Supplemental Information

RNA-RNA Interactomes of ProQ and Hfq

Reveal Overlapping and Competing Roles

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Supplemental Information

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A total | unbound | IP

B

	Library #	WT LB (1)	WT LB (7)	ProQ-3XFLAG LB (2)	ProQ-3XFLAG LB (8)	Hfq-FLAG LB (3)	Hfq-FLAG LB (9)	WT M63 (4)	WT M63 (10)	ProQ-3XFLAG M63 (5)	ProQ-3XFLAG M63 (11)	Hfq-FLAG M63 (6)	Hfq-FLAG M63 (12)
WT LB	1		0.49	0.56	0.59	0.50	0.50	0.34	0.50	0.46	0.49	0.40	0.41
	7	0.84		0.79	0.50	0.78	0.54	0.79	0.56	0.77	0.57	0.74	0.59
ProQ-3XFLAG LB	2	0.85	0.49		0.93	0.84	0.80	0.25	0.45	0.73	0.70	0.71	0.71
	8	0.86	0.80	0.97		0.86	0.78	0.67	0.47	0.83	0.70	0.74	0.68
Hfq-FLAG LB	3	0.77	0.53	0.88	0.81		0.94	0.25	0.53	0.65	0.64	0.81	0.81
	9	0.76	0.78	0.85	0.84	0.98		0.64	0.55	0.75	0.64	0.84	0.79
WT M63	4	0.73	0.39	0.67	0.26	0.64	0.26		0.37	0.33	0.35	0.29	0.29
	10	0.77	0.83	0.71	0.72	0.74	0.75	0.82		0.77	0.49	0.72	0.47
ProQ-3XFLAG M63	5	0.73	0.54	0.83	0.72	0.76	0.64	0.80	0.43		0.83	0.77	0.77
	11	0.76	0.81	0.83	0.84	0.78	0.77	0.81	0.81	0.93		0.82	0.73
Hfq-FLAG. M63	6	0.64	0.58	0.75	0.68	0.85	0.79	0.69	0.46	0.85	0.73		0.94
	12	0.65	0.75	0.76	0.74	0.85	0.84	0.69	0.73	0.84	0.82	0.98	

Supp. Figure S1. Evaluation of conditions for RIL-seq and correlation between RIL-seq libraries
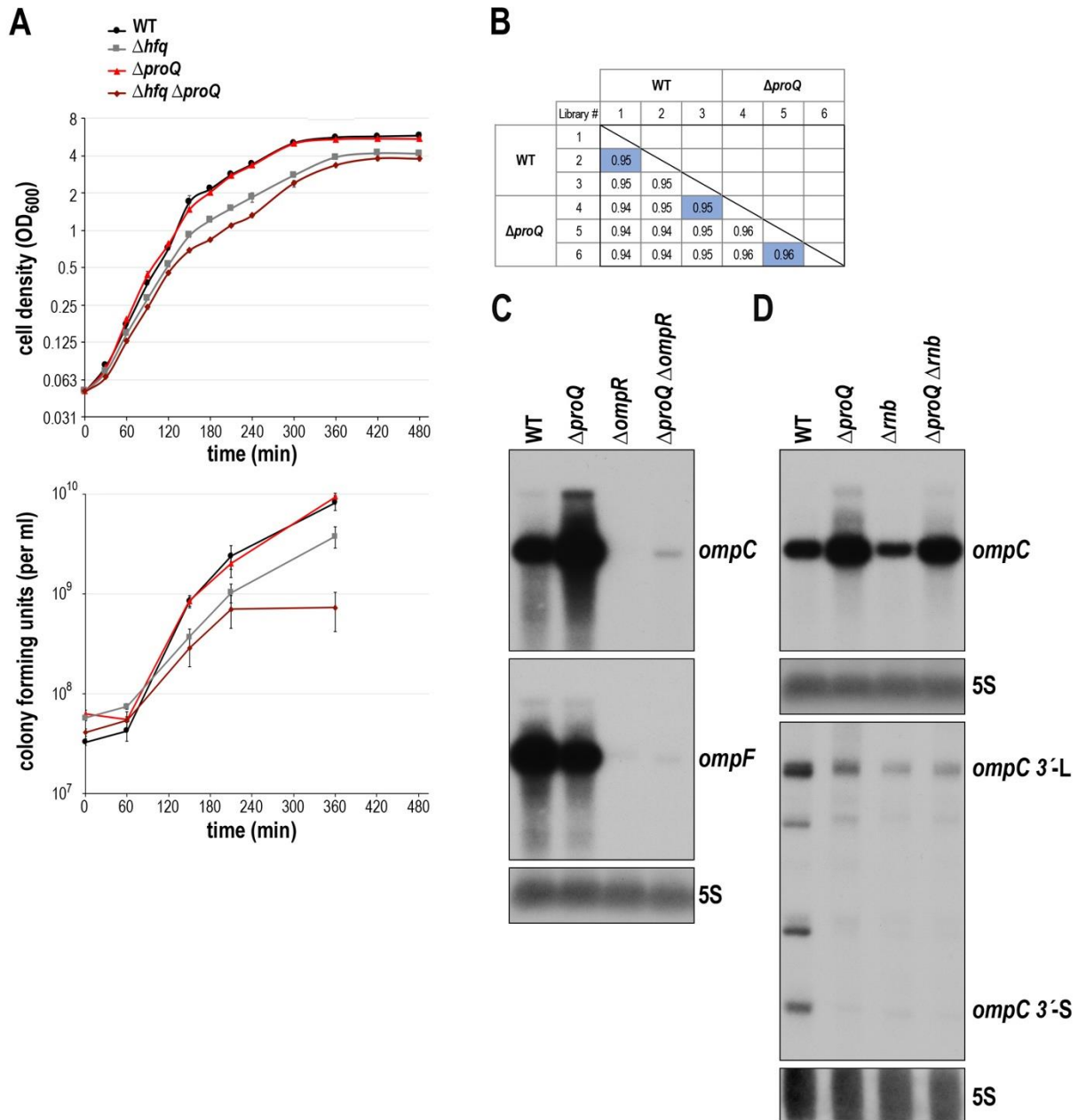
(related to Figure 1)

(A) Immunoblot assay of Hfq-FLAG and ProQ-3XFLAG immunoprecipitated after cross-linking. Strains expressing Hfq-FLAG (HM34) and ProQ-3XFLAG (GSO953) as well as a WT control strain (GSO983) were grown to OD_{600}~1.0, the cells were exposed to 80,000 $\mu J/cm^2$ UV irradiation to generate protein-RNA crosslinks, and cell lysates were prepared. The lysates were subjected to IP assay using magnetic beads carrying M2 anti-FLAG monoclonal antibody. The lysates, unbound fraction, and bound fraction (IP) were examined by immunoblot analysis using anti-FLAG antibody.

(B) Correlation in number of mapped sequenced fragments in corresponding genomic windows between same-condition libraries (blue shading). The reproducibility of the results within same-condition libraries was evaluated for all statistically significant chimeric fragments (S-chimeras)

(cells above the diagonal) and for all single and chimeric fragments (cells below the diagonal). The numbers of fragments mapped to a 100 nt long region of the genome in two libraries were analyzed. The Spearman correlation coefficients are reported for each cell. The libraries correspond to those listed in Table S1.

Supp. Figure S2. Growth of Δ*hfq*, Δ*proQ* and Δ*hfq* Δ*proQ* mutants and effects of Δ*proQ* on RNA expression
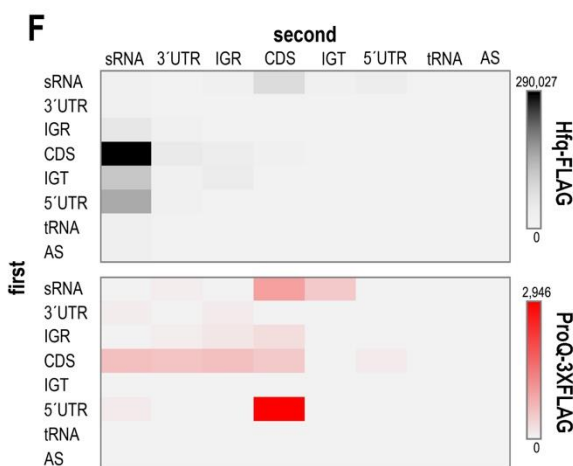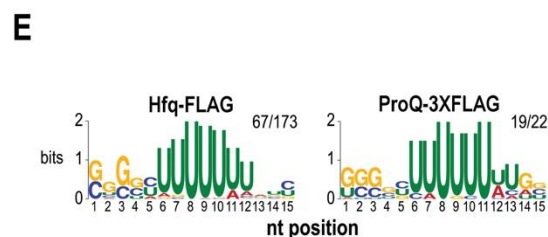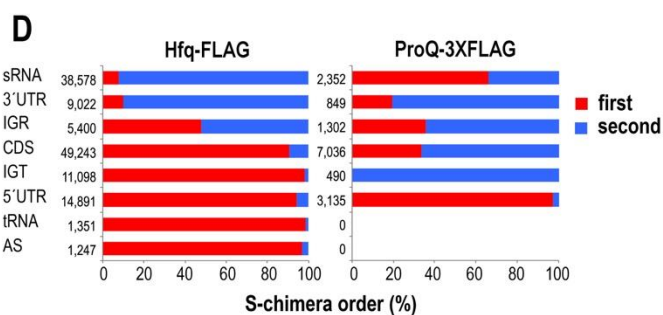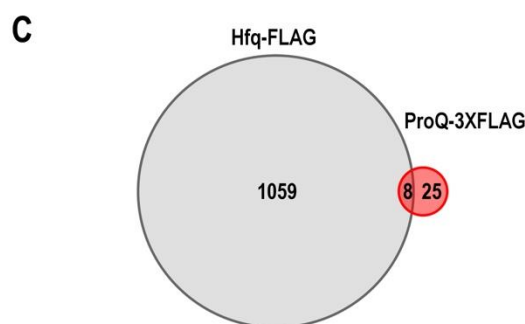
(related to Figure 2)
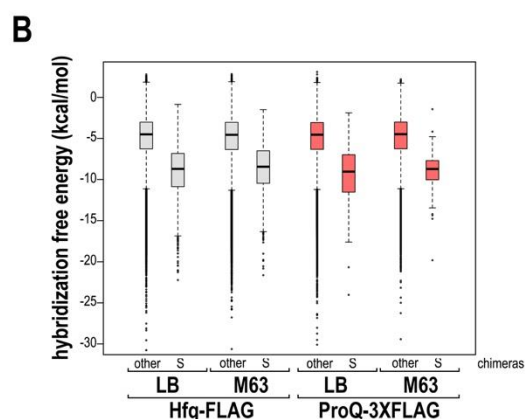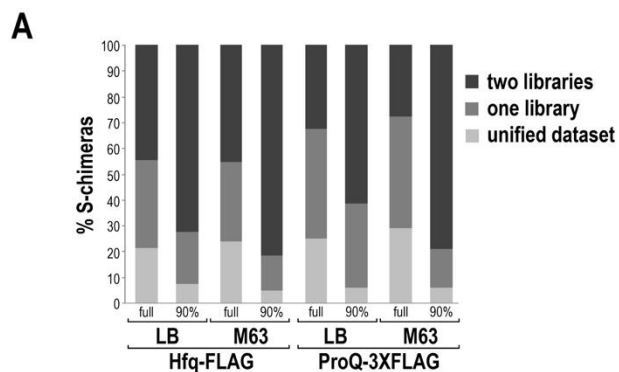
(A) Growth curves of WT (GSO982), Δ*hfq* (GSO954), Δ*proQ* (GSO956) and Δ*hfq* Δ*proQ* (GSO957) in LB medium. Overnight cultures were diluted to $OD_{600} = 0.05$ and cell growth was monitored for 480 min by OD_{600} measurements. Viable counts were determined at 0, 60, 180,

240 and 360 min. All points give the average of three biological replicates with the standard deviation.

(B) Correlation in number of mapped sequenced fragments in corresponding genomic windows between same-condition libraries (blue shading). The reproducibility of the results within same-condition libraries was evaluated at the level of mapped fragments. Comparison of the sequenced fragments between three libraries is shown. The numbers of fragments mapped to a 100 nt long region of the genome in three libraries were analyzed. The Spearman correlation coefficients are reported for each cell. The libraries correspond to those listed in Table S4.

(C) Northern analysis showing that some effects of Δ*proQ* on *ompC* mRNA levels were independent of OmpR. Total RNA was extracted from WT (GSO982), Δ*proQ* (GSO958), Δ*ompR* (GSO967), Δ*proQ* Δ*ompR* (GSO963) strains after 150 min after dilution of the overnight culture, separated on an agarose gel and sequentially probed for the *ompC*, *ompF* and 5S RNAs.

(D) Control northern analysis for 5S RNA levels for the membranes probed in Figure 2E. The *ompC* and *ompC* 3´ panels of Figure 2E are repeated here.

Supp. Figure S3. Impact of 90% threshold on RIL-seq chimera data and results for cells grown in M63

(related to Figure 3)

(A) The percentage of S-chimeras that were found in two libraries (dark gray), one library (gray), or the unified dataset (light gray) for all four samples of RIL-seq experiment 1 for the full datasets and after the application of a "90% cutoff". The cutoff corresponds to the number of chimeras for which 90% of the S-chimeras in the control libraries (*E. coli* with untagged Hfq and ProQ) are eliminated when table is sorted according to the number of chimeric fragments. We used this threshold for further analysis of S-chimeras in the ProQ-3XFLAG or Hfq-FLAG libraries and thus only considered interactions supported by 39 chimeric fragments or more for cells grown in LB and 60 chimeric fragments or more for cells grown in M63 (see STAR methods for more details). The chart illustrates how the cutoff enriches for the chimeras found in two libraries.

(B) Summary of computed hybridization free energy (kcal/mol) using RNAup for statistically significant chimeras (≥39 chimeric fragments in LB dataset and ≥60 in M63 dataset) and other chimeras that did not pass the threshold for statistical significance.

(C) Venn diagram showing ~24% of the RNA pairs found on ProQ-3XFLAG in M63 are shared with Hfq-FLAG M63 dataset. Only chimeras with unique names were counted.
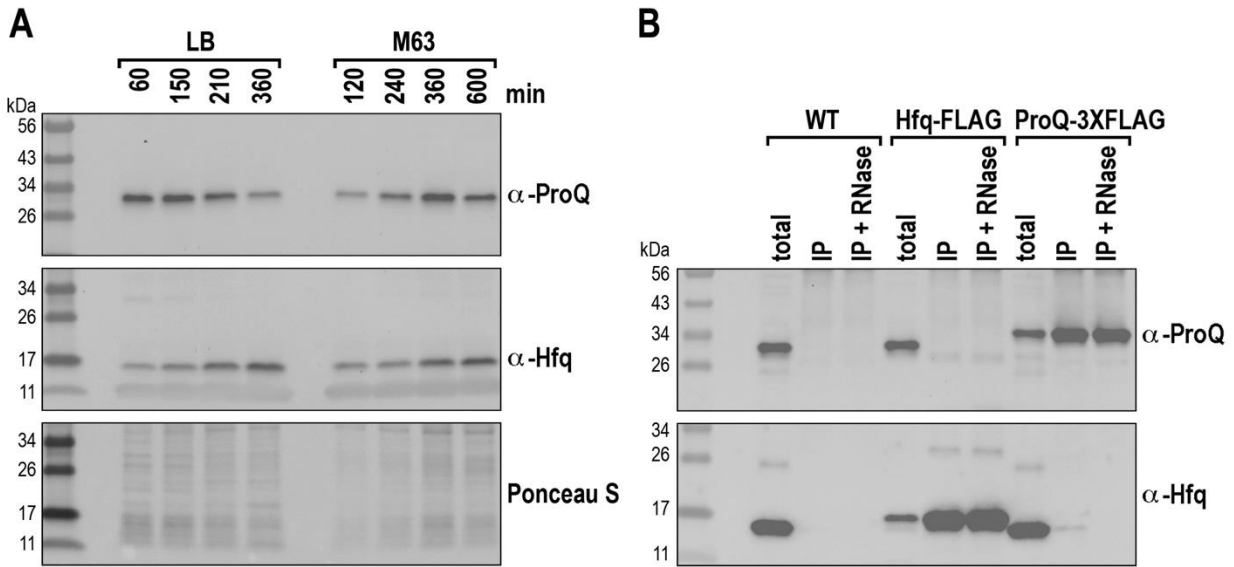
(D) Distribution of RNA locations as first (red) and second (blue) in chimeric fragments for RNAs derived from various genomic elements in Hfq M63 and ProQ M63 datasets.

(E) Motifs found for second RNA in Hfq M63 (E = 1.7 x 10^{-40}) and ProQ M63 (E = 3.5 x 10^{-17}) datasets. Fractions correspond to number of sequences containing motif, over the total number analyzed.

(F) Total number of chimeric fragments for each combination of genomic elements in the M63 chimera dataset of Hfq-FLAG (top) or ProQ-3XFLAG (bottom). Mapped fragments were classified as in Figure 1A. Rows represent the first RNA in the chimera and columns represent the second RNA in the chimera. In the Hfq dataset, the most prominent pairs are sRNAs with CDS or 5´ UTR whereas in the ProQ dataset other combinations are also abundant.

(G) The top 15 chimeras in Hfq and ProQ M63 datasets, when table is sorted by the number of chimeras, are different. The only pair that found in both sets is *yebO*-CyaR. The enrichment value is given with the boxes for the top 15 most-enriched RNAs shaded according to the key. For (E), (F) and (G) classifications are as in Figure 1A.

A

	LB				M63				
kDa	60	150	210	360	120	240	360	600	min

α-ProQ

α-Hfq

Ponceau S

B

	WT			Hfq-FLAG			ProQ-3XFLAG		
	total	IP	IP + RNase	total	IP	IP + RNase	total	IP	IP + RNase

α-ProQ

α-Hfq

C

	Library #	WT		Hfq-FLAG		ProQ-3XFLAG		ΔproQ Hfq-FLAG		Δhfq ProQ-3XFLAG	
		1	2	3	4	5	6	7	8	9	10
WT	1										
	2	0.79									
Hfq-FLAG	3	0.58	0.64		0.86	0.42	0.40	0.79	0.76	0.28	0.29
	4	0.60	0.66	0.96		0.41	0.40	0.82	0.79	0.27	0.29
ProQ-3XFLAG	5	0.66	0.72	0.82	0.83		0.78	0.36	0.36	0.56	0.56
	6	0.65	0.72	0.83	0.83	0.95		0.36	0.35	0.57	0.57
ΔproQ Hfq-FLAG	7	0.53	0.59	0.92	0.92	0.77	0.77		0.86	0.24	0.26
	8	0.52	0.57	0.91	0.91	0.75	0.76	0.96		0.24	0.25
Δhfq ProQ-3XFLAG	9	0.61	0.68	0.79	0.79	0.89	0.90	0.74	0.74		0.76
	10	0.61	0.67	0.79	0.79	0.89	0.90	0.74	0.74	0.96	

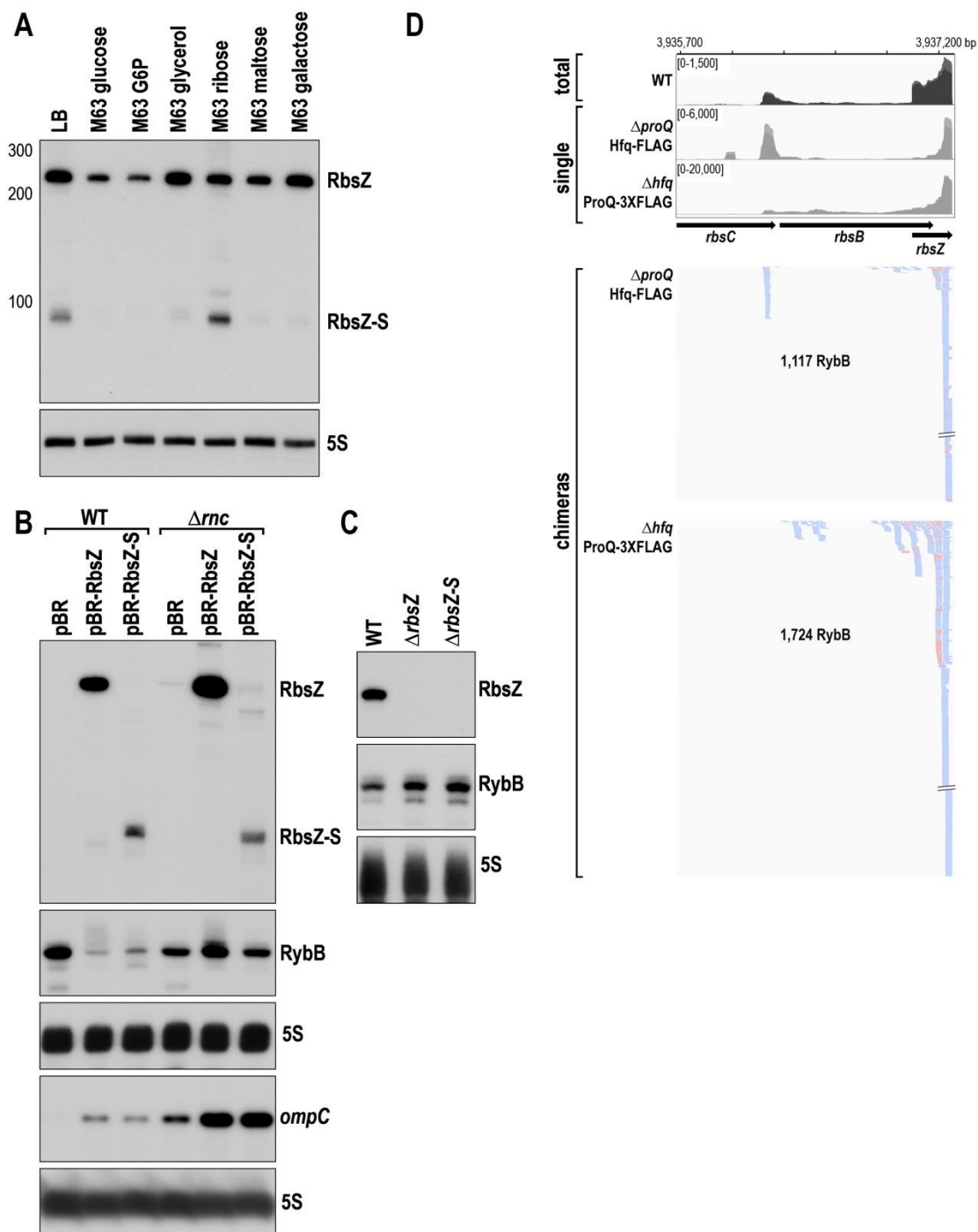**Supp. Figure S4. Hfq and ProQ do not interact directly**

(related to Figure 4)

(A) Relative levels of ProQ and Hfq for cells grown 60, 150, 210 and 360 min after dilution in LB and for 120, 240, 360 and 600 min after dilution in M63 with 0.2% glucose were determined by immunoblot analysis using α-ProQ or α-Hfq antibodies.

(B) Immunoblot analysis using α-ProQ or α-Hfq antibodies of immunoprecipitated Hfq-FLAG (HM34) or ProQ-3XFLAG (GSO953) imply that there is no direct interaction between Hfq and

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ProQ for samples taken at 360 min. Some of the IP samples were also subjected to treatment with a mix of RNase A and RNase T1.

(C) Correlation in number of mapped sequenced fragments in corresponding genomic windows between same-condition libraries (blue shading). The reproducibility of the results within same-condition libraries was evaluated as described in the legend of Figure S1B. Each cell above the diagonal shows these results for S-chimeras. The Spearman correlation coefficients are reported for each cell. The name of a library includes the condition and library number listed in Table S7.

Supp. Figure S5. Elevated RbsZ-S in ribose and effects of RbsZ-S and ΔrbsZ (for Figures 5, 6 and 7)

(A) Total RNA was extracted from WT (GSO982) grown to exponential phase (OD$_{600}$~0.6) in LB medium or M63 minimal medium supplemented with 0.2% of glucose, glucose-6-phosphate

(G6P), ribose, maltose or galactose or 0.4% glycerol, separated on an acrylamide gel and sequentially probed for the RbsZ and 5S RNAs.

(B) Total RNA was extracted from WT (GSO982), Δ*rnc* (GSO971) harboring the indicated plasmids after 360 min after dilution of the overnight culture. RNA was separated on either an acrylamide gel, transferred to a membrane, and sequentially probed for the RbsZ, RybB, and 5S RNAs or an agarose gel, transferred to a membrane, and sequentially probed for the *ompC* and 5S RNAs.

(C) Total RNA was extracted from WT (GSO982), Δ*rbsZ* (GSO965) and Δ*rbsZ-S* (GSO966) strains 360 min after dilution of the overnight culture, separated on an acrylamide gel and sequentially probed for the RbsZ, RybB and 5S RNAs.

 (D) Browser image for Hfq-FLAG data in Δ*proQ* mutant strain and ProQ-3XFLAG data in Δ*hfq* mutant strain from RIL-seq experiment 2 for 3,935,700-3,937,200 region of the *E. coli* chromosome. Top: Signal for WT total RNA (dark gray) and Hfq/ProQ RIL-seq enriched fragments in two biological repeats are overlaid (light gray). Read count ranges are shown in the upper left of each frame. Bottom: chimeras with RybB in unified datasets. Red and blue lines indicate RbsZ is first or second RNA in the chimera, respectively.