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Supplemental Information

Hierarchy in Hfq Chaperon Occupancy of Small RNA Targets Plays a Major Role in Their Regulation

Raya Faigenbaum-Romm, Avichai Reich, Yair E. Gatt, Meshi Barsheshet, Liron Argaman, and Hanah Margalit

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Supplementary Figures

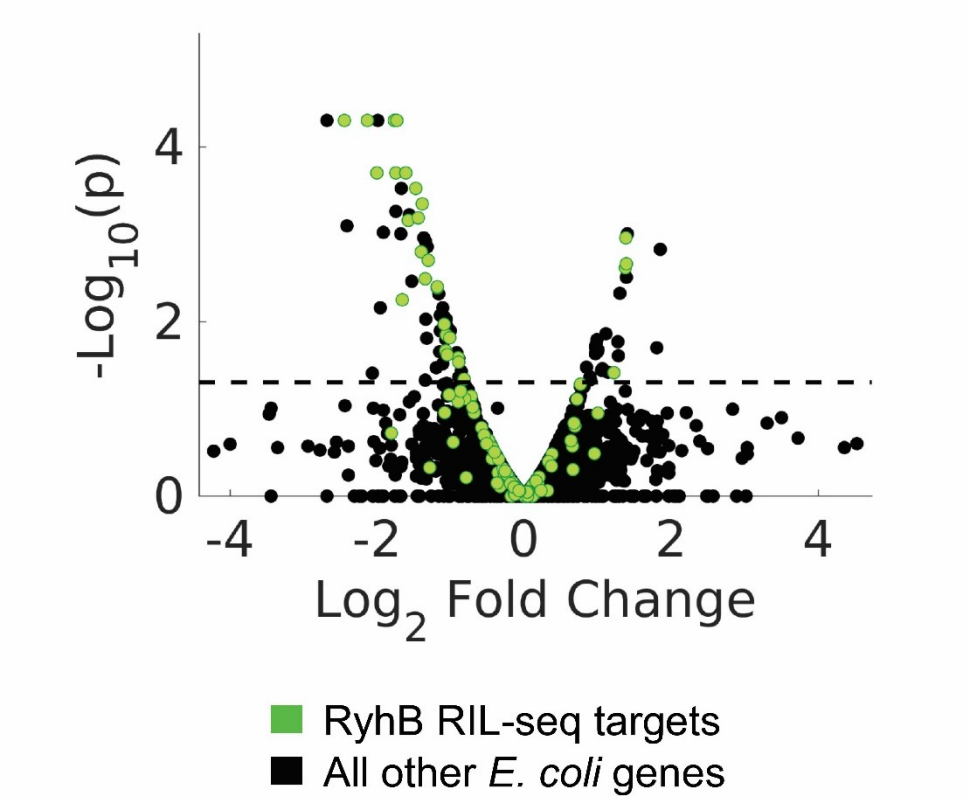
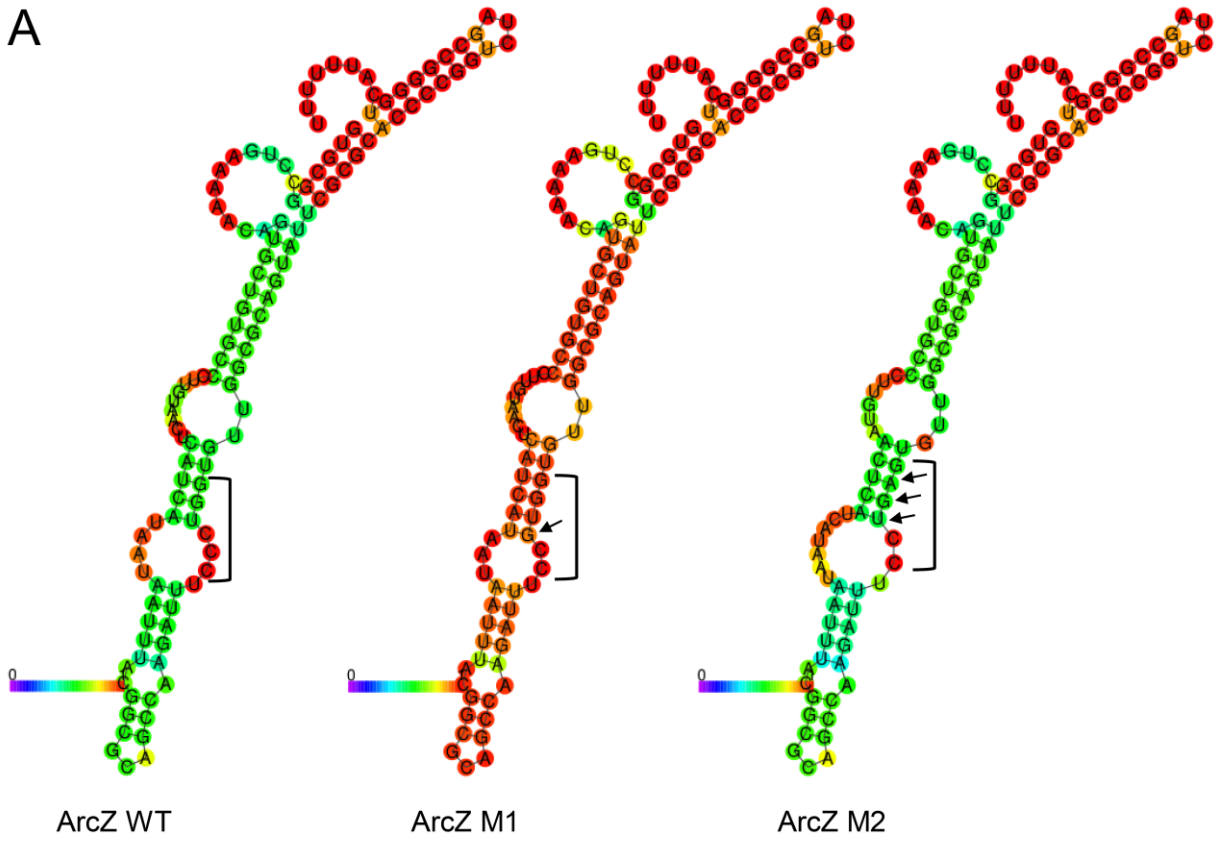
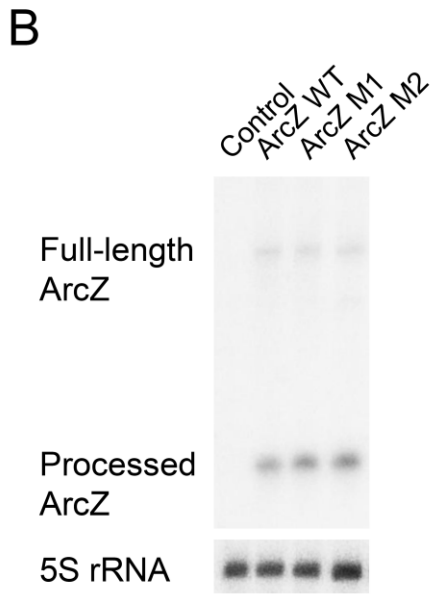


Figure S1 (related to Figure 2). Some RyhB targets are unaffected both at the mRNA and the translational level. Shown is a Volcano plot of ribosome-profiling results of translational level change following RyhB overexpression, based on ribosomal-profiling data of Wang et al. (2015). The translational level change of each gene is represented by the Log_2 Fold Change (Wang et al., 2015). The statistical significance of the change is represented as $-\log_{10}p$ (y axis). Green dots represent the RyhB targets detected by RIL-seq applied to *E. coli* grown to exponential phase under iron limitation. Black dots represent the rest of *E. coli* genes. The dashed line represents the statistical significance threshold set by Wang et al. (2015) ($p \leq 0.05$).


A
ArcZ WT
ArcZ M1
ArcZ M2


B
Control
ArcZ WT
ArcZ M1
ArcZ M2
Full-length ArcZ
Processed ArcZ
5S rRNA

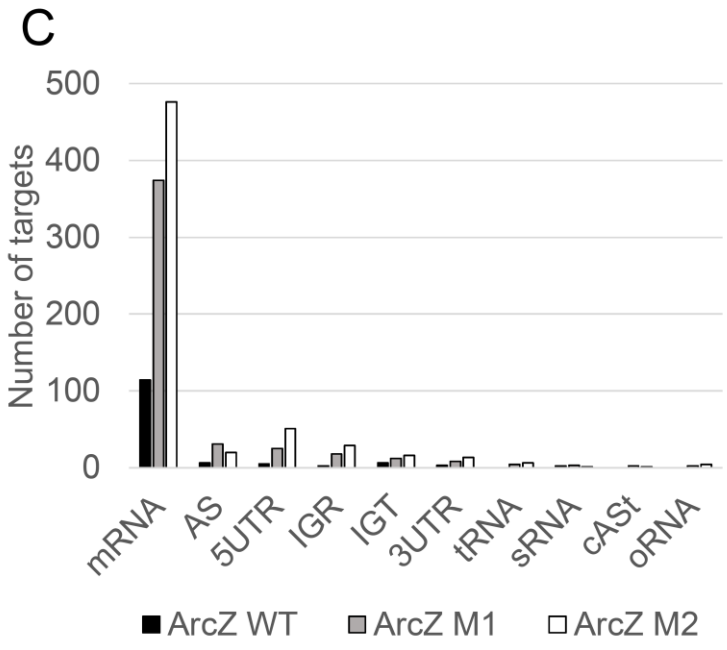

C
Number of targets
500
400
300
200
100
0
mRNA
AS
5UTR
IGR
IGT
3UTR
tRNA
sRNA
cASt
oRNA
ArcZ WT
ArcZ M1
ArcZ M2

Figure S2 (related to Figure 3). Analysis of ArcZ mutants. (A) The predicted secondary structures of ArcZ wild type and mutants are similar. The secondary structures of ArcZ WT, ArcZ M1 and ArcZ M2 were predicted using RNAfold (Lorenz et al., 2011). Colors represent computed base pairing probabilities, with red color for the highest probability. The binding site is marked with a bracket; the mutations are marked with arrows. (B) The cellular levels of ArcZ WT, ArcZ M1 and ArcZ M2 are similar, as shown by northern blot analysis using total RNA extracted from a Δ*arcZ* strain carrying pJV300 (Control), pZE12-ArcZ WT, pZE12-ArcZ M1 or pZE12-ArcZ M2 plasmids grown to OD_{600}=1.0 and induced with IPTG for 20 min. 5S rRNA was probed as a loading control. (C) Classification of RIL-seq targets of ArcZ WT, ArcZ M1 and ArcZ M2 into ten major categories: 5UTR (5' untranslated region), CDS (coding sequence), 3UTR (3' untranslated region), tRNA, sRNA, oRNA (other non-coding RNAs), AS (antisense), cASt (cis antisense with putative trans target), IGR (intergenic region), and IGT (intergenic within transcript). Bars represent the number of targets classified to each category.

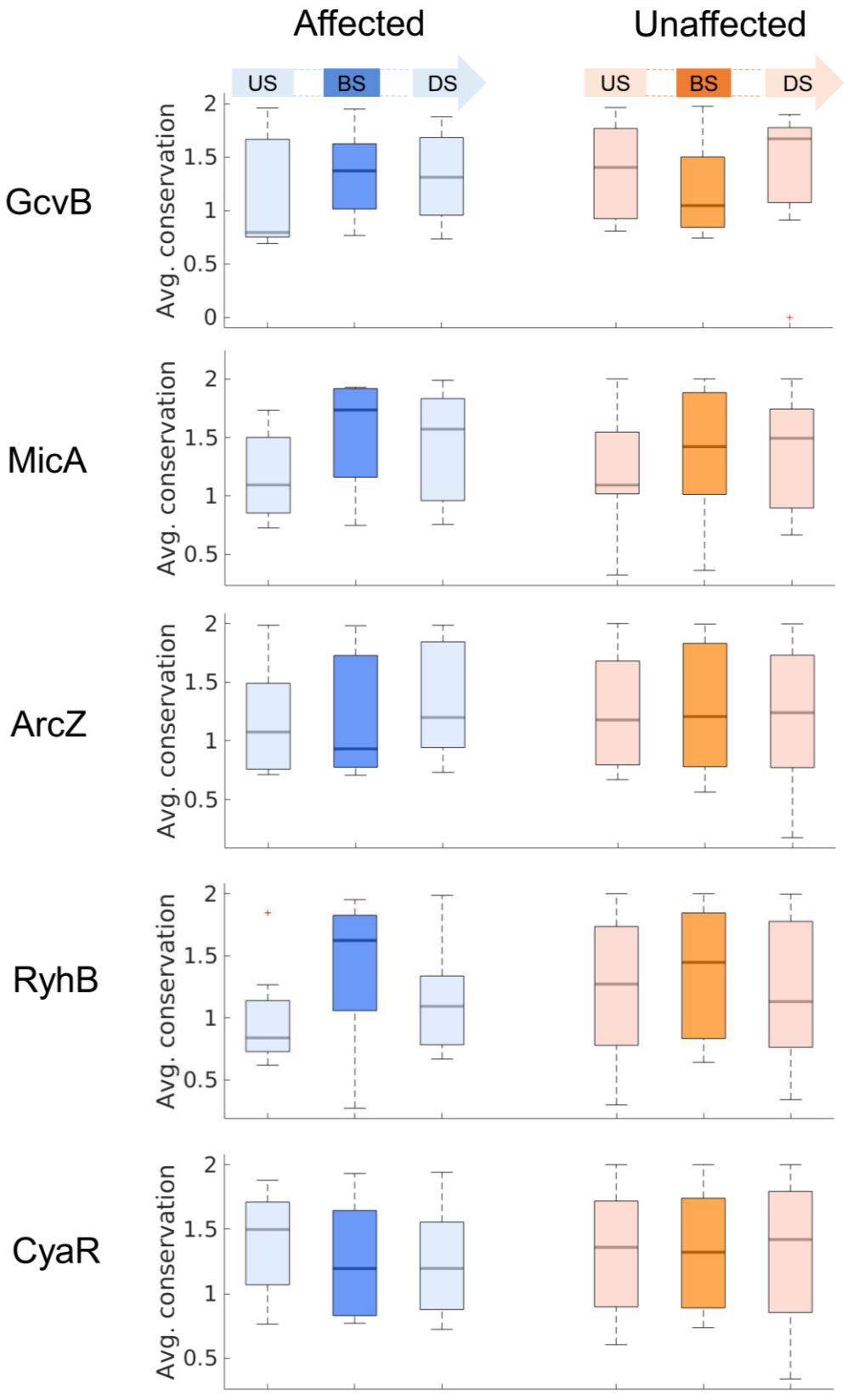
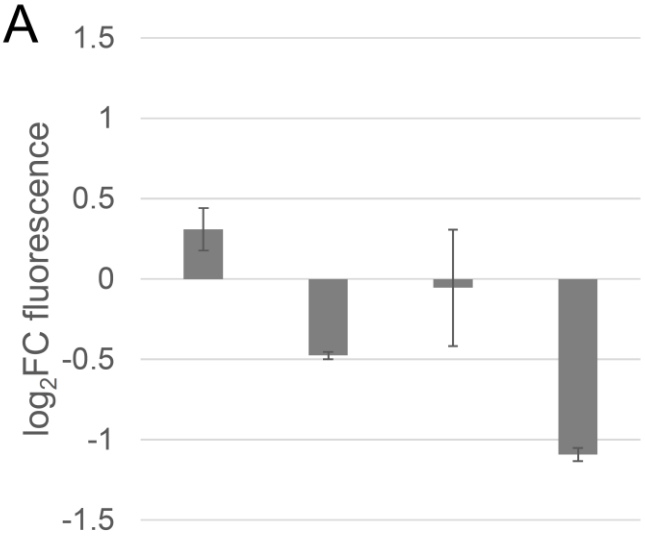

Affected
Unaffected
US
BS
DS
US
BS
DS
GcvB
MicA
ArcZ
RyhB
CyaR
Avg. conservation
0
0.5
1
1.5
2

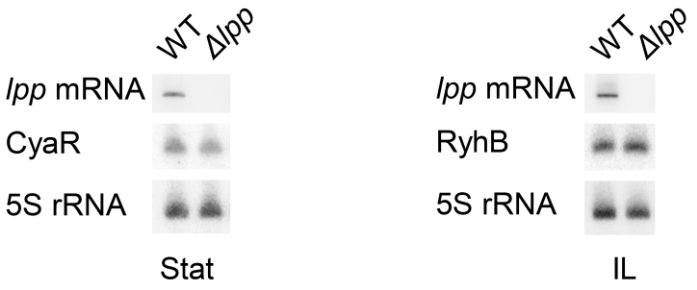
Figure S3 (related to Figure 2). sRNA binding sites of affected and unaffected targets show similar evolutionary conservation degrees. For all tested sRNAs, the conservation degrees (represented by the average information content) of the affected targets (blue) and the unaffected targets (orange) were high and did not differ statistically significantly between the two subsets. The conservation degrees of their flanking sequences (light blue for affected targets and light orange for unaffected targets) were used as a control. For all sRNAs both flanking regions did not differ statistically significantly in their conservation degrees from the binding sites for both affected and unaffected subsets. Statistical significance was assessed by Wilcoxon rank sum test. US, upstream sequence, BS, binding site, and DS, downstream sequences.

A

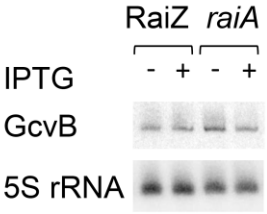


sRNA	CyaR	RyhB	GcvB	GcvB
Fused target	*lpp*	*lpp*	*raiA*	*gatY*

B



C



D

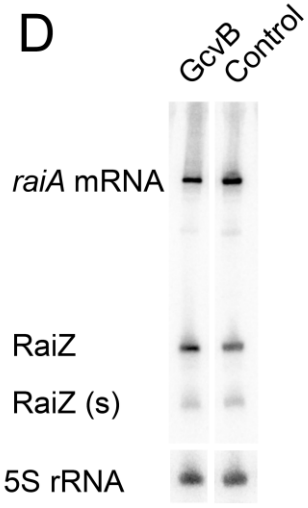


Figure S4 (related to Figures 1 and 2): Detailed experiments exploring steady interactions that do not lead to changes in the target RNA level. (A) Testing the effect of a sRNA on its target protein level using target-*gfp* translational fusion: bacteria carrying a target-*gfp* fusion expressing plasmid and a sRNA overexpressing plasmid or a control plasmid were grown at 30 ºC to exponential phase (OD_{600} = 0.5) and the GFP intensities were measured. The log_2 fold change values (log_2FC) between GFP fluorescence in cells overexpressing the sRNA and cells carrying a control plasmid are presented. Error bars indicate standard deviations based on three independent repeats. (B) *lpp*-mRNA levels do not affect the levels of CyaR and RyhB. BW25113 and BW25113 Δ*lpp* cells were grown to stationary phase (Stat; OD_{600}=1.0) or to exponential phase (OD_{600}=0.5) subjected to iron limitation (IL, 200 μM 2,2'-dipyridyl for 30 min). Total RNA was extracted and 30 μg RNA were analyzed by northern blots, using radiolabeled probes for *lpp*, CyaR, RyhB and 5S rRNA. (C) RaiZ and *raiA*-mRNA levels do not affect the levels of GcvB. Δ*raiA* cells carrying pZE12-RaiZ or pZE12-*raiA* plasmids were grown to exponential phase (OD_{600}=0.5) and induced with IPTG (1mM, 30 min). Total RNA was extracted and analyzed as in (B) using radiolabeled probes for GcvB sRNA and 5S rRNA. (D) GcvB sRNA does not affect RaiZ or *raiA* mRNA. Δ*gcvB* cells carrying pZA12-GcvB or pTP011 (control) plasmids were grown to exponential phase (OD_{600}=0.5) and induced with IPTG for 15 min. Total RNA was extracted and analyzed as in (B) using radiolabeled probes for *raiA*/RaiZ and 5S rRNA (loading control). RaiZ-S is a processing product of RaiZ.