**SUPPORTING INFORMATION**

# Grad-seq guides the discovery of ProQ as a major small RNA binding protein

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## SI Materials and Methods

### *Bacteria and media*

For all experiments, *Salmonella enterica enterica* serovar Typhimurium SL1344 (see the complete list of strains in Tables S2 and S3) were streaked on LB plates with appropriate antibiotics and grown overnight at 37°C. Individual colonies were inoculated in liquid LB medium with the same antibiotics for subculturing overnight at 37°C with shaking at 220 rpm, and then diluted 1:100 in fresh LB medium with antibiotics for culturing to the desired density (exponential phase – $OD_{600}$ = 0.5, transition phase – $OD_{600}$ = 2, stationary phase – 6 h after the culture reached $OD_{600}$ = 2).

Deletion and FLAG-tagged strains were generated as described in (1, 2) with the use of gene-specific oligonucleotides (Tables S2 and S3). All initial deletion strains were transduced into fresh WT background with the use of P22 phage, and the kanamycin resistance cassettes were removed by pCP20 transformation to avoid polar effects, as described in (1). The *proQ* deletion removes most of the ORF (including the initiator codon) without disrupting the internal *prc* promoter (3).

For total RNA sample preparation, 4 $OD_{600}$ of culture were mixed with 1/5 volume of the 95% ethanol, 5% phenol, snap-frozen in liquid nitrogen and thawed on ice, and RNA was extracted with the TriZOL reagent (Life Technologies). For total protein preparation, 0.4-0.5 $OD_{600}$ of culture were pulse-pelleted and the cell pellet was resuspended in 100 µl 1×Laemmli buffer.

### *Protein gel electrophoresis and Western blotting*

Protein samples were resolved by 12% SDS-PAGE followed by Coomassie staining. For Western blotting, 15% SDS-PAGE was used, followed by semi-dry transfer on nitrocellulose membranes and probing with protein-specific antisera. FLAG-tagged proteins were detected with monoclonal anti-FLAG antibodies (Sigma, #F1804). Antisera for ribosomal proteins, ProQ, MukB/RNase E are kind gifts of Matthias Springer, Daniel Sheidy, and Agamemnon Carpousis, respectively. GroEL was detected with commercial antibodies (Sigma G6532).

### *RNA gel electrophoresis and Northern blotting*

RNA samples were resolved by 8% PAGE in 1×TBE and 7M urea and stained with ethidium bromide and/or transferred onto a Hybond+ membrane (GE Healthcare Life Sciences) and probed with RNA-specific oligonucleotides (Table S3). 5S rRNA was probed for as a loading control.

### *Glycerol gradient fractionation*

For lysis, 200 $OD_{600}$ of JVS-01338 (*hfq-3×FLAG*) bacterial culture grown to $OD_{600}$=2 (transition phase) were harvested by centrifugation at 4000 g at 4°C for 15 min, washed thrice with ice-cold 1×TBS and resuspended in 500 µl of the lysis buffer (20 mM Tris-HCl, pH7.5, 150 mM KCl, 1 mM $MgCl_2$, 1 mM DTT, 1 mM PMSF, 0.2% Triton X100, 20 U/ml DNase I, Thermo Scientific, 200 U/ml SUPERase-IN, Life Technologies). Lysis was carried out on a Retsch MM400 machine at 30 Hz for 10 min in the presence of 750 µl 0.1 mm glass beads (BioSpec Products). The lysate was cleared by centrifugation at 14,000 g at 4°C for 10 min and layered on a linear 10%-40% (w/v) glycerol gradient in the same buffer without DNase I nor SUPERase-IN, formed in a Beckman SW40Ti

tube with the use of the Gradient Station model 153 (Biocomp). The gradient was centrifuged at 100,000 g (23,700 rpm) for 17 h at 4°C and fractionated in 20 equal fractions by pipetting. $OD_{260}$ for each fraction was measured with Nanodrop. For protein analysis, 90 µl of each fraction were mixed with 30 µl of the 5× Laemmli loading buffer (for the pellet, 20 µl per 30 µl of Laemmli buffer were taken). Samples were stored at -20°C. For RNA isolation, the rest of each fraction was deproteinized by addition of 1% SDS and 1 volume of hot phenol and shaking at 1,500 rpm at 55°C for 5 min. Phases were separated by centrifugation at 12,000 g for 15 min at 4°C. The aqueous phases were added glycogen to 50 µg/ml and RNA was precipitated with 60% isopropanol. The RNA pellets were dissolved in 35 µl of DEPC-treated sterile MilliQ water and stored at -80°C.

***RNA-seq***

For Grad-seq, two biological replicates have been analyzed. 85 pg/µl of the spike-in RNA (5'P-CUCGUCCGACGUCACCUAGA, IBA) had been added to each fraction prior the library preparation. RNA-seq libraries were prepared by Vertis AG (Freising-Weihenstephan, Germany). Briefly, RNA was polyadenylated with poly(A) polymerase, 5'-triphosphates were removed with tobacco acid pyrophosphatase followed by ligation of a 5'-adapter. First-strand cDNA synthesis was performed with the use of an oligo(dT) barcoded adapter primer and the M-MVL reverse transcriptase. The resulting cDNA was PCR-amplified with a high fidelity DNA polymerase. cDNA was purified with the Agencourt AMPure XP kit (Beckman Coulter Genomics) and sequenced on an Illumina HiSeq2000 platform. The resulting reads were mapped to the S. Typhimurium SL1344 genome with the use of the READemption pipeline version 0.3.4 (4).

For in-gradient profiling, fraction-wise gene-specific RNA-seq read counts were normalized by corresponding spike-in counts. For few fractions, biases uniformly distorting all

profiles were evident: those were manually readjusted which allowed reconstitution of smooth profiles. To compare shapes of distributions by PCA, all profiles with ≥30 reads in at least one fraction were power-transformed to improve linearity and standardized to the range from 0 to 1. To derive averaged distributions for RNA classes, profiles of individual ncRNAs were standardized to the range from 0 to 1, summed up and averaged fraction-wise. For PCA, RNA profiles were power-adjusted to improve linearity (the exponent value – 0.8268 - was derived from the regression analysis of RNA mass vs spike-in reads fraction-wise). PCA was performed in R and visualized in R and Python. For sRNAs, PC1 (~44% of variance) mostly reflects the influence of few mRNA-derived and/or mRNA-overlapping sRNAs (following, like mRNAs, strong ribosomal components) and, therefore, is uninformative for our purpose of revealing groups of noncoding RNAs. We opted for PC2 (~22% of variance) and PC3 (~13% of variance) analysis instead, which allowed a better resolution and clustering of typical sRNAs. Both PC1 vs PC2 and PC2 vs PC3 plots are available on Figs S4. All molecular weight estimates provided in this work were made assuming a most frequently encountered moderately elongated shape of particles (5).

RNA-seq data are available in Gene Expression Omnibus (accession number GSE62988). The workflow implemented as Shell script and the analysis-specific tools are deposited at Zenodo at DOI:10.5281/zenodo.35176.

### *Affinity chromatography of MS2 aptamer-tagged sRNAs*

Affinity chromatography was performed on cell lysates from *Salmonella* cultures grown to the transition phase as described in (6) with addition of *in vitro* transcribed MS2-aptamer-tagged sRNAs. Templates for the latter were created by overlapping PCR with sRNA- and MS2-specific primers (see Table S3 for a detailed description). Each sRNA was assayed at least twice, usually

with both 3'- and 5'-positioning of the MS2 tag. For each series of experiments, MS2 RNA was used as a negative control. Proteins co-purified with bait sRNAs were visualized by silver staining and identified by LC-MS/MS. Those reproducibly found in replicate experiments and absent from respective MS2 control samples were considered specific binding partners.

***RNA co-immunoprecipitation***

For RNA coIP, 50 $OD_{600}$ of bacteria were grown to the desired growth stage and harvested by centrifugation. They were lysed as described in the 'Glycerol gradient fractionation' section. After clearing the lysate, the equivalent of 0.5 $OD_{600}$ was diluted to 90 µl with 1× Laemmli buffer (lysate protein sample) and stored at -20°C. The equivalent of 5 $OD_{600}$ was saved for RNA extraction with TriZOL (lysate RNA sample). The lysate was added 35 µl of monoclonal anti-FLAG M2 antibody (Sigma, #F1804) and rocked for 30 min at 4°C. Then 75 µl of prewashed Protein A sepharose (Sigma, #P6649) were added and the mixture was rocked for additional 30 min. Afterwards, beads were washed extensively with the lysis buffer, and similar flow-through and wash protein and RNA samples were collected. Beads were resuspended in the lysis buffer, mixed with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1, pH4.5, Roth) for 20 s and incubated at room temperature for 3 min. After centrifugation, the aqueous phase was precipitated with isopropanol (coIP RNA sample) and the organic phase was precipitated with acetone (coIP protein sample). The purified RNA samples were treated with DNase I (Thermo Scientific) to remove the residual DNA and reisolated with phenol:chloroform:isoamyl alcohol. The protein coIP samples were soaked in 1× Laemmli buffer and denatured at 95°C for 10 min. For Western blotting, the equivalents of 0.05 $OD_{600}$ (for lysate, flow-through and wash samples) or 5 $OD_{600}$ (for coIP samples) were loaded on the gel. For Northern blotting, the equivalents of 0.3 $OD_{600}$ and 0.03 $OD_{600}$ were analyzed, respectively. Independent RNA co-immunoprecipitation

experiments were performed in *E. coli* and in *Salmonella* in the stationary phase twice, in *Salmonella* in the exponential phase twice, in *Salmonella* in the transition phase four times, with very similar results.

***Analysis of coIP enrichment data***

Gene-wise read counts were normalized by the total number of mapped reads. Enrichment is then calculated as a quadruple ratio of the lysate and coIP read counts in the FLAG-tagged and WT (untagged) strains, thus accounting for both the input transcript abundance and the background nonspecific interaction with beads:

$$E = \frac{[FLAGcoIP][WTlysate]}{[FLAGlysate][WTcoIP]}$$

Only genes with at least 5 reads in each of the four samples were used for enrichment calculations. The read countings of coIP libraries and lysate libraries were compared for samples from exponential, transition and stationary growth phases in 2, 4, and 2 biological replicates, respectively (which was expected to ensure the statistical power of 0.5-0.8, based on previously reported guidelines (7)). Enrichment factors were calculated with DESeq2 (8) but using the total number of aligned reads for the normalization. Transcripts enriched with $\log_2$(enrichment) values over two medians (i.e. 0.91, 1.13, and 0.75 for exponential, transition, and stationary phases, respectively, resulting in cut-off fold-changes of 3.76-fold, 4.38-fold, and 3.36-fold, respectively.) and an FDR-adjusted *P*-value below 0.05 were declared “ProQ-bound”.

***Mass spectrometry***

Each sample (gradient fraction or affinity chromatography pull-down) was subjected to one-dimensional gel electrophoresis followed by LC-MS/MS analysis. We followed the protocol published in (9) with minor modifications. In brief, each of the 20 gel lanes was cut into 10 equidistant pieces and subjected to tryptic digestion. Eluted peptides were then loaded and desalted on a self-packed reversed phase C18 column using a Proxeon EasynLC II. Peptides were separated in a binary gradient of 85 minutes from 1 to 99% buffer B (0.1% (v/v) acetic acid in acetonitrile; buffer A: (0.1% (v/v) acetic acid)) with a constant flow rate of 300 nL/min. MS and MS/MS data were recorded with an LTQ Orbitrap (Thermo) coupled online to the LC- setup. Each scan cycle consisted of a survey scan with a resolution of R = 30,000 in the Orbitrap section followed by dependent scans (MS/MS) of the five most abundant precursor ions. Database searching of the MS/MS '*.raw' data was done with Sorcerer-SEQUEST (ThermoFinnigan; version v.27, rev. 11) against the *S. enterica* strain SL1344 using a target decoy protein sequence database (complete proteome set of *S. enterica* strain SL1344 with a set of common laboratory contaminants). The resulting out files were compiled with Scaffold 4. Proteins were only considered as identified if at least 2 unique peptides matching quality criteria (delta cN > 0.1 and XCorr > 2.2; 3.5; 3.75 for doubly, triply or higher charged peptides) have been identified. For sedimentation profiling of proteins in glycerol gradients, two biological replicates have been analyzed. For reconstruction of in-gradient profiles, only proteins with ≥5 spectral counts in the peaking fraction were retrieved. The raw spectral counts were then normalized by the total number of spectral counts in each fraction and multiplied by the intensity of the Coomassie staining across the corresponding lane. The mass spectrometry proteomics data are deposited to the ProteomeXchange Consortium via the PRIDE partner repository (dataset identifier PXD003360).

### *ProQ purification and electrophoretic mobility shift assay (EMSA)*

The S. Typhimurium *proQ* gene was cloned into pTYB11 plasmid (NEB) to allow the intein-based expression and purification (IMPACT) of a tagless protein, as described in the manufacturer's protocol. For EMSA, 5 nM $^{32}$P-5'-end labeled *in vitro* transcribed RNA (MEGAscript T7 transcription kit, Life Technologies) were incubated with varying concentrations of ProQ (5 nM to 1 μM) in the reaction buffer (25 mM Tris-HCl, pH7.4, 100 mM NaCl, 1 mM $MgCl_2$) at 37°C for 20 min. After binding, the samples were resolved by native 8% PAGE in 0.5× TBE at 4°C, the gel was vacuum-dried and bands were visualized with Phosphorimager. Apparent $K_d$ values were estimated with the Scatchard procedure which accounts for partial inactivation of the protein. For competition assays, 1-1,000-fold molar excess of yeast tRNA (LifeTechnologies) or the same cold RNA were used as nonspecific and specific competitors, respectively.

### *RNA-seq analysis of ΔproQ*

For total RNA sequencing, RNA samples from WT carrying the empty pJV300 plasmid, Δ*proQ* strain carrying the empty pJV300 plasmid, and Δ*proQ* strain complemented with a pZE12-proQ plasmid ($n$ = 3 for each strain, which was expected to ensure the statistical power of ~0.7, based on previously reported guidelines (7)) were collected at $OD_{600}$ = 2, as described above. cDNA preparation, RNA-seq and read mapping were performed as described in the 'RNA-seq' section. Gene expression changes were analyzed with RUV-edgeR (10) to account for nonuniform RNA fragmentation across replicates during cDNA library generation.

***RNA in vivo stability assay***

Bacterial cultures were grown in a water bath to the stationary phase, added 500 μg/ml rifampicin, and 4 $OD_{600}$ samples were collected 0, 1, 2, 4, 8, 16, and 32 min later. Each sample was immediately mixed with 1/5 volume of 95% ethanol and 5% phenol and frozen in liquid nitrogen. RNA was isolated with TriZOL (Life Technologies) and analyzed by Northern blotting with the use of ImageQuant Tools. 5S rRNA was used as loading control.

***Statistical and other analyses***

Most descriptive statistical analyses have been performed in Excel. Most statistical tests used in this work are nonparametric to avoid the assumptions of normal distribution and homoscedasticity, with big sample sizes ensuring adequate statistical power. Fisher's exact test was done with the use of GraphPad QuickCalcs (www.graphpad.com/quickcalcs/). Mann-Whitney test, Pearson's and Spearman's correlations and regression analysis were carried out with the use of Free Statistics and Forecasting Software v1.1.23-r7 (11). Wilcoxon matched-pairs signed-ranks test calculator is available on http://www.fon.hum.uva.nl/Service/ank_Test.html. Kolmogorov-Smirnov test is performed with Statistics to use (12). All tests were two-sided. KEGG enrichment analysis was performed with the R. package "clusterProfiler".

Multiple alignment of selected sequences of the ProQ/FinO family was carried out with COBALT (13). It included the following species: γ-proteobacteria - *ECO* FinO, *Escherichia coli* FinO, *ECO* ProQ, *Escherichia coli* ProQ, *SEN*, *Salmonella enterica* ProQ, *VCH*, *Vibrio cholerae* ProQ, *LPN*, *Legionella pneumophila* ProQ, *MRH*, *Marinobacterium rhizophilum* ProQ, *SAM, Succinomonas amylolytica* ProQ, Phage ΦEa104, *Erwinia* phage phiEa104 FinO-like protein; α-proteobacteria – *SME, Sinorhizobium meliloti* FinO-like protein, *MAL, Mesorhizobium alhagi* FinO-like protein, *AFE,*

*Afipia felis* FinO-like protein, *OTH*, Candidatus *Odyssella thessalonicensis* FinO-like protein; β-proteobacteria – *BVI, B. vietnamiensis* ProQ, *NME, Neisseria meningitidis* 1681 (14).

The aligned sequences of the ProQ/FinO family proteins (InterPro IPR016103) were downloaded from PFAM and redundant sequences removed. Based on this non-redundant alignment a phylogenetic tree was constructed with the use of PhyML (with 600 bootstraps rounds), Phylip (Version 3.2), Cladisitics 5, and Clann (15). The resulting tree in Newick format was visualized using iTOL (16).

Predictions of RNA secondary structures are done with *RNAfold* (17). For analyses shown in Fig. S9E,F, only sRNAs with well-defined termini were retained, which was necessary for high-confidence folding energy predictions. This criterion was pre-established.

## SI figures

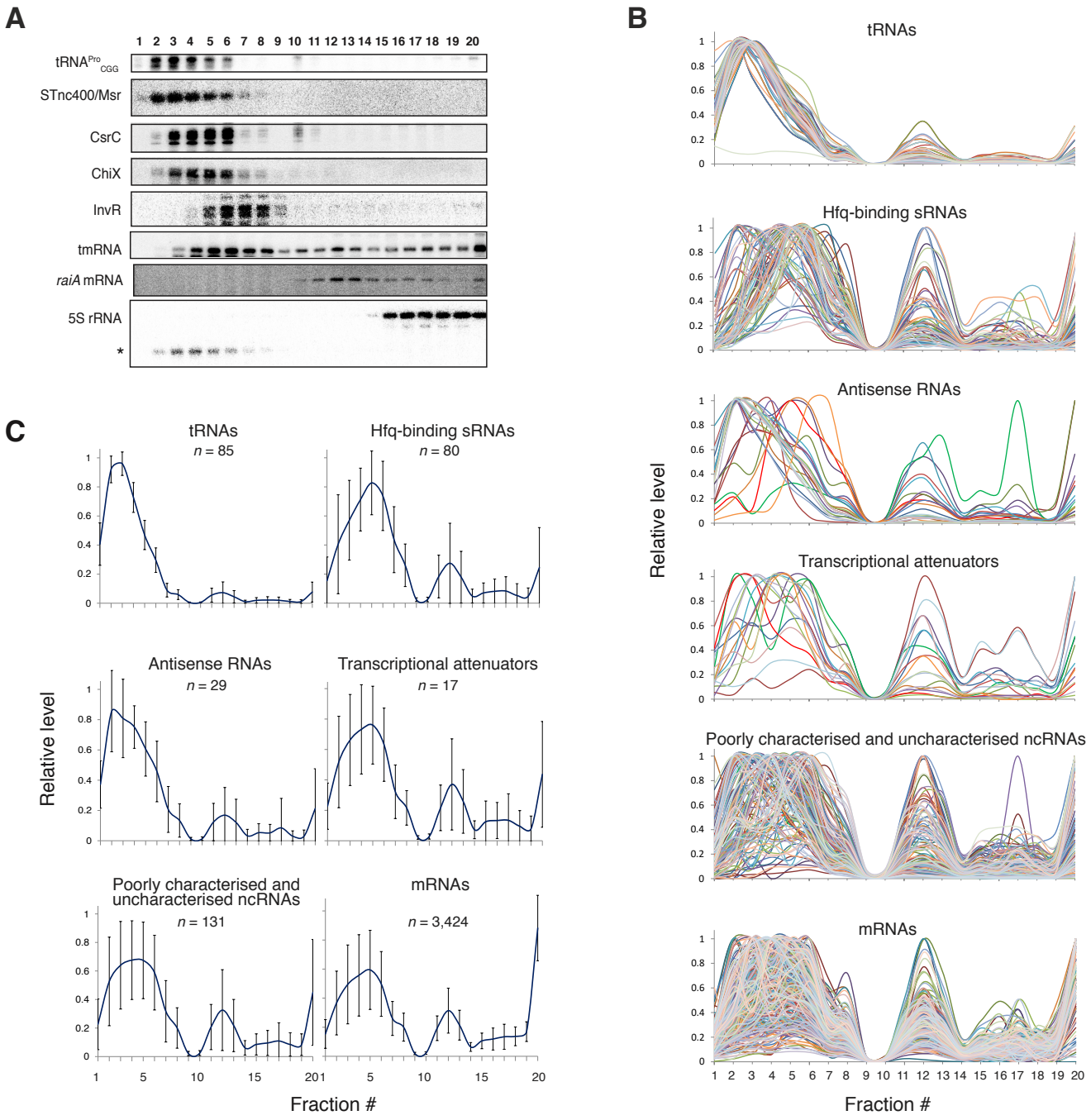


**Figure S1. Grad-seq profiles reveal diverse biochemical behavior of bacterial RNA classes. (A)** Northern blots of samples shown on Figs. 1 and S3 probed with RNA-specific oligonucleotides. Retron-encoded STnc400/Msr, CsrA-sequestering CsrC, Hfq-dependent ChiX and InvR, and tmRNA represent different groups of bacterial ncRNAs. A subpopulation of 5S rRNA not assembled into 50S ribosomal subunits is marked with an asterisk. **(B)** Grad-seq in-gradient distributions of major known bacterial RNA classes, as determined by RNA-seq (all profiles are standardized to the range from 0 to 1). **(C)** Averaged Grad-seq profiles of major known *Salmonella* RNA classes. All individual profiles of RNAs from each class were cumulated and presented as an average along the gradient ±SD.

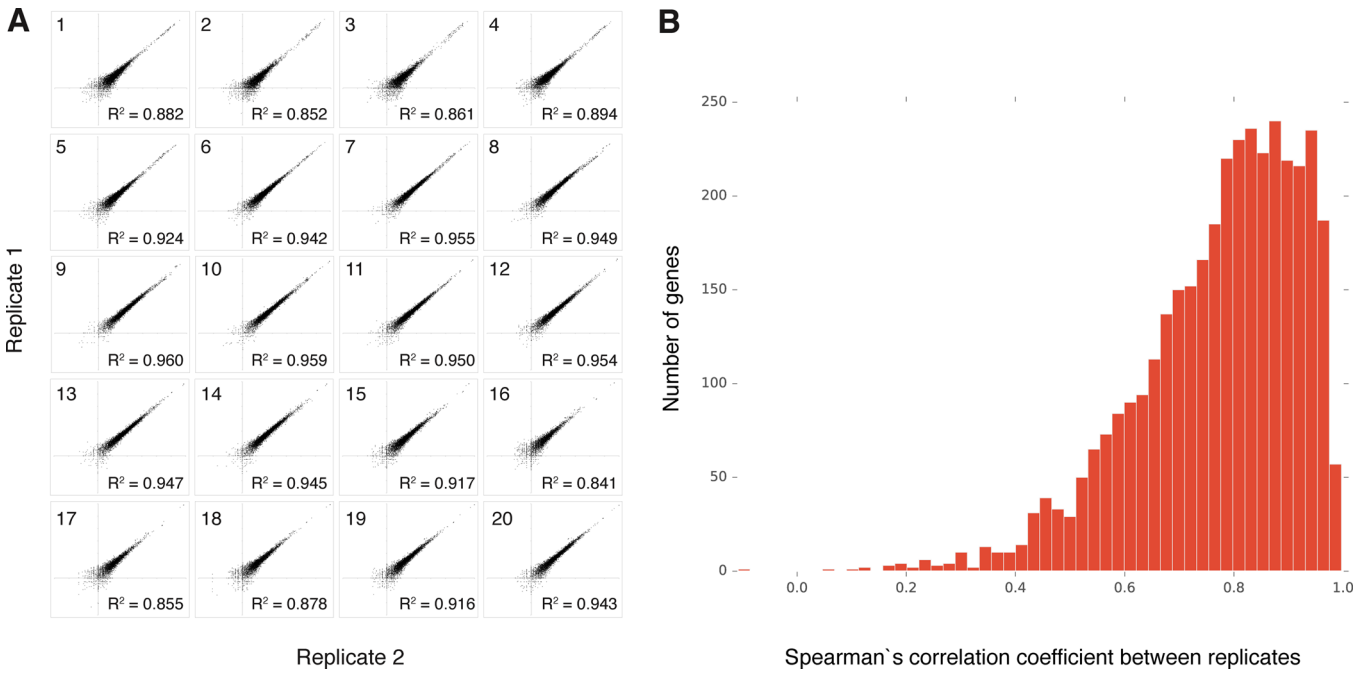


**Figure S2. Evaluation of Grad-seq reproducibility. (A)** Fraction-wise reproducibility of RNA-seq data used for reconstruction of Grad-seq RNA distributions. Pearson's determination coefficients are computed on log-transformed RNA-seq read counts for two biological gradient replicates. **(B)** Distribution of Spearman's correlation coefficients of individual Grad-seq profiles between the two replicates. All individual profiles of RNAs from each class were cumulated and presented as an average along the gradient ±SD.

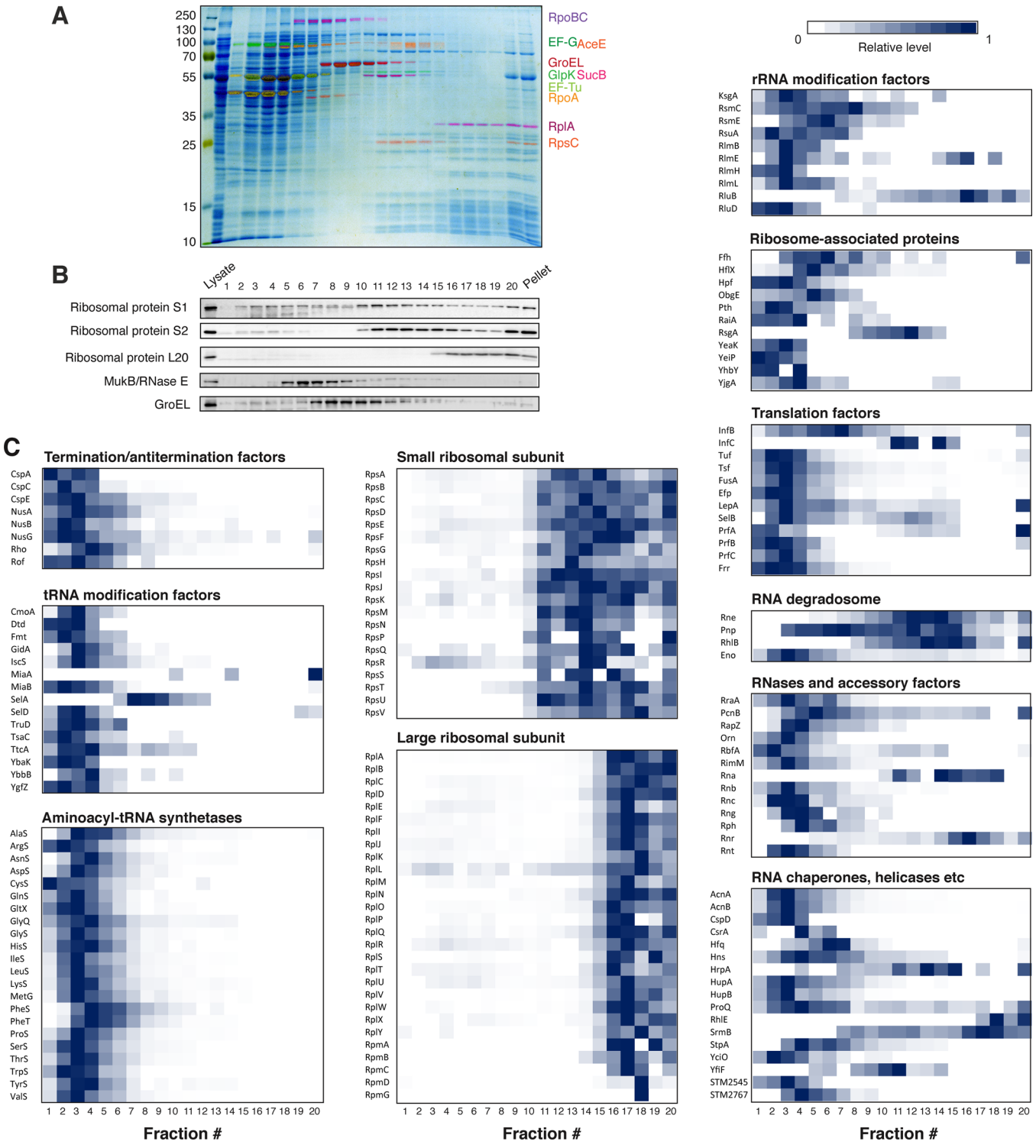


**Figure S3. Complexes formed by Salmonella proteins as visualized by glycerol gradient sedimentation followed by mass-spectrometric analysis of fractions. (A,B)** Distributions of major *Salmonella* proteins in a glycerol gradient analyzed by conventional techniques. *Salmonella* Typhimurium SL1344 cells grown to the transition phase were lysed and natively resolved on a 10-40% glycerol gradient. Fractions are numbered from the top to the bottom of the gradient. **(A)** Coomassie-stained SDS gel showing the protein profile of the gradient. Selected bands identified with LC-MS/MS are highlighted in false color and identified on the right. The ladder (in kDa) is shown on the left. **(B)** Western blots of samples shown on panel (A) probed with protein-specific antisera. **(C)** In-gradient distributions of major groups of known and putative RBPs, their complexes and associated factors, as determined by LC-MS/MS. All profiles are standardized to the range from 0 to 1.

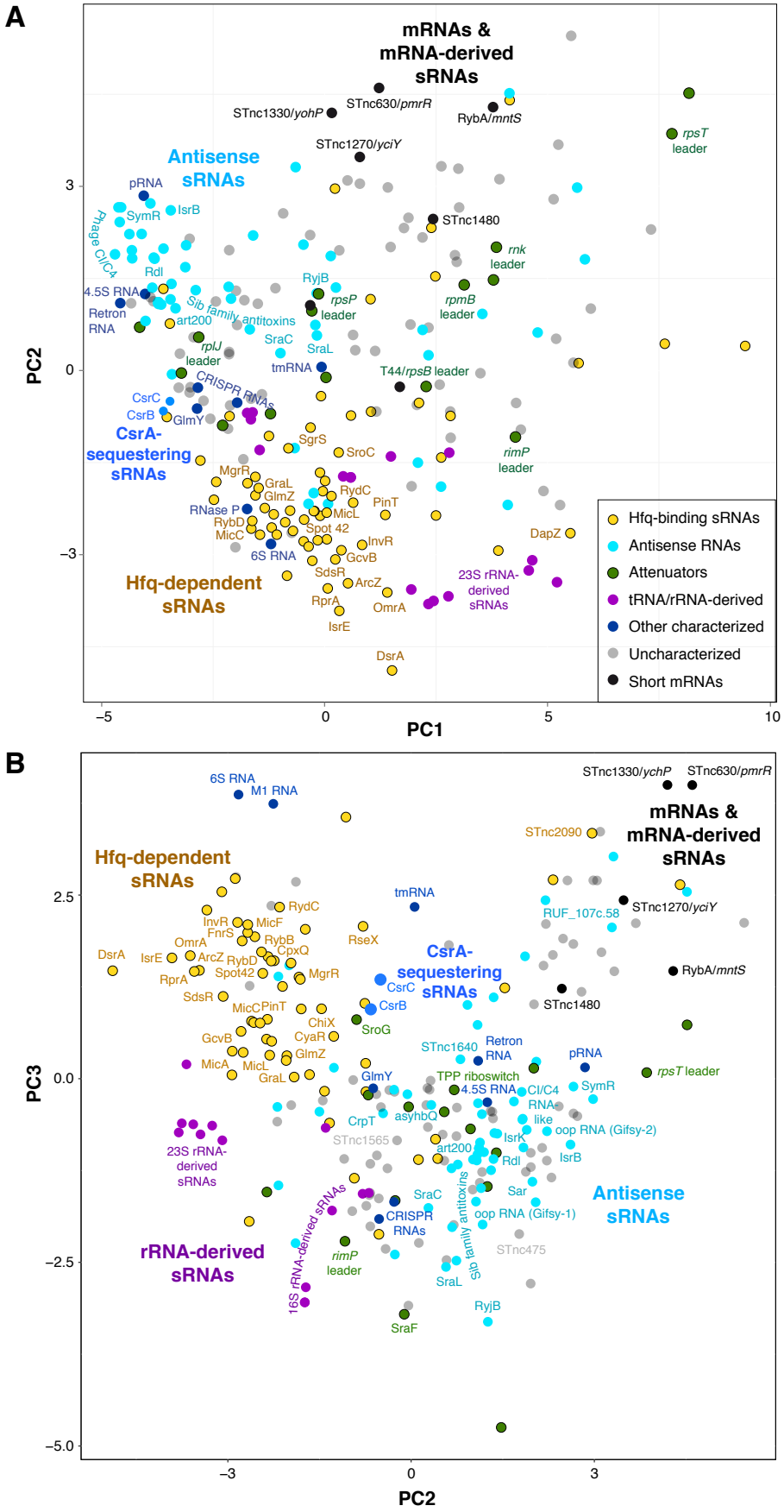


**Figure S4. Principal component analyses (PCA) of Grad-seq profiles of *Salmonella* sRNAs.** Grad-seq PCA plots of 238 *Salmonella* sRNAs with detailed annotation. PC1 (~44% of variance) vs PC2 (~22% of variance, panel A) and PC2 vs PC3 (~13% of variance, panel B) plots are shown.

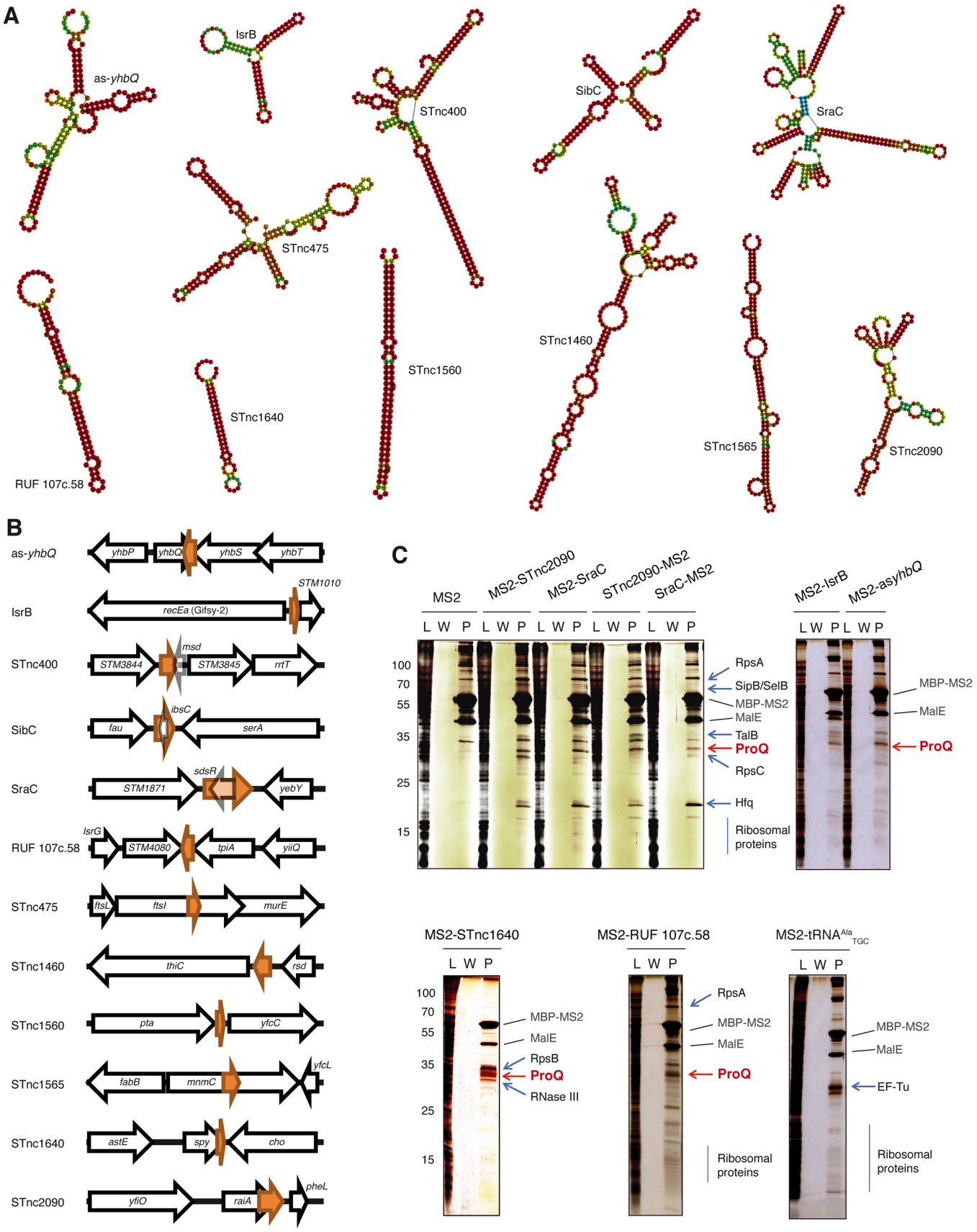


**Figure S5. Pull-down of protein partners interacting with select unusually distributed MS2 aptamer-tagged sRNAs from *Salmonella* cell lysates. (A,B)** Predicted secondary structures (panel A) and the genomic context (panel B) of the selected sRNAs (orange arrows). **(C)** Examples of affinity chromatography experiments. Lysate (L), wash (W) and pull-down (P) fractions are resolved on SDS gels and silver-stained. Specific protein bands which were not observed in MS2 aptamer controls are identified by LC-MS/MS. MBP-MS2 is maltose-binding protein fused with the MS2 phage coat protein, MalE is the endogenous maltose-binding protein. The upper left panel presents experiments with both 5'- and 3'-MS2-tagged sRNAs. tRNA on the bottom right panel is shown as an additional control. Ladder (in kDa) is shown on the left.

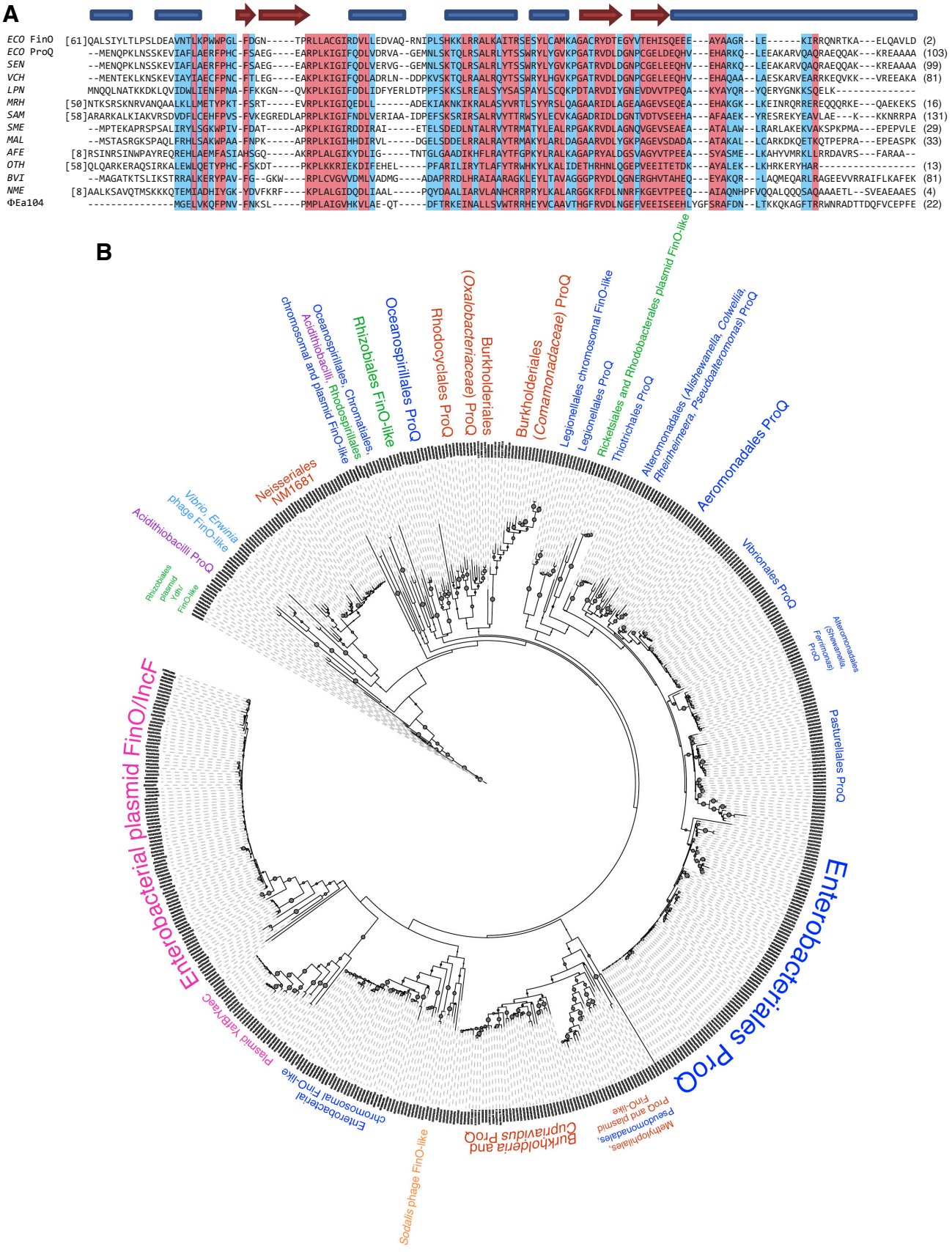


**Figure S6. ProQ/FinO protein family. (A)** Alignment of FinO-like domains of selected members of the ProQ/FinO family from various proteobacteria (see SI Materials and Methods for further detail). Conserved residues are red, no-gap positions are blue. FinO structural elements (α-helices: blue boxes, β-sheets: red arrows) are mapped according to (18). **(B)** Phylogenetic tree of known representatives of the ProQ/FinO family. α-, β-, γ-proteobacteria and Acidithiobacilli groups are shown in blue, red, green and purple, respectively. Other colors are used to highlight diverse plasmid- and phage-encoded members found in γ-proteobacteria (mostly enterobacteria).

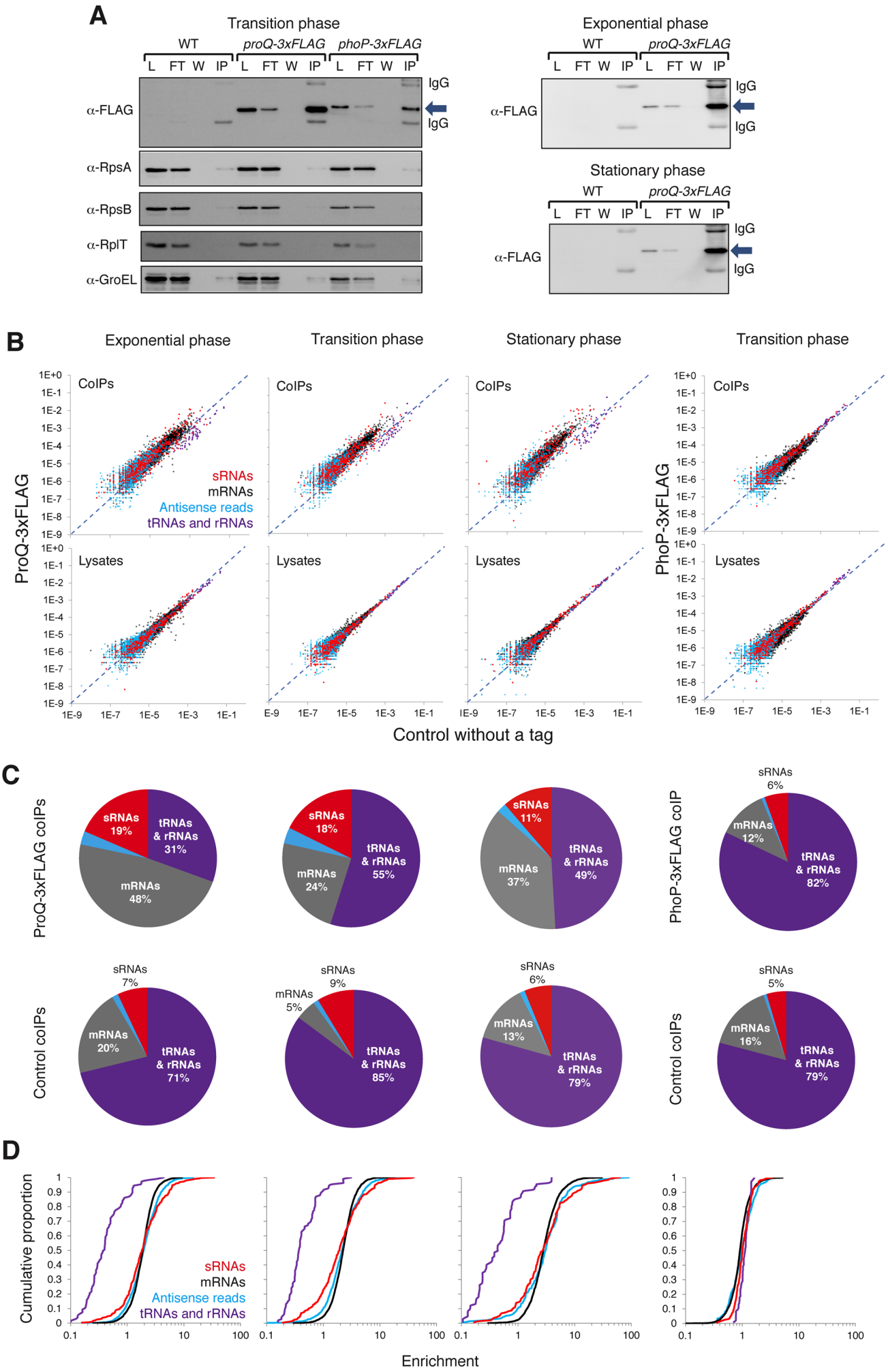
A
Transition phase
WT
proQ-3xFLAG
phoP-3xFLAG
L FT W IP L FT W IP L FT W IP
α-FLAG
α-RpsA
α-RpsB
α-RplT
α-GroEL
IgG
IgG
Exponential phase
WT
proQ-3xFLAG
L FT W IP L FT W IP
α-FLAG
IgG
IgG
Stationary phase
WT
proQ-3xFLAG
L FT W IP L FT W IP
α-FLAG
IgG
IgG
B
Exponential phase
Transition phase
Stationary phase
Transition phase
CoIPs
Lysates
sRNAs
mRNAs
Antisense reads
tRNAs and rRNAs
ProQ-3xFLAG
PhoP-3xFLAG
Control without a tag
C
ProQ-3xFLAG coIPs
sRNAs 19%
tRNAs & rRNAs 31%
mRNAs 48%
sRNAs 18%
tRNAs & rRNAs 55%
mRNAs 24%
sRNAs 11%
tRNAs & rRNAs 49%
mRNAs 37%
PhoP-3xFLAG coIP
sRNAs 6%
mRNAs 12%
tRNAs & rRNAs 82%
Control coIPs
sRNAs 7%
mRNAs 20%
tRNAs & rRNAs 71%
sRNAs 9%
mRNAs 5%
tRNAs & rRNAs 85%
sRNAs 6%
mRNAs 13%
tRNAs & rRNAs 79%
Control coIPs
sRNAs 5%
mRNAs 16%
tRNAs & rRNAs 79%
D
Cumulative proportion
sRNAs
mRNAs
Antisense reads
tRNAs and rRNAs
Enrichment

**Figure S7. ProQ is a global RNA-binding protein. Co-immunoprecipitation of *Salmonella* ProQ-associated RNAs.** **(A)** Chromosomally FLAG-tagged ProQ (blue arrow) was detected on Western blot with FLAG-specific antibodies in lysate (L), flow-through (FT), wash (W) and immunoprecipitation (IP) fractions. An untagged WT strain and a strain with a chromosomally FLAG-tagged DNA-binding protein, PhoP, were used as negative controls. The absence of contamination with other abundant proteins was verified with specific antisera. **(B)** Normalized read counts before (Lysates) and after (coIPs) pull-downs performed in three growth phases are plotted versus WT negative control. **(C)** Read distributions between RNA classes in ProQ-3×FLAG, PhoP-3×FLAG and WT control coIPs for the corresponding experiments. **(D)** Cumulative enrichment distributions for the corresponding experiments. The figure shows representatives of at least two independent experiments in each growth phase.

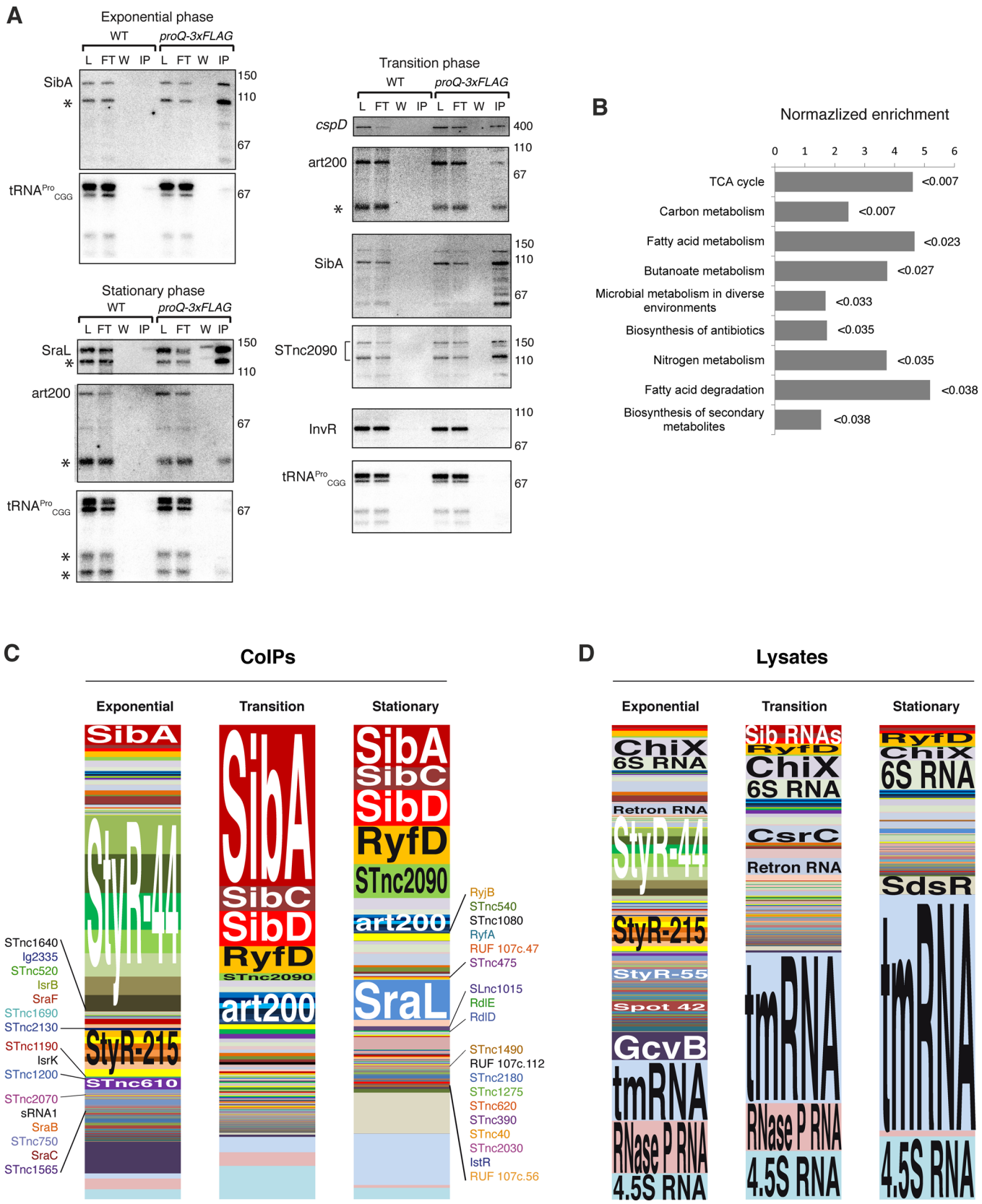


**Figure S8. Diversity of ProQ-binding RNAs revealed by RNA co-immunoprecipitation. (A)** Northern blot detection of ProQ-associated RNAs in co-immunoprecipitation experiments shown in Fig. S7. RNA was isolated from lysate (L), flow-through (FT), wash (W) and immunoprecipitation (IP) fractions. Noncoding RNAs SibA, SraL, art200 and STnc2090 as well as the *cspD* mRNA are top ProQ targets. An Hfq-dependent sRNA, InvR, and a tRNA are shown here as examples of non-enriched transcripts. Stable RNA fragments are marked with asterisks. The positions of size markers (in nt) are shown on the right of each blot. **(B)** KEGG enrichment analysis of the mRNAs co-immunoprecipitated with ProQ. Adjusted *P*-values are provided on the right of each category. Only significantly enriched pathways (FDR < 0.05, $P_{adj} < 0.05$) are shown. **(C)** Dynamics of the noncoding ProQ interactome over growth. The chart shows sRNA read distributions in ProQ coIPs over three growth phases in *Salmonella*. StyR-44 and StyR-215 are families of ncRNAs derived from rRNA operons. art200 is a family of IS200 transposon-derived antisense RNAs. SibACD are antitoxins in Sib/*ibs* type I toxin-antitoxin systems. **(D)** sRNA read distributions in the corresponding lysates. Most prominent sRNAs are named. sRNAs are plotted in the same order in all columns on panels (B) and (C).

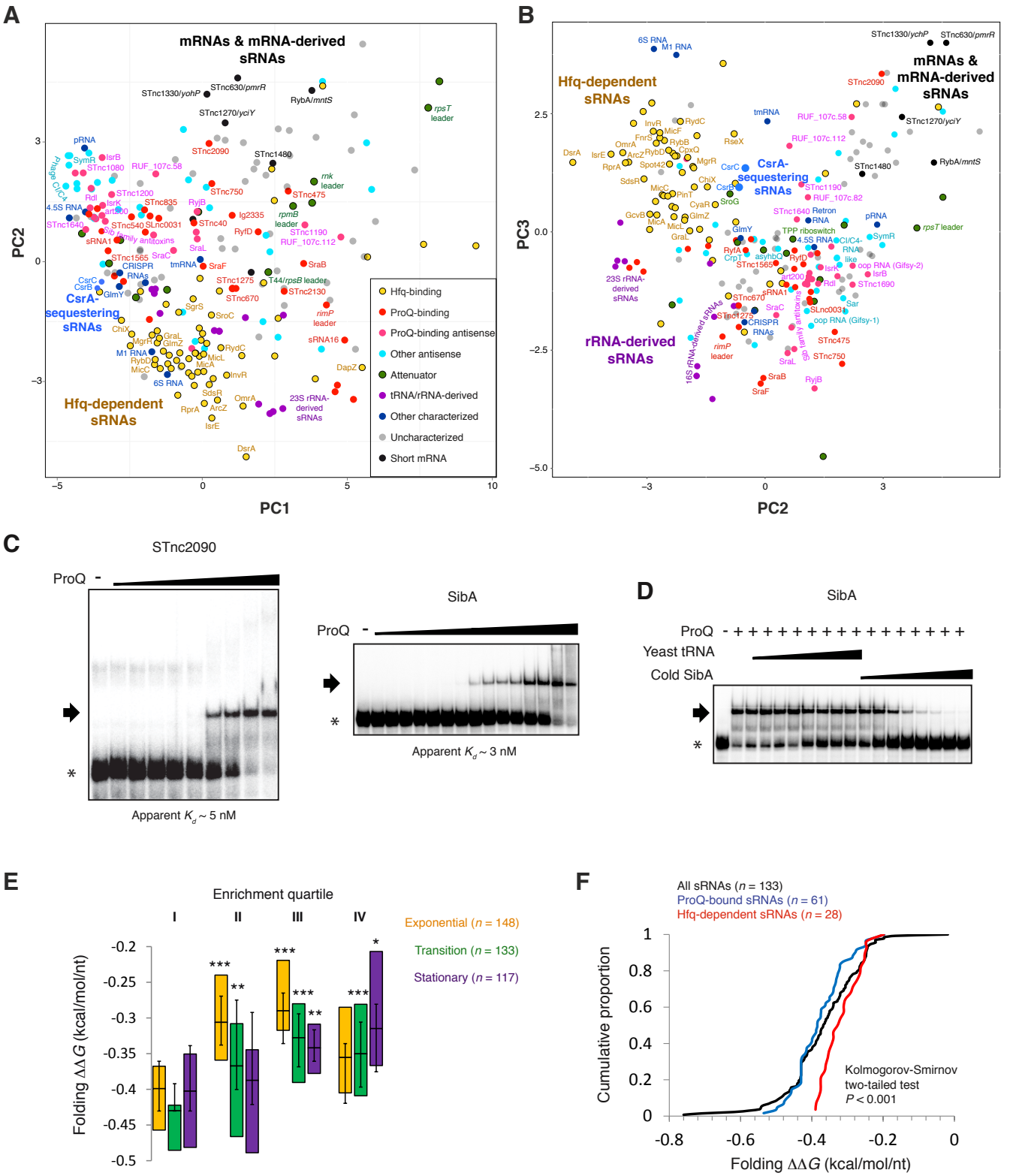


**Figure S9. ProQ-interacting sRNAs form a distinct class of highly structured transcripts. (A,B)** Grad-seq PCA of 238 *Salmonella* sRNAs showing the group of ProQ-associated RNAs with detailed annotation on PC1 vs PC2 (panel A) and PC2 vs PC3 (panel B) plots (cf. Figs. 4C and S4). **(C)** EMSAs performed with purified ProQ (0-1,000 nM) and two of its highly enriched ligands. Asterisks and arrows mark free RNA and ProQ-RNA complexes, respectively. Apparent $K_d$ values were estimated by Scatchard procedure. **(D)** Competition assay of the ProQ-SibA complex with yeast tRNA and cold SibA (0-1,000-fold molar excess) as a nonspecific and a specific competitor, respectively. **(E)** ProQ prefers structured RNAs. Shown are the median predicted thermodynamic ensemble free energies of folding (normalized by the transcript length), interquartile ranges (boxes) and approximate 95% CIs of the medians (whiskers) for sRNAs binned by enrichment quartiles in three growth phases (I corresponds to most highly enriched species, IV groups worst binders). * $P$ = 0.08, ** $P$ < 0.04, *** $P$ < 0.003 (Mann-Whitney test, FDR-adjusted, all comparisons with quartile I). **(F)** ProQ-associated sRNAs are significantly more structured than Hfq-dependent sRNAs. Cumulative distributions of predicted length-normalized thermodynamic ensemble folding free energies for sRNA groups are shown.

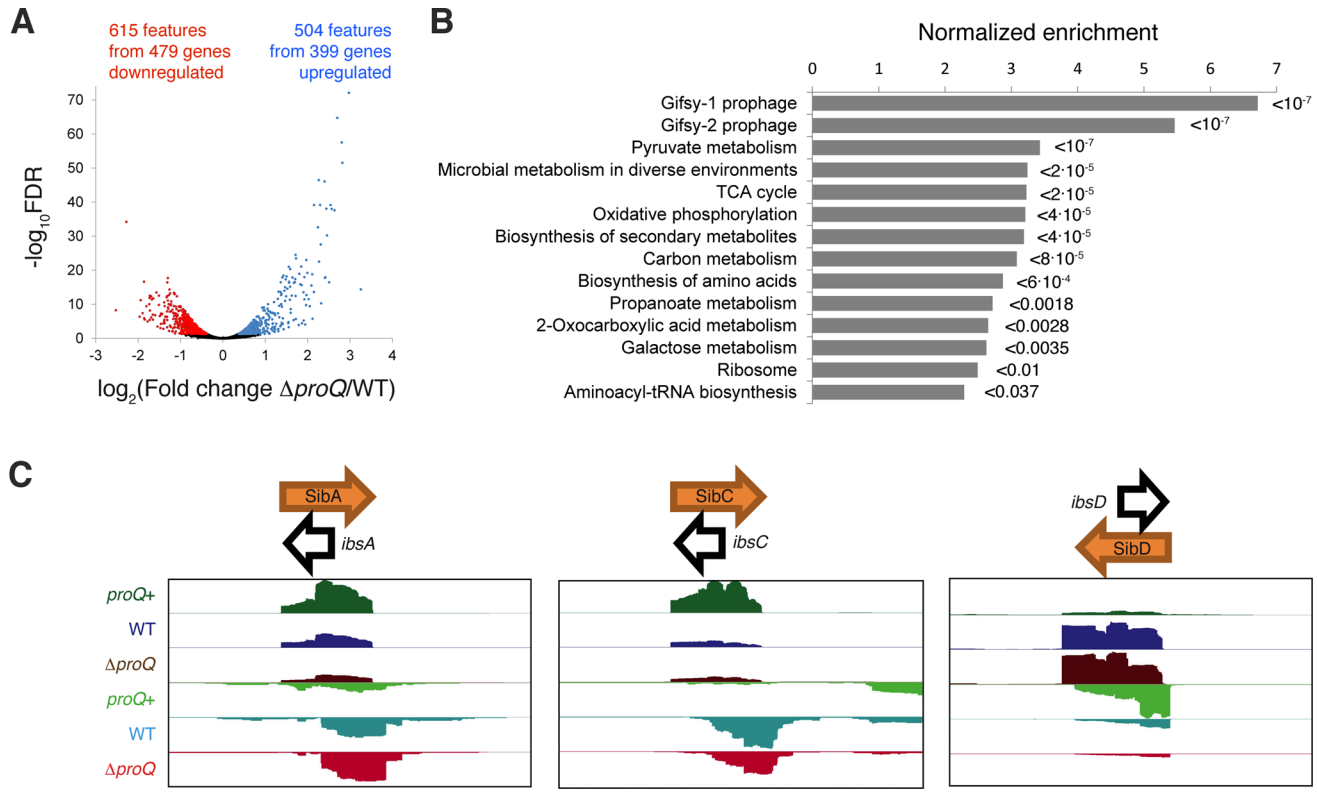


**Figure S10. ProQ is a global gene expression regulator in *Salmonella*. (A)** Deletion of *proQ* globally affects the gene expression in *Salmonella*. The volcano plot shows the differential expression changes of 8,249 genomic features (coding sequences, UTRs, ncRNAs) plotted against the corresponding FDR values. Features with FDR < 0.05 are highlighted with color. **(B)** KEGG enrichment analysis of the genes differentially affected by *proQ* deletion. Adjusted *P*-values are provided on the right of each category. Only significantly enriched pathways (FDR < 0.05, $P_{adj}$ < 0.05) are shown. **(C)** Examples of *Salmonella* sRNAs (Sib family antitoxins) whose abundance and ability to repress *cis*-encoded mRNA targets (*ibs* mRNAs) depend on ProQ. Read distributions on both strands of three Sib/*ibs* loci in three strains (WT, Δ*proQ* and *proQ*+) are shown scaled to the same height.

## SI Tables

## Table S1. ProQ-associated sRNAs

| sRNA | Genomic coordinates | Features | Enriched in* |
|---|---|---|---|
| **art200a** | 981624<981711 | Family of IS200 transposon-associated *cis*-antisense sRNAs | E, T |
| **art200b** | 1270920<1271007 | | E, T |
| **art200c** (SLnc1010) | 1872588>1872675 | | E, T |
| **art200d** (SLnc1011) | 2003753>2003840 | | E, T |
| **art200e** (SLnc1020) | 2577497>2577584 | | E, T |
| **art200f** | 3485887>3485974 | | E, T |
| **art200g** | 3657129>3657216 | | E, T |
| **Ig2335** | 4162241>4162392 | | E, T, S |
| **IsrB** | 1060741>1060834 | Prophage *cis*-antisense? | E, S |
| **IsrF** | 1586723<1587018 | | E, T |
| **IsrJ** | 2759644<2759718 | Prophage-encoded | S |
| **IsrK** | 2760478<2760556 | Prophage *cis*-antisense | (E), (T), (S) |
| **IstR-1** | 4019328<4019404 | Antitoxins from *tisAB*/IstR type I TA locus | E, T, S |
| **IstR-2** | 4019328<4019458 | | E, T, S |
| **RdlD** (STnc710, RdlB, StyR-207) | 3829661>3829727 | Family of antitoxins from *ldr*/Rdl type I TA loci | (T), (S) |
| **RdlE** (STnc1060, RdlA, RdlB, StyR-207) | 466718<466783 | | E, T, S |
| **RyfA** (tp1, PAIR3) | 2672651>2672945 | | E, T, S |
| **RyfD** | 2805082<2805222 | | (E), (T), (S) |
| **RyjB** (STnc1120) | 1662190<1662282 | *Cis*-antisense? | E, T, S |
| **SibA** (tp11, RyeC, QUAD1a) | 2211623>2211781 | Family of antitoxins from *ibs*/Sib type I TA loci | E, T, S |
| **SibC** (t27, RygC, QUAD1c) | 3243669>3243814 | | E, T, S |
| **SibD** (tp8, C0730, RygD, QUAD1d, StyR-229) | 3382993<3383144 | | E, T, S |
| **SraB** (pke20, PsrD) | 1231270>1231378 | *Trans*-base-paring? | E, S |
| **SraC** (RyeA, tpke79, IS091, SLnc0050) | 1925543>1925852 | *Cis*-antisense? | S |
| **SraF** (tpk1, IS160, PsrN, PRE-element) | 3412774>3412960 | Attenuator | E, S |
| **SraG** (PsrO) | 3472128>3472312 | *Trans*-/*cis*- base-paring | E |
| **SraL** (RyjA) | 4526162<4526302 | *Trans*-/*cis*(?)-base-paring | T, S |
| **RUF_107c.19** | 223450>223563 | | T |
| **RUF_107c.23** | 563424<563507 | *Cis*-antisense? | T |
| **RUF_107c.47** | 3497979<3498072 | | E, T, S |
| **RUF_107c.58** | 4311608<4311707 | *Cis*-antisense? | T, S |
| **RUF_107c.82** | 4463078>4463228 | *Cis*-antisense? | T |
| **RUF_107c.83** | 4470487>4470622 | *Cis*-antisense? | T |
| **RUF_107c.92** | 4660039<4660173 | *Cis*-antisense? | T |
| **RUF_107c.102** | 4856253<4856395 | *Cis*-antisense? | T |
| **RUF_107c.105** | 5014>5162 | *Cis*-antisense? | T |
| **RUF_107c.112** | 2368367>2368466 | *Cis*-antisense? | E |
| **RUF_111** | 2285557<2285752 | | E |
| **SLnc0031** | 3543523<3543651 | | (T), (S) |
| **SLnc1015** | 2459769>2459846 | *Cis*-antisense? | E, T, S |
| **sRNA1** | 257717>257816 | | E, S |
| **sRNA16** | 1291395<1291605 | | T |
| **STnc40** | 161417>161558 | Attenuator? | T, S |
| **STnc310** | 3413966<3414055 | *Cis*-antisense? | E, T |
| **STnc320** | 3425410>3425649 | *Cis*-antisense? | E, T |
| **STnc420** | 4272916<4272977 | | E |
| **STnc475** | 142801>142976 | | T |
| **STnc520** | 1290590<1290674 | | T, S |
| **STnc540** | 1376238>1376364 | | E, T, S |
| **STnc610** (*rimP*-leader) | 3479058<3479185 | Attenuator? | E, S |
| **STnc620** | 4498083>4498195 | | E, T, S |
| **STnc670** | 1170291>1170591 | | E |

| | | | |
|---|---|---|---|
| **STnc750** (sRNA8) | 3261186<3261273 | | S |
| **STnc835** | 817257>817293 | | (S) |
| **STnc1030** | 350913>351102 | | E |
| **STnc1080** (oop RNA Gifsy-2) | 1062745<1062806 | Prophage *cis*-antisense? | T, S |
| **STnc1190** | 4601021>4601437 | *Cis*-antisense? | (E), (T), (S) |
| **STnc1200** | 924756<924838 | *Cis*-antisense? | (T), (S) |
| **STnc1275** | 1992503<1992663 | | T |
| **STnc1500** | 138100<138274 | *Cis*-antisense? | (S) |
| **STnc1550** | 2437665>2437808 | *Cis*-antisense? | E, T |
| **STnc1565** | 2488465>2488645 | | E, T |
| **STnc1640** (StyR-288) | 1345903>1345970 | *Cis*-antisense? | E, T, S |
| **STnc1650** | 2133038>2133100 | *Cis*-antisense? | E |
| **STnc1690** (STnc1140, RUF_175c.3) | 1931467>1931545 | *Cis*-antisense? | S |
| **STnc1990** | 1294843>1294986 | *Cis*-antisense? | E |
| **STnc2030** | 1784648>1784727 | *Cis*-antisense? | S |
| **STnc2070** | 2672472<2672567 | | E, S |
| **STnc2090** | 2808307>2808466 | *Trans*-base-paring? | E, T, S |
| **STnc2130** (SLnc0038) | 4475779>4475869 | *Trans*-base-paring? | E, T |
| **STnc2180** (3'ETS(leuZ)) | 1992864<1992915 | sRNA sponge | E |
| **STnc3070** | 2851167<2851225 | *Cis*-antisense? | T |
| **STnc3160** | 2807823<2807900 | *Cis*-antisense? | S |
| **STnc3310** | 1009199<1009379 | | E, S |
| **STnc3330** | 1024493<1024591 | *Cis*-antisense? | T |
| **STnc3420** | 1289667<1289799 | *Cis*-antisense? | T |
| **STnc3430** | 1294946<1295057 | *Cis*-antisense? | T |
| **STnc3440** | 1303294>1303380 | | T, S |
| **STnc3460** | 1363845>1363959 | *Cis*-antisense? | E, S |
| **STnc3480** | 1519287>1519550 | *Cis*-antisense? | (E) |
| **STnc3510** | 1506478<1506597 | | T |
| **STnc3640** | 1915824>1916082 | | E |
| **STnc3700** | 2131885>2131995 | *Cis*-antisense? | T |
| **STnc3720** | 2264259>2264328 | *Cis*-antisense? | T |
| **STnc3880** | 3305410>3305514 | | E |
| **STnc3970** | 3507594<3507677 | *Cis*-antisense? | T |
| **STnc4130** | 4796788<4796863 | *Cis*-antisense? | E, T, S |
| **STnc4140** | 4846444<4846540 | *Cis*-antisense? | (T), (S) |
| **STnc4190** | 209868<209971 | *Cis*-antisense? | E |
| **STnc4220** | 1464776<1464883 | | E |
| **STnc4230** | 2831959>2832046 | | E, T, S |
| **STnc4250** | 4069296>4069505 | | E, S |
| **StyR-24a** | 982113>982272 | Family of IS200 transposon-associated sRNAs | T |
| **StyR-24d** | 2003194<2003351 | | T |
| **StyR-24e** | 2576935<2577095 | | T |
| **StyR-24g** | 3656569<3656727 | | T |
| **StyR-44e** | 4219508>4219651 | 23S rRNA-derived sRNAs | E |
| **StyR-44f** | 4374472>4374615 | | E |
| **StyR-44g** | 4417752>4417894 | | E |
| **StyR-150** | 241227>241270 | *Cis*-antisense? | T |
| **StyR-199** | 120107>120156 | *Cis*-antisense? | S |
| **StyR-219** | 3221493>3221564 | *Cis*-antisense? | E, T |
| **StyR-243** | 4365232<4365397 | *Cis*-antisense? | E |
| **StyR-248** | 1502537>1502747 | *Cis*-antisense? | T |
| **StyR-291** | 1883981<1884041 | *Cis*-antisense? | T |
| **StyR-305** | 847583>847697 | *Cis*-antisense? | E |
| **StyR-329b** (RUF_107c.80) | 4426270>4426340 | | T |
| **StyR-358** | 751572>751684 | *Cis*-antisense? | T |

*E - exponential, T - transition, S - stationary phase (based on DESeq2); symbols in parentheses correspond to manually retrieved entries (based on analysis of at least two independent experiments).

## Table S2. Bacterial strains used in this study

| Strain ID | Genotype | Plasmid | Description |
|---|---|---|---|
| JVS-00007 | WT | - | *Salmonella* Typhimurium SL1344 WT |
| JVS-10315 | Δ*proQ::Kan* | - | *Salmonella* Typhimurium SL1344 *proQ* deletion strain created with the use of the lambda-Red system (oligonucleotides JVO-08461/JVO-08462) |
| JVS-10317 | Δ*proQ* | - | JVS-10315 healed with the use of the pCP20 plasmid |
| JVS-10473 | Δ*proQ* | pJV300 | JVS-10317 transformed with the control plasmid, used throughout the study as the *proQ* deletion strain |
| JVS-10474 | Δ*proQ* | pZE12-ProQ | JVS-10317 transformed with the complementation plasmid, used throughout the study as the *proQ* complementation strain. The pZE12-luc plasmid contains the *proQ* gene under control of its native promoter (insert created with primers JVO-08523/JVO-08524) cloned into XbaI site in the same orientation as the plasmid-encoded terminator. |
| JVS-01338 | *hfq-3×FLAG* | - | *Salmonella* Typhimurium SL1344 *hfq-3×FLAG* strain |
| JVS-10314 | *proQ-3×FLAG* | - | *Salmonella* Typhimurium SL1344 *proQ-3×FLAG* strain created with the use of the lambda-Red system (oligonucleotides JVO-08728/JVO-08729) |
| JVS-04317 | *csrA-3×FLAG::Kan* | - | *Salmonella* Typhimurium SL1344 *csrA-3×FLAG* strain created with the use of the lambda-Red system (oligonucleotides JVO-03591/JVO-03592) |
| JVS-10136 | *phoP-3×FLAG::Kan* | - | *Salmonella* Typhimurium SL1344 *phoP-3×FLAG* strain created with the use of the lambda-Red system (oligonucleotides JVO-08380/JVO-08381) |

## Table S3. Oligonucleotides used in this study

| Oligo ID | Sequence | Description |
|---|---|---|
| JVO-00322 | CTACGGCGTTTCACTTCTGAGTTC | Northern blot probe 5S rRNA |
| JVO-01003 | GAATCTCCGAGATGCCG | Northern blot probe 6S RNA |
| JVO-02181 | AAGCAGATTGATAAATGCAACG | Northern blot probe InvR |
| JVO-03260 | CATCTTGCGGTCTGGCA | Northern blot probe art200 |
| JVO-03562 | GTGTCTGAAAAACGTACCCTGAT | Northern blot probe MS2 aptamer |
| JVO-03591 | CCAGCGTATCCAGGCTGAAAAATCCCAGCAG TCCAGTTACGACTACAAAGACCATGACGG | Sense oligo for 3×FLAG-tagging CsrA with the use of pSUB11 (2) (to be used with JVO-03592) |
| JVO-03592 | ACCATATCAACAGTGAGGTTGAAAAAAGTCA TGAAGGGACCCATATGAATATCCTCCTTAG | Antisense oligo for 3×FLAG-tagging CsrA with the use of pSUB11 (2) (to be used with JVO-03591) |
| JVO-03722 | ACGTTGACTGGTATAAACCTGGC | Northern blot probe RdlD |
| JVO-04201 | GTTTTTTTTAATACGACTCACTATAGGTCGT ACACCATCAGGGTAC | Universal sense oligo for 5'-MS2 aptamer tagging, carries T7 promoter (to be used with JVO-04203 to create template for MS2 aptamer control on pRR05 (6)) |
| JVO-04202 | GTTTTTTTTAATACGACTCACTATAGG | Universal sense oligo for the second PCR step in MS2 aptamer tagging, carries T7 promoter (to be used with an sRNA-specific oligo in 5'-MS2 tagging, or with JVO-04943 in 3'-MS2 tagging) |

| | | |
|---|---|---|
| JVO-04203 | GTGACCAGACCCTGATGG | Universal antisense oligo for 3'-MS2 aptamer tagging (to be used with JVO-04201 to create template for MS2 aptamer control on pRR05 (6)) |
| JVO-04943 | ACAGACCCTGATGGTGTCT | Antisense oligo to create the MS2 PCR product for 3'-MS2 tagging (to be used with JVO-04943), and a universal antisense oligo for the second PCR in 3'-MS2 tagging (to be used with JVO-04202) |
| JVO-04944 | GTCACCGTACACCATCAGGGTAC | Sense oligo to create the MS2 PCR product for 3'-MS2 tagging (to be used with JVO-04943) |
| JVO-05974 | GGCGATACACTCAATGTAAGGG | Northern blot probe STnc2090 |
| JVO-07694 | GTGTCTGGCGAAACGCT | Northern blot probe Msr/STnc400 |
| JVO-08016 | CACCATCAGGGTCTGGTCACGAAAAGGCTGAGACCGTTA | Sense oligo for the first PCR in 5'-MS2 tagging STnc1460 (*thi*-box part) (to be used with JVO-08017) |
| JVO-08017 | ATTACTCTTGTTCCCTTCGC | Antisense oligo for 5'-MS2 tagging STnc1460 (*thi*-box part) (to be used with JVO-08016 for the first PCR, and with JVO-04202 for the second PCR) |
| JVO-08018 | GTTTTTTTTAATACGACTCACTATAGGGAAAAGGCTGAGACCGTTA | Sense oligo for the first PCR in 3'-MS2 tagging STnc1460 (*thi*-box part), carries T7 promoter (to be used with JVO-08019) |
| JVO-08019 | GTACCCTGATGGTGTACGGTGACATTACTCTTGTTCCCTTCGC | Antisense oligo for the first PCR in 3'-MS2 tagging STnc1460 (*thi*-box part) (to be used with JVO-08018) |
| JVO-08044 | CACCATCAGGGTCTGGTCACCGCATAATTTAAGACCGC | Sense oligo for the first PCR in 5'-MS2 tagging STnc1640 (to be used with JVO-08045) |
| JVO-08045 | ATAAAGAAAACCGCCGAT | Antisense oligo for 5'-MS2 tagging STnc1640 (to be used with JVO-08044 for the first PCR, and with JVO-04202 for the second PCR) |
| JVO-08046 | GTTTTTTTTAATACGACTCACTATAGGCGCATAATTTAAGACCGC | Sense oligo for the first PCR in 3'-MS2 tagging STnc1640, carries T7 promoter (to be used with JVO-08047) |
| JVO-08047 | GTACCCTGATGGTGTACGGTGACATAAAGAAAACCGCCGAT | Antisense oligo for the first PCR in 3'-MS2 tagging STnc1640 (to be used with JVO-08046) |
| JVO-08075 | CACCATCAGGGTCTGGTCACACTCTTTAGCGTTAGGCTTTG | Sense oligo for the first PCR in 5'-MS2 tagging STnc400 (to be used with JVO-08076) |
| JVO-08076 | AAAAGTACTCAATAAGTTAGTGTCTGG | Antisense oligo for 5'-MS2 tagging Pre-Msr/STnc400 (to be used with JVO-08151 for the first PCR, and with JVO-04202 for the second PCR) |
| JVO-08077 | GTTTTTTTTAATACGACTCACTATAGGACTCTTTAGCGTTAGGCTTTG | Sense oligo for the first PCR in 3'-MS2 tagging Pre-Msr/STnc400, carries T7 promoter (to be used with JVO-08078) |
| JVO-08078 | GTACCCTGATGGTGTACGGTGACAAAAGTACTCAATAAGTTAGTGTCTGG | Antisense oligo for the first PCR in 3'-MS2 tagging Pre-Msr/STnc400 (to be used with JVO-08152) |
| JVO-08149 | CACCATCAGGGTCTGGTCACCCCCCTCTTCGGAGG | Sense oligo for the first PCR in 5'-MS2 tagging STnc1460 (to be used with JVO-08150) |
| JVO-08150 | GTTTTTTTTAATACGACTCACTATAGGCCCCCTCTTCGGAGG | Antisense oligo for 5'-MS2 tagging STnc1460 (to be used with JVO-08149 for the first PCR, and with JVO-04202 for the second PCR) |
| JVO-08164 | TTCATCGTTATTATTATCCCG | Northern blot probe ChiX |
| JVO-08376 | CACCATCAGGGTCTGGTCACAATTGATCAACAAGCTGGAAC | Sense oligo for the first PCR in 5'-MS2 tagging STnc2090 (to be used with JVO-08377) |
| JVO-08377 | CGCGCCCGAAGGCGCGTTGG | Antisense oligo for 5'-MS2 tagging STnc2090 (to be used with JVO-08376 for the first PCR, and with JVO-04202 for the second PCR) |
| JVO-08378 | GTTTTTTTTAATACGACTCACTATAGGAATTGATCAACAAGCTGGAAC | Sense oligo for the first PCR in 3'-MS2 tagging STnc2090, carries T7 promoter (to be used with JVO-08379) |
| JVO-08379 | GTACCCTGATGGTGTACGGTGACCGCGCCCGAAGGCGCGTTGG | Antisense oligo for the first PCR in 3'-MS2 tagging STnc2090 (to be used with JVO-08378) |
| JVO-08380 | TACCACCGTACGCGGACAAGGATATCTTTTTGAATTGCGCGACTACAAAGACCATGACGG | Sense oligo for 3×FLAG-tagging PhoP with the use of pSUB11 (2) (to be used with JVO-08381) |

| | | |
|---|---|---|
| JVO-08381 | CGCAGCGACAGCGGCAGAAAATGGCGAGCAA ATTTATTCACATATGAATATCCTCCTTAG | Antisense oligo for 3×FLAG-tagging PhoP with the use of pSUB11 (2) (to be used with JVO-08380) |
| JVO-08405 | TTTGCGCTGGCGACCAGATG | Northern blot probe *raiA* mRNA |
| JVO-08461 | CAACGGATAACGTAGCAATTACTGATGGCGT CATTATAATGTGTAGGCTGGAGCTGCTTC | Sense oligo for deletion of *proQ* in *Salmonella* with the use of pKD4 (1) (to be used with JVO-08462) |
| JVO-08462 | CGGTTTATCAGCGCGAGGTTTACGTTCAGCG CCTTCTTTACATATGAATATCCTCCTTAG | Antisense oligo for deletion of *proQ* in *Salmonella* with the use of pKD4 (1) (to be used with JVO-08461) |
| JVO-08493 | CATACTGGTGATACTCTTAGTG | Northern blot probe SibA |
| JVO-08495 | ATGCAACGGCCTCTGCTT | Northern blot probe *cspD* mRNA |
| JVO-08497 | TGTTAATCATCGCTTGCCCA | Northern blot probe SibC |
| JVO-08498 | GAGGTTCCGGTTTGTGTTGAT | Northern blot probe SraL |
| JVO-08515 | GGTGGTTGCTCTTCCAACATGGAAAATCAAC CTAAGTTGAATAGC | Sense oligo to amplify *Salmonella proQ* ORF for cloning into pTYB11, SapI site (to be used with JVO-08516) |
| JVO-08516 | GGTGGTCTGCAGTCATCAGAACACCAGGTGT TCTGCGCG | Antisense oligo to amplify *Salmonella proQ* ORF for cloning into pTYB11, PstI site (to be used with JVO-08515) |
| JVO-08523 | AGGCGTCTCTAGATACCGAAGAAGATGAACA CGGCC | Sense oligo to amplify *Salmonella proQ* ORF for cloning into pZE12-luc, XbaI site (to be used with JVO-08524) |
| JVO-08524 | AGGCGTCTCTAGAAAAAAAAGTGTTCATGCC AGGCC | Antisense oligo to amplify *Salmonella proQ* ORF for cloning into pZE12-luc, XbaI site (to be used with JVO-08523) |
| JVO-08540 | CCTCCGACCCCTTCG | Northern blot probe $tRNA^{Pro}_{CGG}$ |
| JVO-08543 | GTTTTTTTTAATACGACTCACTATAGGTTGA CATTATTCTTGATGTGGC | Sense oligo to create template for SibA *in vitro* transcription, carries T7 promoter (to be used with JVO-08544) |
| JVO-08544 | AAAATAAGGAAAAGGGTTATGATGAAGG | Antisense oligo to create template for SibA *in vitro* transcription, (to be used with JVO-08543) |
| JVO-08551 | CCCAAAACGTGAGTAAGTATCTA | Northern blot probe STnc475 |
| JVO-08720 | CACCATCAGGGTCTGGTCACAGACCGAATAC GATTCC | Sense oligo for the first PCR in 5'-MS2 tagging SraC (to be used with JVO-08721) |
| JVO-08721 | CGCAAACTGGAAAACCTGG | Antisense oligo for 5'-MS2 tagging SraC (to be used with JVO-08720 for the first PCR, and with JVO-04202 for the second PCR) |
| JVO-08722 | GTTTTTTTTAATACGACTCACTATAGGAGAC CGAATACGATTCC | Sense oligo for the first PCR in 3'-MS2 tagging SraC, carries T7 promoter (to be used with JVO-08723) |
| JVO-08723 | GTACCCTGATGGTGTACGGTGACCGCAAACT GGAAAACCTGG | Antisense oligo for the first PCR in 3'-MS2 tagging SraC (to be used with JVO-08722) |
| JVO-08728 | GGGTATGTCTTTGATTGTACGCGCAGAACAC CTGGTGTTCGACTACAAAGACCATGACGG | Sense oligo for 3×FLAG-tagging ProQ in *Salmonella* with the use of pSUB11 (2) (to be used with JVO-08729) |
| JVO-08729 | AAGCCTAAAAAAAGTGTTCATGCCAGGCCTG GCCTCCGTTCATATGAATATCCTCCTTAG | Antisense oligo for 3×FLAG-tagging ProQ in *Salmonella* with the use of pSUB11 (2) (to be used with JVO-08728) |
| JVO-10325 | TGGTGGAGCTGGCGGGAGTT | Northern blot probe tmRNA |
| JVO-10346 | GTTTTTTTTAATACGACTCACTATAGGATTG ATCAACAAGCTGGAACG | Sense oligo to create template for STnc2090 *in vitro* transcription, carries T7 promoter (to be used with JVO-10347) |
| JVO-10347 | AAAAAACGCGCCCGAAG | Antisense oligo to create template for STnc2090 *in vitro* transcription, (to be used with JVO-10346) |
| JVO-10931 | GCTTTGGGAACTAGCGAATC | Northern blot probe SLnc1015 |
| JVO-11060 | GCCAATAATTCGCACACATTGC | Northern blot probe SraB |
| JVO-11395 | CACCATCAGGGTCTGGTCACAGTCGAGTAAC GTCGGTG | Sense oligo for the first PCR in 5'-MS2 tagging STnc475 (to be used with JVO-11396) |
| JVO-11396 | AGAGAAGGTGGCCCTCTC | Antisense oligo for 5'-MS2 tagging STnc475 (to be used with JVO-11395 for the first PCR, and with JVO-04202 for the second PCR) |

| | | |
|---|---|---|
| JVO-11397 | GTTTTTTTTAATACGACTCACTATAGGAGTC GAGTAACGTCGGTG | Sense oligo for the first PCR in 3'-MS2 tagging STnc475, carries T7 promoter (to be used with JVO-11398) |
| JVO-11398 | GTACCCTGATGGTGTACGGTGACAGAGAAGG TGGCCCTCTC | Antisense oligo for the first PCR in 3'-MS2 tagging STnc475 (to be used with JVO-11397) |
| JVO-11399 | CACCATCAGGGTCTGGTCACCTTTCCGCGCC CTGGTTTTAC | Sense oligo for the first PCR in 5'-MS2 tagging STnc1565 (to be used with JVO-11400) |
| JVO-11400 | AAAGCGCGCCCTGTCG | Antisense oligo for 5'-MS2 tagging STnc1565 (to be used with JVO-11399 for the first PCR, and with JVO-04202 for the second PCR) |
| JVO-11401 | GTTTTTTTTAATACGACTCACTATAGGCTTT CCGCGCCCTGGTTTTAC | Sense oligo for the first PCR in 3'-MS2 tagging STnc1565, carries T7 promoter (to be used with JVO-11402) |
| JVO-11402 | GTACCCTGATGGTGTACGGTGACAAAGCGCG CCCTGTCG | Antisense oligo for the first PCR in 3'-MS2 tagging STnc1565 (to be used with JVO-11401) |
| JVO-11518 | CTCCTGACCCTCATCCTGAGTCG | Northern blot probe CsrC |
| JVO-11694 | CACCATCAGGGTCTGGTCACGGGGCTATAGC TCAGCTGGG | Sense oligo for the first PCR in 5'-MS2 tagging $tRNA^{Ala}_{TGC}$ (to be used with JVO-11695) |
| JVO-11695 | TGGAGCTATGCGGGATCG | Antisense oligo for 5'-MS2 tagging $tRNA^{Ala}_{TGC}$ (to be used with JVO-11694 for the first PCR, and with JVO-04202 for the second PCR) |
| JVO-12159 | CACCATCAGGGTCTGGTCACTTTCTCTGAGA TGTTTGCAAGCGGGCCAG | Sense oligo for the first PCR in 5'-MS2 tagging 6S RNA (to be used with JVO-12160) |
| JVO-12160 | GAATCTCCGAGATGCCGCCGC | Antisense oligo for 5'-MS2 tagging 6S RNA (to be used with JVO-12159 for the first PCR, and with JVO-04202 for the second PCR) |
| JVO-12161 | GTTTTTTTTAATACGACTCACTATAGGTTTC TCTGAGATGTTTGCAAGCGGGCCAG | Sense oligo for the first PCR in 3'-MS2 tagging 6S RNA, carries T7 promoter (to be used with JVO-12162) |
| JVO-12162 | GTACCCTGATGGTGTACGGTGACGAATCTCC GAGATGCCGCCGC | Antisense oligo for the first PCR in 3'-MS2 tagging 6S RNA (to be used with JVO-12161) |
| JVO-12249 | CACCATCAGGGTCTGGTCACGAAGGGTGAGG GAGGCG | Sense oligo for the first PCR in 5'-MS2 tagging SibC (to be used with JVO-12250) |
| JVO-12250 | GGGAAAGCCCCTACCGAGGC | Antisense oligo for 5'-MS2 tagging SibC (to be used with JVO-12249 for the first PCR, and with JVO-04202 for the second PCR) |
| JVO-12255 | CACCATCAGGGTCTGGTCACGCCTTAACAGC ACCCCGATATATC | Sense oligo for the first PCR in 5'-MS2 tagging IsrB (to be used with JVO-12256) |
| JVO-12256 | GAAAACGCCCACCGAAGCGGGC | Antisense oligo for 5'-MS2 tagging IsrB (use with JVO-12255 for the first PCR, and with JVO-04202 for the second PCR) |
| JVO-12261 | CACCATCAGGGTCTGGTCACTTTTAAGCACC GGCGTTTGC | Sense oligo for the first PCR in 5'-MS2 tagging as*yhbQ* (to be used with JVO-12262) |
| JVO-12262 | TTAACGCTGGCGTTTGCGGC | Antisense oligo for 5'-MS2 tagging as*yhbQ* (to be used with JVO-12261 for the first PCR, and with JVO-04202 for the second PCR) |
| JVO-12267 | CACCATCAGGGTCTGGTCACCTAATGCCGGA TGGCGGCG | Sense oligo for the first PCR in 5'-MS2 tagging STnc1560 (to be used with JVO-12268) |
| JVO-12268 | AGAATGCCGGATGGCGATGC | Antisense oligo for 5'-MS2 tagging STnc1560 (to be used with JVO-12267 for the first PCR, and with JVO-04202 for the second PCR) |
| JVO-12270 | CACCATCAGGGTCTGGTCACCTTAATTGCCA ATCAATGTCTGATGGC | Sense oligo for the first PCR in 5'-MS2 tagging RUF 107c.58 (to be used with JVO-12271) |
| JVO-12271 | ATAAATGCCGGATGGCGGC | Antisense oligo for 5'-MS2 tagging RUF 107c.58 (to be used with JVO-12270 for the first PCR, and with JVO-04202 for the second PCR) |

## SI Datasets

**Dataset S1. Grad-seq profiles of *Salmonella* Typhimurium SL1344 transcripts in 10-40% glycerol gradient, based on the fraction-wise RNA-seq.** Only the profiles with ≥30 reads in at least one fraction are shown. All profiles are standardized to the range from 0 to 1.

**Dataset S2. Sedimentation profiles of *Salmonella* Typhimurium SL1344 proteins in 10-40% glycerol gradient, based on the fraction-wise LC-MS/MS.** Only profiles with ≥5 spectral counts in the peaking fraction are retained. All profiles are standardized to the range from 0 to 1.

**Dataset S3. Enrichments of *Salmonella* Typhimurium SL1344 transcripts in ProQ coIP experiments.** Transcripts significantly enriched over two medians (according to DESeq2) in each growth phase are highlighted.

**Dataset S4. Differential gene expression analysis of the *Salmonella* Typhimurium SL1344 Δ*proQ* strain vs the parental WT.**

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