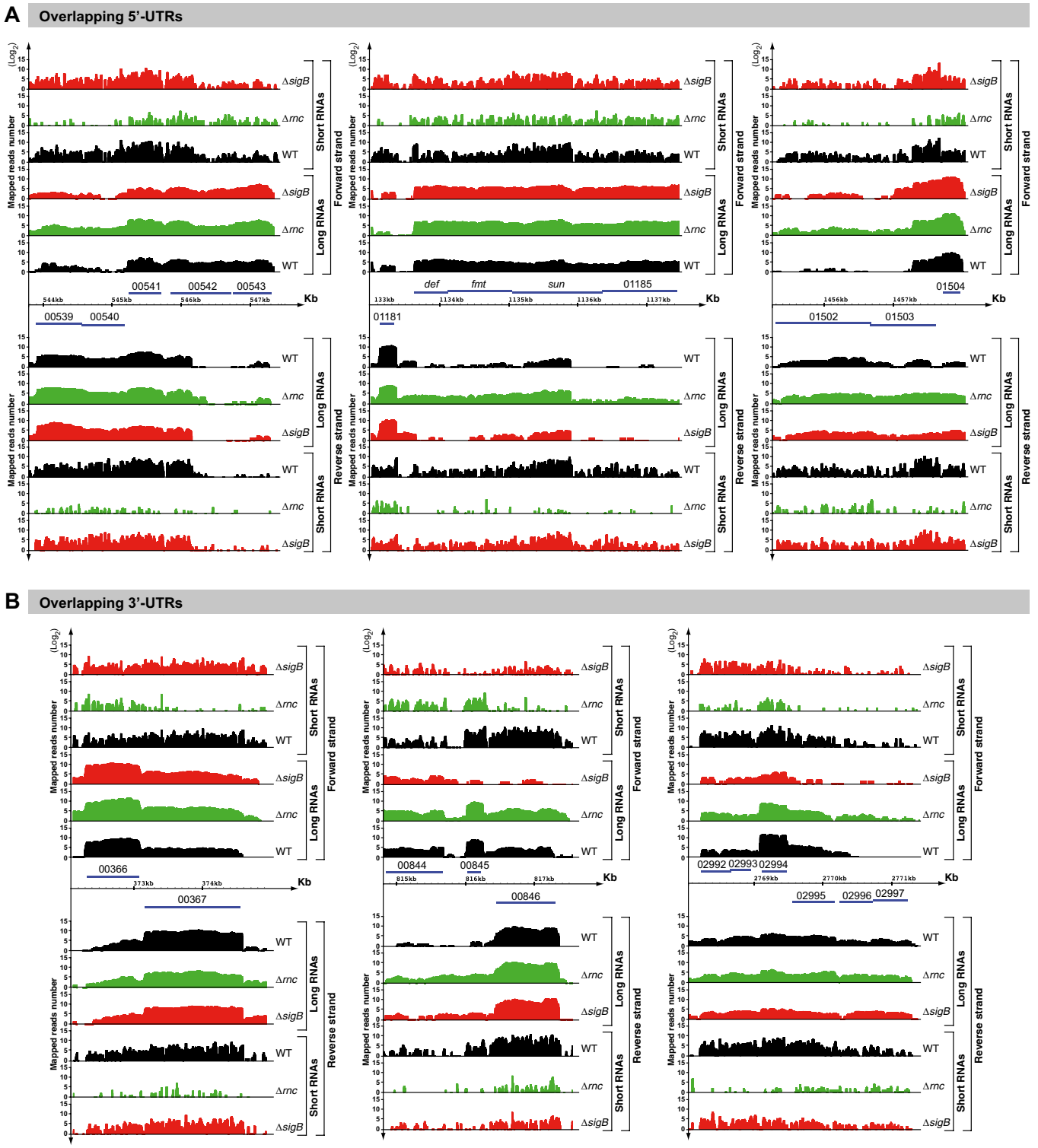


Fig. S2. Examples of mapped reads distribution in regions with 5′ and 3′ UTRs with overlapping transcription in *S. aureus*. (*A*) Examples of 5′ UTRs with overlapping transcription. (*B*) Examples of 3′ UTRs with overlapping transcription. Drawings are images from IGB software showing different regions of the genome of *S. aureus* NCTC 8325. Genomic coordinates denote the position in kilobases of the *S. aureus* NCTC 8325 genome. Annotated ORFs are shown as blue lines. The number on the ORF indicates the gene identification. Long RNAs show the distribution of uniquely mapped reads of long RNA libraries in *S. aureus* 15981 (black), *S. aureus* 15981 Δ*rnc* (green), and *S. aureus* 15981 Δ*sigB* (red). Short RNAs shows the distribution of uniquely mapped reads of short RNA libraries using the same pattern of colors as described for long RNAs. The scale (log_2) indicates the number of mapped reads per nucleotide position.

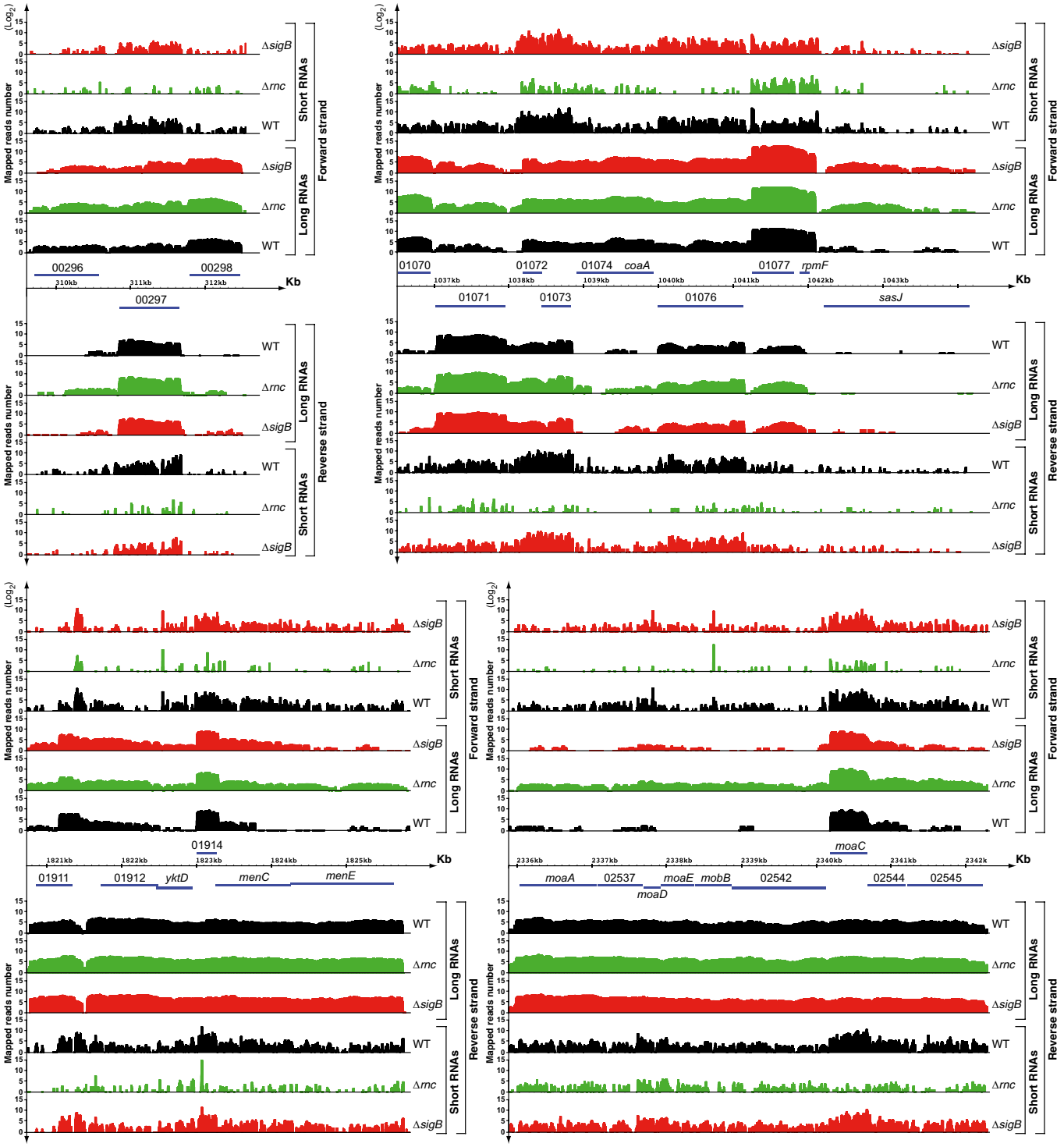


Fig. S3. Examples of mapped reads distribution in regions with "overlapping operons" in *S. aureus*. Drawings are images from IGB software showing different regions of the genome of *S. aureus* NCTC 8325. Genomic coordinates denote the position in kilobases of the *S. aureus* NCTC 8325 genome. Annotated ORFs are shown as blue lines. The number on the ORF indicates the gene identification. Long RNAs show the distribution of uniquely mapped reads of long RNA libraries in *S. aureus* 15981 (black), *S. aureus* 15981 Δ*rnc* (green), and *S. aureus* 15981 Δ*sigB* (red). Short RNAs shows the distribution of uniquely mapped reads of short RNA libraries using the same pattern of colors as described for long RNAs. The scale (log_2) indicates the number of mapped reads per nucleotide position.

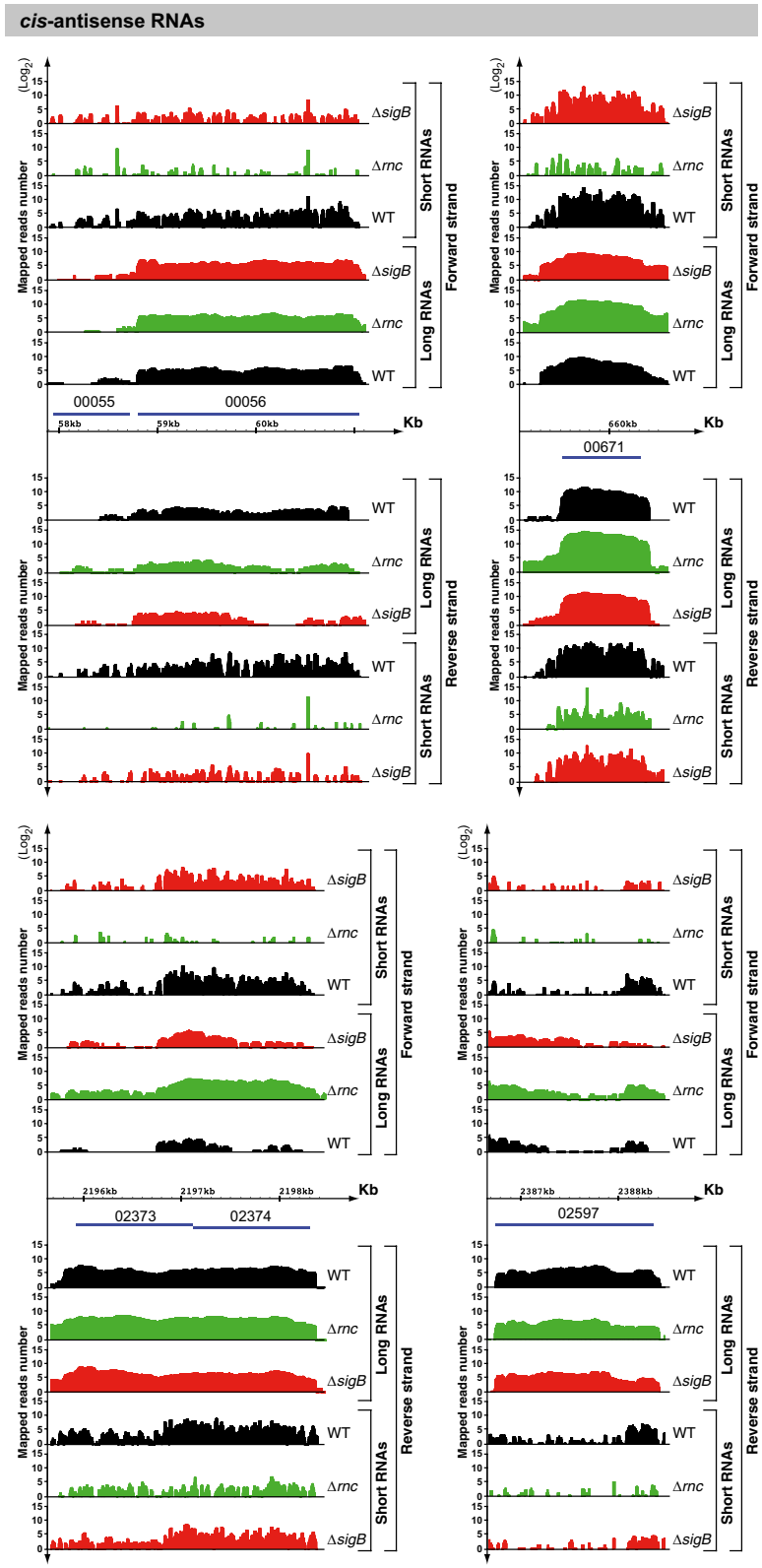


Fig. S4. Examples of mapped reads distribution in regions with *cis*-antisense RNAs in *S. aureus*. Drawings are images from IGB software showing different regions of the genome of *S. aureus* NCTC 8325. Genomic coordinates denote the position in kilobases of the *S. aureus* NCTC 8325 genome. Annotated ORFs are shown as blue lines. The number on the ORF indicates the gene identification. Long RNAs show the distribution of uniquely mapped reads of long RNA libraries in *S. aureus* 15981 (back), *S. aureus* 15981 Δ*rnc* (green), and *S. aureus* 15981 Δ*sigB* (red). Short RNAs shows the distribution of uniquely mapped reads of short RNA libraries using the same pattern of colors as described for long RNAs. The scale (log_2) indicates the number of mapped reads per nucleotide position.

A

Antisense RNA type		ORF target	SigB promoter* of the antisense RNA
Overlapping 3'-UTR	Long 3'-UTR of SAOUHSC_00356	SAOUHSC_00357	TAT**GAATAA**AATTAAAAACAAG--**GGGTAA**-TACAATCTA
Overlapping 3'-UTR	Long 3'-UTR of SAOUHSC_00569	SAOUHSC_00571	AGA**GTTACA**AACTCATCTTTT---**GGGTAT**-AGAATACCT
Antisense RNA		SAOUHSC_00056	AAT**GTTTAA**TGCCAGTGTGTCC--**GTGATA**TCTATATCAT
Antisense RNA		SAOUHSC_00573	TAT**GTTTCA**AATATTTCAAATA--**GGTTAA**AATAATATTA
Overlapping 3'-UTR	Long 3'-UTR of SAOUHSC_00569	SAOUHSC_00572	TAT**GTTTCA**AATATTTCAAATA--**GGTTAA**AATAATATTA
Overlapping 3'-UTR	Long 3'-UTR of SAOUHSC_00845	SAOUHSC_00846	AAT**GTTTAG**ATAATTTTTCAGT--**GGGTAA**GTATTATATA
Overlapping 3'-UTR	Long 3'-UTR of SAOUHSC_00134	SAOUHSC_00133	AAA**GAGTAT**AAAAATCATGTTA--**GGGTAT**AAGTAAATAA
Overlapping 3'-UTR	Long 3'-UTR of SAOUHSC_02994	SAOUHSC_02995	ATT**GATTCA**TTTTTATAGAAAC--**GGGTAA**AAATGATAAT
Antisense RNA		*murI*	TAG**GTTTAT**AGAGCAATGGATAGT**GGGTAC**-ATCCTAAAA
Overlapping 3'-UTR	Long 3'-UTR of SAOUHSC_00792	*clpP*	AAT**GCTTTA**AATTGATGTATAAT-**GGGAAT**AAGAAATAAA
Antisense RNA		*lexA*	ATT**GTTTTT**GGCATGAGATTAAA-**GGGTAA**TGTTTGTCTG
Antisense RNA		SAOUHSC_02571	CAC**GTTTAA**CTTAATGTGAGCG--**TGGAAT**ATATATAATA
Overlapping 3'-UTR	Long 3'-UTR of SAOUHSC_00826	SAOUHSC_00825	TTA**GTTTAA**AAGATAATGTGACG-**GGGTAA**-AACGCAATG

*The SigB promoter consensus sequence of *S. aureus* is GTTT[AT][AT]<N_{12-15}>GGG[AT]A[AT]

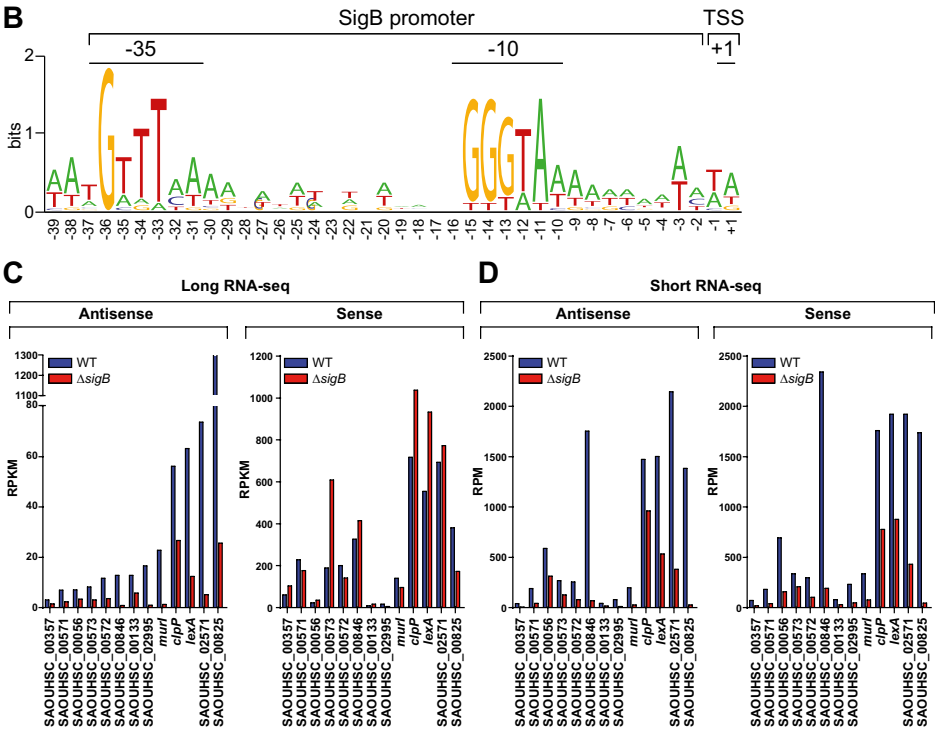


Fig. S5. Relationship between the amount of short RNAs and the level of antisense transcript. (*A*) The table shows the consensus sequence for Sigma B transcription factor in the promoter region of the 13 selected antisense transcripts. The conserved consensus sequence according to Bischoff et al. (1) is shown in bold. (*B*) Consensus logo generated from the conserved sequence in the promoters of the SigB regulated transcripts created using Weblogo tool (http://weblogo.berkeley.edu/). (*C*) Long RNA-seq data were used to select those ORFs for which the antisense transcript signal was reduced by at least a factor of 2 in Δ*sigB* (Δ*sigB*/WT < 0.5). Histogram shows the number of long RNA reads for sense and antisense transcripts of each selected ORF in wild type and Δ*sigB*, normalized to the length of the ORF and the number of uniquely mapped reads in millions (RPKM). (*D*) Histogram shows the number of short RNAs that mapped uniquely in sense and antisense transcripts of each ORF in wild type and Δ*sigB*, normalized to the number of uniquely mapped reads in millions (RPM). In all ORFs, the numbers of short RNAs in the sense and antisense strand decrease significantly in Δ*sigB* because of the reduction in long antisense RNA levels.

1. Bischoff M, et al. (2004) Microarray-based analysis of the *Staphylococcus aureus* sigmaB regulon. *J Bacteriol* 186:4085–4099.

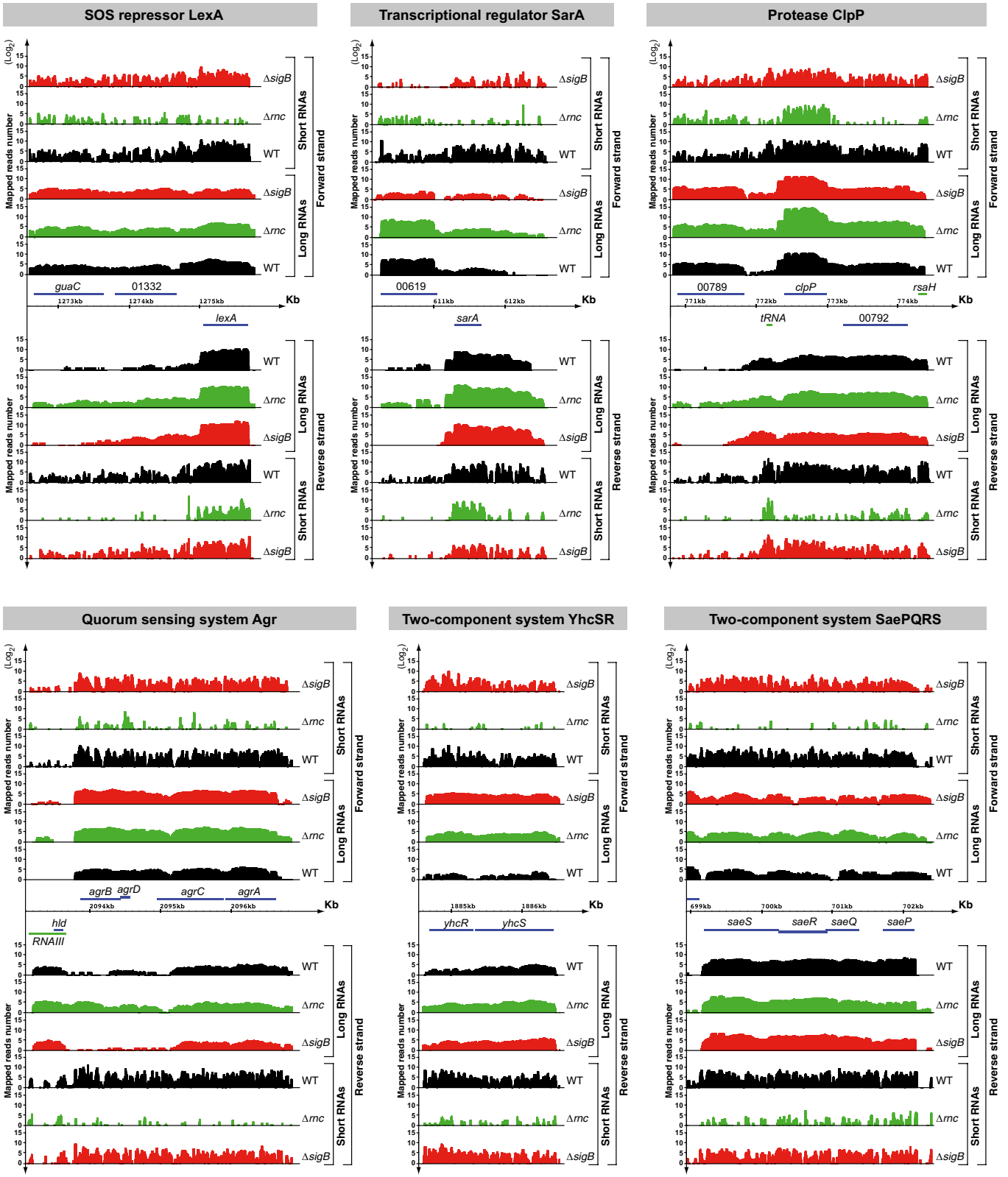


Fig. S6. Examples of mapped reads distribution for genes important for virulence and biology of *S. aureus*. Drawings are images from IGB software showing different regions of the genome of *S. aureus* NCTC 8325. Genomic coordinates denote the position in kilobases of the *S. aureus* NCTC 8325 genome. Annotated ORFs are shown as blue lines. The number on the ORF indicates the gene identification. Long RNAs show the distribution of uniquely mapped reads of long RNA libraries in *S. aureus* 15981 (black), *S. aureus* 15981 Δ*rnc* (green), and *S. aureus* 15981 Δ*sigB* (red). Short RNAs show the distribution of uniquely mapped reads of short RNA libraries using the same pattern of colors as described for long RNAs. The scale (log_2) indicates the number of mapped reads per nucleotide position.

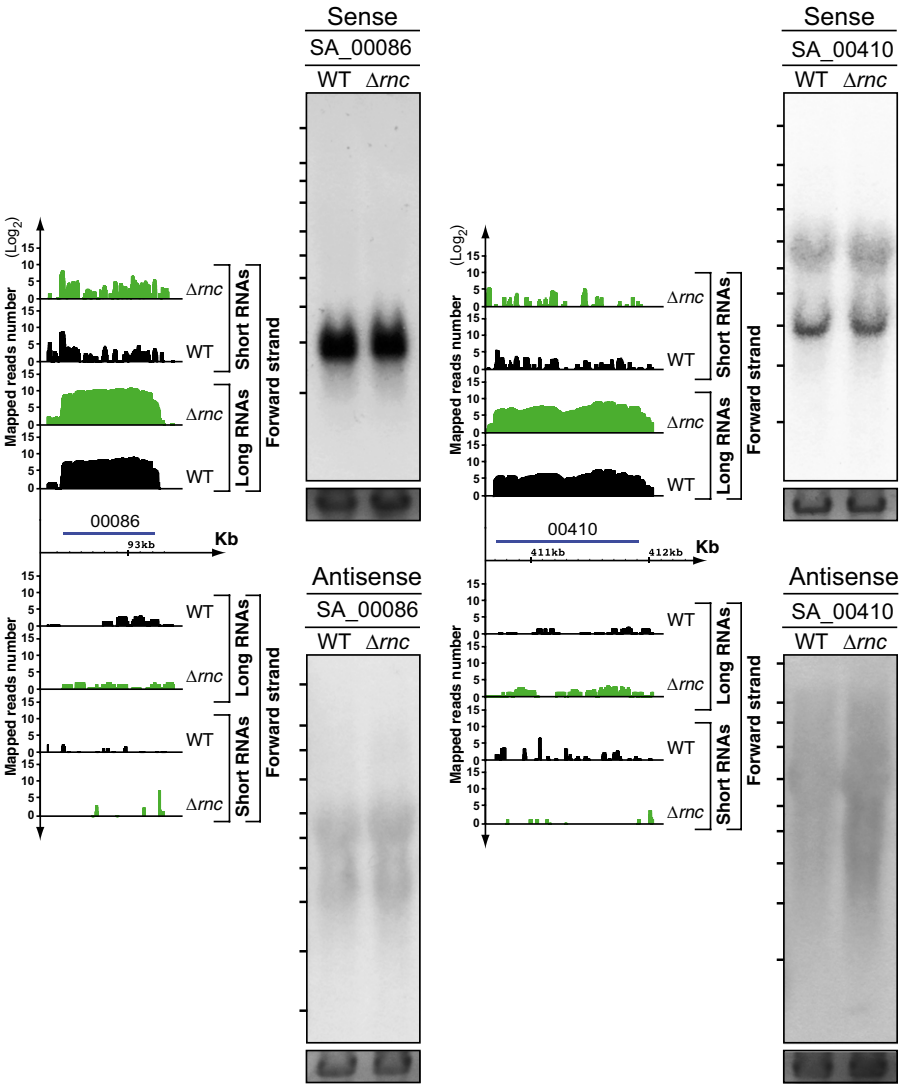


Fig. S7. Expression levels of sense/antisense transcripts. The drawings show the IGB software image for these particular regions of the genome of *S. aureus* NCTC 8325. Northern blot analysis of RNA harvested from *S. aureus* 15981 wild type and its corresponding *S. aureus* 15981 Δ*rnc* using specific riboprobes against sense and antisense strands of SAOUHSC_00086 and SAOUHSC_00410 ORFs. The transcription levels of the sense strand remained unaltered in the RNase III mutant consistent with the absence of an antisense transcript in the RNase III mutant.

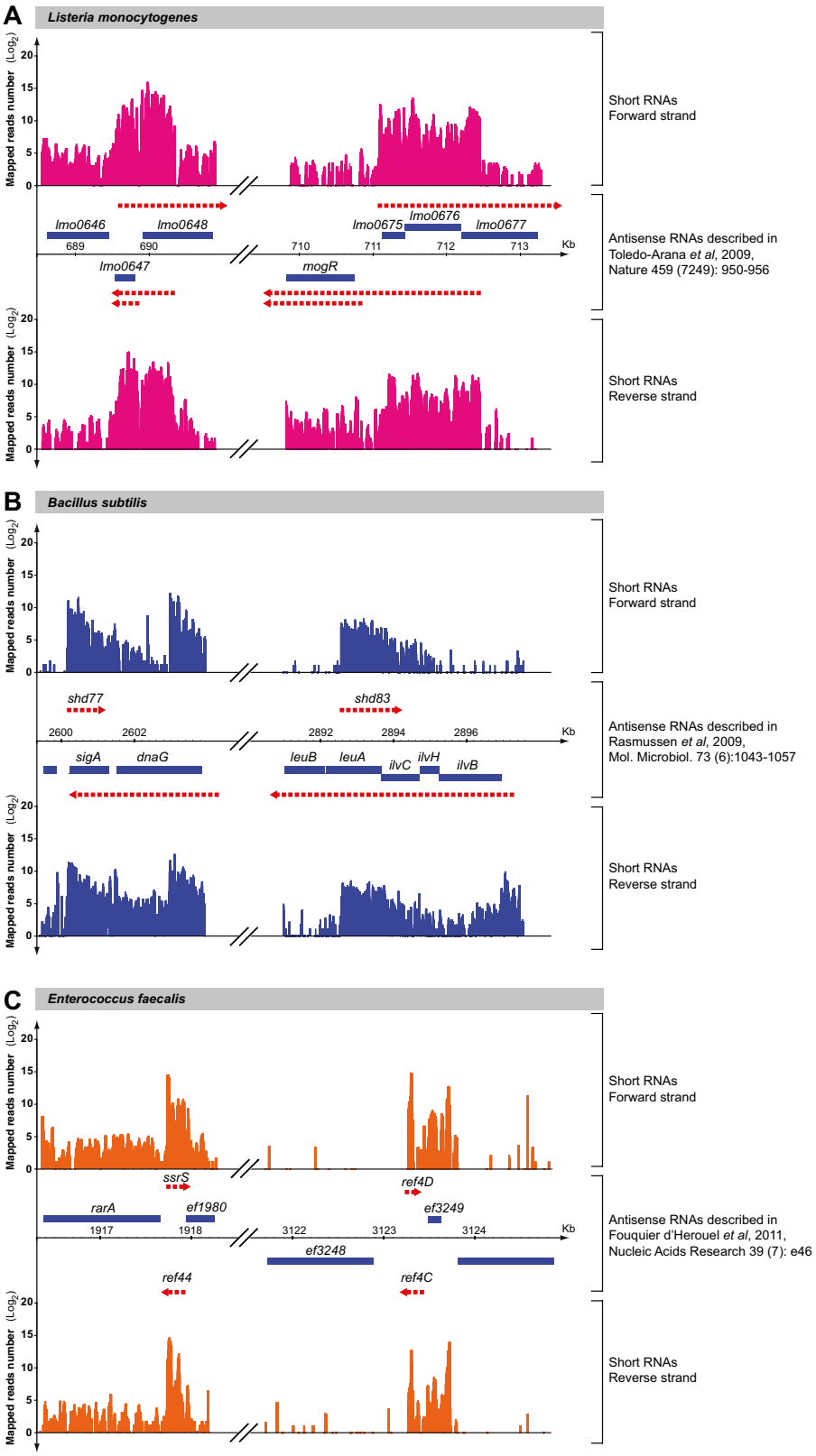


Fig. S8. Examples of mapped reads distribution of short RNA libraries in regions with overlapping transcription in diverse Gram-positive bacteria. Drawings are images from IGB software showing the distribution of uniquely mapped reads of short RNA libraries in regions with known antisense transcription in *L. monocytogenes* (*A*), *B. subtilis* (*B*), and *E. faecalis* (*C*). The dashed red lines show the location of the transcripts previously described. Genomic coordinates denote the position in kilobases of the *L. monocytogenes* EGD-e, *B. subtilis* 168, and *E. faecalis* V583 genomes, respectively. Annotated ORFs are shown as blue lines. The number on the ORF indicates the gene identification. The scale (log_2) indicates the number of mapped reads per nucleotide position.

Table S1. Strains and oligonucleotides used in this study

Name	Description or sequence
Strains	
S. aureus 15981	Clinical isolate (1)
15981 Δ*rnc*	15981 with deletion of the *rnc* gene (this study)
15981 Δ*SAOUHSC_01251*	15981 with deletion of the *SAOUHSC_01251 (pnp)* gene (this study)
15981 Δ*sigB*	15981 with deletion of the *sigB* gene (1)
Enterococcus faecalis JH2-2	Plasmid free wild-type strain (2)
Bacillus subtilis MIC1982	Our laboratory collection (this study)
Listeria monocytogenes EGD	Serotype 1/2a (3)
Salmonella enteritica ser. Enteritidis 3934	Clinical isolate (4)
Oligonucleotides	
SAOUHSC_01251 (pnp) deletion	
pnp-A	CAGAAGTACAAATCGC
pnp-B	TATAAATCTCCTCTCT
pnp-C	TTCAAGAGAGGAGATTTATAGCATCACACAGAGCATTAGAAGAA
pnp-D	TGCTCATGTCCGTGTG
Small RNA library sequencing	
3′ ModBan	AMP-5′p-5′pCTGTAGGCACCATCAAT-ddC
5′ Illumina RNA adapter	OH-rArCrArCrUrCrUrUrUrCrCrCrUrArCrArCrGrArCrGrCrUrCrUrUrCrCrGrArUrC-OH
RT-PCR	ATTGATGGTGCCTACAG
Sol_5_SBS3	aatgatacggcgaccaccgaACACTCTTTCCCTACACGACG
Sol_3_ModBan	CAAGCAGAAGACGGCATACGATTGATGGTGCCTACAG
Riboprobe synthesis	
RP T7 antisense sarA	**TAATACGACTCACTATAGGG**TAGGGAGGTTTTAAACATGG
RP antisense sarA	CCAAATTGCGCTAAACAAAA
RP sense sarA	TAGGGAGGTTTTAAACATGG
RP T7 sense sarA	**TAATACGACTCACTATAGGG**CCAAATTGCGCTAAACAAAA
RP T7 antisense lexA	**TAATACGACTCACTATAGGG**TTGTAAGTGATCAAACAAATG
RP antisense lexA	TTACATTTCGCGGTACAAAC
RP sense lexA	TTGTAAGTGATCAAACAAATG
RP T7 sense lexA	**TAATACGACTCACTATAGGG**TTACATTTCGCGGTACAAAC
RP T7 antisense clpP	**TAATACGACTCACTATAGGG**TTATTGAAACAACAAACCG
RP antisense clpP	TGTGATTTGCAGCAATTTCG
RP sense clpP	GTTATTGAAACAACAAACCG
RP T7 sense clpP	**TAATACGACTCACTATAGGG**TGTGATTTGCAGCAATTTCG
RP T7 antisense SaeS	**TAATACGACTCACTATAGGG**ACACTAACTTTGACCTTAAC
RP antisense SaeS	GTTGATAATCTGTTTGCTTG
RP sense SaeS	ACACTAACTTTGACCTTAAC
RP T7 sense SaeS	**TAATACGACTCACTATAGGG**GTTGATAATCTGTTTGCTTG
RP T7 antisense SA_00086	**TAATACGACTCACTATAGGG**GCAGTTGTTGATTTCAATGA
RP antisense SA_00086	AATGCATTCACAGTAATACC
RP sense 86	GCAGTTGTTGATTTCAATGA
RP T7 sense SA_00086	**TAATACGACTCACTATAGGG**AATGCATTCACAGTAATACC
RP T7 antisense SA_00410	**TAATACGACTCACTATAGGG**TATCGATAAAGATCTTGTCG
RP antisense SA_00410	ATCAAAACGCTGCGTATTCA
RP sense SA_00410	TATCGATAAAGATCTTGTCG
RP T7 sense SA_00410	**TAATACGACTCACTATAGGG**ATCAAAACGCTGCGTATTCA
RP T7 sense recF	**TAATACGACTCACTATAGGG**AGGGATTATTGAATCTGGTG
RP sense recF	CGTGCCGTGTCTATAACTAA
RP T7 antisense recF	**TAATACGACTCACTATAGGG**CGTGCCGTGTCTATAACTAA
RP antisense recF	AGGGATTATTGAATCTGGTG
RP T7 sense agr	**TAATACGACTCACTATAGGG**ATTTTATTGGTATAGGTTGC
RP sense agr	TAACAAAATTGACCAGTTTG
RP T7 antisense agr	**TAATACGACTCACTATAGGG**TAACAAAATTGACCAGTTTG
RP antisense agr	ATTTTATTGGTATAGGTTGC
RP T7 sense yhcSR	**TAATACGACTCACTATAGGG**TTGGCTAGGAAATGCTTTAG
RP sense yhcSR	TGAATTGGAGCGATTTG
RP T7 antisense yhcSR	**TAATACGACTCACTATAGGG**TGAATTGGAGCGATTTG
RP antisense yhcSR	TTGGCTAGGAAATGCTTTAG

In bold, T7 promoter sequence. Underlined, complementary sequence to fuse both AB and CD PCR fragments and generate the mutant allele.

1. Valle J, et al. (2003) SarA and not sigmaB is essential for biofilm development by Staphylococcus aureus. *Mol Microbiol* 48:1075–1087.
2. Jacob AE, Hobbs SJ (1974) Conjugal transfer of plasmid-borne multiple antibiotic resistance in *Streptococcus faecalis* var. zymogenes. *Journal of bacteriology* 117(2):360–372.
3. Mackaness GB (1962) Cellular resistance to infection. *J Exp Med* 116:381–406.
4. Solano C, et al. (2002) Genetic analysis of *Salmonella enteritidis* biofilm formation: critical role of cellulose. *Mol Microbiol* 43:793–808.