Supplemental Information

Supplemental Materials and Methods

Protein sample preparation and Western blot analysis

For Hfq detection, we used the sample buffer under highly reduced conditions (containing 5% β-mercaptoethanol), and, for RpoS detection, sample buffer with 42mM DTT was used. Samples were loaded onto NuPAGE 12% Bis-Tris gel (Invitrogen) and electrophoresis was performed. Samples were transferred onto cellulose membrane and probed with the specific antibody. 1:5000 dilution of both RpoS and Hfq antiserums were used (Zhang *et al.*, 2002). In particular, Hfq antiserum was prepared by pre-absorption with the cell extract of the *hfq* deletion mutant (NM22562) to reduce nonspecific background bands. In order to measure Hfq molecules per cell, a standard curve of the known amount of Hfq was obtained for each experiment. Purified Hfq used for the standard curve was quantitated by a Bradford assay (Bio-Rad) against a standard curve of BSA.

Co-Immunoprecipitation

The supernatant of samples after centrifugation was transferred into a clean tube and precleared with protein A sepharose beads for 30 min at 4ºC with rotation. The

agarose beads were removed by centrifugation and the supernatants were collected in a new tube. 100 μl of precleared supernatant was mixed with 500 μl binding buffer and 55 μl of either Hfq antiserum or prebleed antiserum conjugated to protein A sepharose beads, respectively, and then incubated for one hour at 4ºC with rotation. The beads from each sample were collected by centrifugation at 1000rpm at 4ºC and washed with high salt, low salt, Lithium buffers and two washes of TE (pH8), respectively. The beads were resuspended with 250 μl elution buffer. 25 μl of beads were saved for Hfq western blot analysis and RNA was extracted from the rest of the beads. For total input samples, 100 μl extract was used for the RNA purification. After ethanol precipitation, RNA was resuspended with 15 μl of DEPC water and 1.5 μl of RNA was loaded for further Northern blot analysis. For the western blot analysis, 25 μl of SDS sample buffer was added into each sample and 15 μl of each sample was used for further analysis.

sRNA library screening

After transformation in a microtiter dish, cells were spotted on LB plates with ampicillin. Each spot represented a strain with a particular sRNA. These spots were directly used to inoculate into LB containing ampicillin, IPTG, and arabinose and cultured at 37ºC with aeration. For IPTG, 100μM of final concentration was used. However, various concentrations of arabinose were used depending on the background strain after checking the suitable concentration of arabinose for each P_{BAD} construction on MacConkey plates: 0.02% for P_{BAD}-*eptB-lacZ*; 0.02% for P_{BAD}-*rpoS-lacZ* and P_{BAD}-lacZ-lacZ (leader, RBS, and start of *lacZ* fused in frame to usual *lacZ* reporter); 0.0005% for P_{BAD}-*dppA-lacZ*, P_{BAD}-*ompX-lacZ*, and P_{BAD}-*yqaE-lacZ*. After approximately 6 hours of incubation, OD was measured at 600nm followed by a β-galactosidase assay.

Table S1. Strains and plasmids used in this study

Strains or plasmids	Description	Reference or Source[a]
Strains		
MG1655	Wild-type *E. coli*	Lab strain
NM18	W3110 Δ*spf*::*cm*	N. Majdalani
NM22540	MG1655 Δ*araBAD*, *leu*$^+$	N. Majdalani
NM22562	MG1655 Δ*araBAD*, *leu*$^+$, Δ*hfq*::cm	N. Majdalani
NM1200	MG1655 mini λ cm^R	N. Majdalani
CF9239	MG1655 Δ*dksA*::*kn*	M. Cashel, NIH
CF9240	MG1655 Δ*dksA*::*tet*	M. Cashel, NIH
GSO149	MG1655 Δ*gcvB*::*kn*	(Hobbs *et al.*, 2010)
PM1205	*mal*::*lacI*^{q}, *araC*$^+$, P$_{BAD}$-*cat*-*sacB*-*lacZ*, mini λ tet^R	(Mandin & Gottesman, 2009)
PM1207	PM1205 Δ*chiX*::*kn*	(Mandin & Gottesman, 2009)
PM1284	PM1205 *lacI′*::P$_{BAD}$-*sfiA*-*lacZ*, Δ*chiX*::*cm*	P. Mandin
PM1409	PM1205 *lacI′*::P$_{BAD}$-*rpoS*-*lacZ*	(Mandin & Gottesman, 2010)
PM1410	PM1205 *lacI′*::P$_{BAD}$-*lacZ*-*lacZ*	P. Mandin (as for PM1051 from (Mandin & Gottesman, 2010)
PM1420	*lacI′*::P$_{BAD}$-*rpoS*-*lacZ* Δ*hfq*::*cm*	P. Mandin; PM1409 + P1 (NM22562)
MG1426	*lacI′*::P$_{BAD}$-*phoP*-*lacZ*, Δ*micA*::*kn*	MG1425{Coornaert, 2010 #3759} + P1(*micA*::*kn*)
NRD352	MG1655 Δ*lacX74 imm*^{21} P$_{cyaR}$-*lacZ* (positions –76 to 64) *crp*::*cat*	(DeLay & Gottesman, 2009)
NRD397	PM1205 *lacI′*::P$_{BAD}$-*yqaE*-*lacZ*	N. De Lay
NRD400	PM1205 *lacI′*::P$_{BAD}$-*yqaE*-*lacZ*, Δ*cyaR*::*cm*	(DeLay & Gottesman, 2009)
KM86	PM1205 *lacI′*::PBAD-*dppA*-*lacZ*	(PM1205 + pBad-dppA F, dppA-lacZR)
KM94	Δ*araBAD*, *leu*$^+$, *araC*$^+$, *araE,* kn-P$_{BAD}$-Oxys	This study (KM100 + OxyS-KnF, pBad-OxyS R)
KM100	*mal*::*lacI*^{q}, Δ*araBAD*, *leu*$^+$, *araC*$^+$, *araE*	(Moon & Gottesman, 2009)
KM125	PM1205 *lacI′*:: P$_{BAD}$-*eptB*-*lacZ*	Moon et al, in preparation

KM132	*mal*::*lacI*^{q}, Δ*araBAD*, *leu*$^{+}$, *araC*$^{+}$, *araE* Δ*mgrR*::*kn*	(Moon & Gottesman, 2009)
KM153	*mal*::*lacI*^{q}, Δ*araBAD*, *leu*$^{+}$, *araC*$^{+}$, *araE* Δ*oxyS*::*kn*	KM100 + P1 (MC4100, Δ*oxyS::kn*) from G. Storz
KM180	MG1655 Δ*araBAD* Δ*mgrR*::*cm*	This study
KM225	*lacI'*:: P_{BAD} *-eptB-lacZ* Δ*mgrR*::*kn*	KM125 + P1 (KM132)
KM255	*mal*::*lacI*^{q}, Δ*araBAD*, *leu*$^{+}$, *araC*$^{+}$, *araE* Δ*chiX*::*kn*	KM100 + P1 (PM1207)
KM328	*lacI'*:: P_{BAD} *-eptB-lacZ* Δ*hfq*::*cm*	KM125+ P1 (NM22562)
KM329	PM1205 *lacI'*:: P_{BAD} *-ybfM-lacZ*	This study
KM331	PM1205 *lacI'*:: P_{BAD} *-ompX-lacZ* (2nd transcriptional start site)	This study
KM333	*lacI'*:: P_{BAD}*-ybfM-lacZ* Δ*chiX*::*kn*	KM329 + P1 (PM1207)
KM334	*lacI'*:: P_{BAD}*-ompX-lacZ* Δ*hfq*::*cm*	KM331 + P1 (NM22562)
KM335	*lacI'*:: P_{BAD}*-yqaE-lacZ* Δ*hfq*::*cm*	NRD397 + P1 (NM22562)
KM336	*lacI'*:: P_{BAD}*-dppA-lacZ* Δ*hfq*::*cm*	KM86 + P1 (NM22562)
KM339	*lacI'*:: P_{BAD}*-dppA-lacZ* Δ*gcvB*::*kn*	KM86 + P1 (GSO149)
KM340	*lacI'*:: P_{BAD}*-ompX-lacZ* Δ*cyaR*::*cm*	KM331 + P1 (NRD352)
KM341	PM1409 Δ*dksA*::*kn*	PM1409 + P1 (CF9239)
KM342	*lacI'*:: P_{BAD}*-ompX-lacZ* Δ*micA*::*kn*	KM331 + P1 (MG1426)
KM344	*lacI'*:: P_{BAD} *-lacZ-lacZ* Δ*hfq*::*cm*	PM1410 + P1 (NM22562)
KM349	*mal*::*lacI*^{q}, Δ*araBAD*, *leu*$^{+}$, *araC*$^{+}$, *araE* Δ*spf*::*cm*	KM100 + P1 (NM18)
KM352	*lacI'*:: P_{BAD}*-dppA-lacZ* Δ*chiX*::*kn*	KM86 + P1 (PM1207)
KM353	*lacI'*:: P_{BAD}*-dppA-lacZ* Δ*gcvB*::*kn,* Δ*chiX*::*cm*	KM339 + P1 (PM1284)
KM354	KM100 Δ*dksA*::*tet*	KM100 + P1 (CF9240)
KM359	*lacI'*:: P_{BAD}*-lacZ-lacZ* Δ*chiX*::*kn*	PM1410 + P1 (PM1207)
KM360	*lacI'*:: P_{BAD}*-lacZ-lacZ* Δ*gcvB*::*kn*	PM1410 + P1 (GSO149)
KM361	*lacI'*::P_{BAD}*-lacZ-lacZ* Δ*mgrR*::*kn*	PM1410 + P1 (KM225)
KM363	*lacI'*:: P_{BAD}*-eptB-lacZ* Δ*chiX*::*kn*	KM125 + P1 (PM1207)
KM364	*lacI'*:: P_{BAD}*-eptB-lacZ* Δ*gcvB*::*kn*	KM125 + P1 (GSO149)
KM366	*lacI'*:: P_{BAD}*-dppA-lacZ* Δ*mgrR*::*kn*	KM86 + P1 (KM225)

KM367	*lacI'*:: P_{BAD}-*dppA-lacZ* Δ*spf*::*cm*	KM86 + P1 (NM18)
KM368	*lacI'*:: P_{BAD}-*lacZ-lacZ* Δ*dksA*::*kn*	PM1410 + P1 (CF9239)
KM381	*lacI'*:: P_{BAD}-*eptB-lacZ* Δ*mgrR*::*cm* Δ*chiX*::*kn*	KM363 + P1 (KM180)
KM390	*lacI'*:: P_{BAD}-*lacZ-lacZ* Δ*mgrR*::*kn* Δ*chiX*::*cm*	KM361 + P1 (PM1284)
KM391	*lacI'*:: P_{BAD}-*lacZ-lacZ* Δ*gcvB*::*kn* Δ*chiX*::*cm*	KM360 + P1 (PM1284)
Plasmids		
pBAD33	Cm^R; araBAD promoter-based expression vector having a pACYC184 origin	(Guzman *et al.*, 1995)
pKMT4	Hfq coding region in pBAD33	K. Thompson
pNM12	Amp^R; pBAD24 derivative vector for sRNAs, pBR322 origin	(Majdalani *et al.*, 1998)
pGFK1014	Amp^R; OxyS cloned under araBAD promoter in pNM12	This study (OxyS-mscI, OxyS-ecoRIR)
pGFK1034	Amp^R; Spot42 cloned under araBAD promoter in pNM12	This study (Spf-mscIF, Spf-ecoRIR)
pGFK1035	Amp^R; ChiX cloned under the araBAD promoter in pNM12	This study (ChiX-mscI, ChiX-ecoRIR)
pSP64-OxyS	Amp^R; OxyS cloned under T7 promoter	A. Zhang (Zhang *et al.*, 1998)

a. Strains listed as from a person without further attribution are from the laboratory strain collection and not previously published. Strains created for this study by P1 transduction are listed as: recipient strain + P1 (donor strain).

Table S2. Oligonucleotides and probes in this study

Oligo name	Sequence (5’ to 3’)
	Oligonucleotides used for cloning plasmids
Spf-mscIF	TGAACTGAACAAAAAAGAGTAAAGTTGGCCATGTAGGGTA
Spf-ecoRIR	GGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGC GATATGTGCACTTCTTACCG
ChiX-mscIF	GTATTGGCAGGATGGTGAGATTGAGCGACAATGGCCATACACCGTC
ChiX-ecoRIR	ACCTGTATGGAGAAGGGAATT-GAATTC-AAATGTTGCGCTAA
OxyS-mscI	ATCAAGCATTCTGACTGATAATTTGGCCATGAAACGGAGCGGCA
OxyS-ecoRIR	CTTTTGCGCAGGCTCGGTTAGGGTAAGAATTCTTATATGTATAAAT
	Oligonucleotides used for constructing PBAD-OxyS and various lacZ fusions
OxyS-KnF	AATAGCAATGAACGATTATCCCTATCAAGCATTCTGACTGTATACGATGTCGCAGAGTAT
pBad-OxyS R	GCAGTGACTTCAAGGGTTAAAAGAGGTGCCGCTCCGTTTCATGGAGAAACAGTAGAGAGT
mgrR-HcmF	TTATTGACGAACAAAATGTGATTAACCGAGTTTAAGCTCCTGTGACGGAAGATCACTTCG
mgrR-HcmR	CAGTAAACCGGCGGTGAATGCTTGCATGGATAGATTTGTGACCAGCAATAGACATAAGCG
pBad-eptBF	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATGCGCGTGTAGATTTTACTTA
eptB-180lacZR	GGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCGAAGGTCACCAGTACGGTGG
pBad-dppA F	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATACGAGGGGCATTTTATGGAG
dppA-lacZR	TAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACCATCCCTGACTTTTTCAAGGA
pBAD-ompX	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATAGGACTTATTTGAATCACAT
ompX-lacZr	GGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCTGCTGAAAGACATGCAATTT

pBAD-ybfM	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATGTAGTCAGCGAGACTTTTCT
ybfM-lacZR	GGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCACTACGTTTGCCACTAAACG
deeplac	TAGGAGCGACCTTATGAGTCAGAATACG
placF	TGTGAGCGGATAACATTGACATTGTG
	Oligos for real time RT-PCR
qlacZ 158f	CAGCCTGAATGGCGAATGG
qlacZ 258r	CGACGACAGTATCGGCCTCA
qssrA F	TTTGTTAGTGGCGTGTCCGT
qssrA R	GAACCCGCGTCCGAAAT
	In vitro transcription
DsrA-VTF	ACGCACGCTGTAATACGACTCACTATAGGAACACATCAGATTTCCTGGTGTAACGAATTTTTTAAGTGCTTCTTGCTTAA GCAAGTTTCATCCCGACCCCCTCAGGGTCGGGATTT
DsrA-VTR	AAATCCCGACCCTGAGGGGGTCGGGATGAAACTTGCTTAAGCAAGAAGCACTTAAAAAATTCGTTACACCAGGAAATC TGATGTGTTCCTATAGTGAGTCGTATTACAGCGTGCGT
Spot42-VTF	ACGCACGCTGTAATACGACTCACTATAGGGTAGGGTACAGAGGTAAGATGTTCTATCTTTCAGACCTTTTACTTCACGTA ATCGGATTTGGCTGAATATTTTAGCCGCCCCAGTCAGTAATGACTGGGGCGTTTTTTA
Spot42-VTR	TAAAAAACGCCCCAGTCATTACTGACTGGGGCGGCTAAAATATTCAGCCAAATCCGATTACGTGAAGTAAAAGGTCTGA AAGATAGAACATCTTACCTCTGTACCCTACCCTATAGTGAGTCGTATTACAGCGTGCGT
ChiX-VTF	ACGCACGCTGTAATACGACTCACTATAGGACACCGTCGCTTAAAGTGACGGCATAATAATAAAAAAATGAAATTCCTCT TTGACGGGCCAATAGCGATATTGGCCATTTTTTT
ChiX-VTR	AAAAAAATGGCCAATATCGCTATTGGCCCGTCAAAGAGGAATTTCATTTTTTTATTATTATGCCGTCACTTTAAGCGACG GTGTCCTATAGTGAGTCGTATTACAGCGTGCGT

	Biotinylated probes used for Northern blots
ArcZ-NB1	CGCCGTAAATTATTATGATGAGTTACAAGGGCACAGCAC
GcvB NB1	CCAGAACACGCATTCCGATAAAACTTTTCGTTCCGGCTCAGG
ChiX NB1	CGGTCCAGGGAAATGGCTCTTGGGAGAGAGCCGTGCGC
IS118-bio	CCAATCATGGCGCGCACAAGCTATAATACCAAC
MgrR-bio	CAGTAAACCGGCGGTGAATGCTTGCATGGATAGAT
OxyS-bio	AAACTCTCGAAACGGGCAGTGACTTCAAGGGTTAAA
CyaR-bio	TGGTTCCTGGTACAGCTAGCATTTTATGGGTTATG
Spot42-bio	GAAGTAAAAGGTCTGAAAGATAGAACATCTTACCTC
DsrA-N2c	TGAGGGGGTCGGGATGAAACTTGCTTAAGCAAGAAGCACT
SsrA-bio	CGCCACTAACAAACTAGCCTGATTAAGTTTTAACGCTTCA

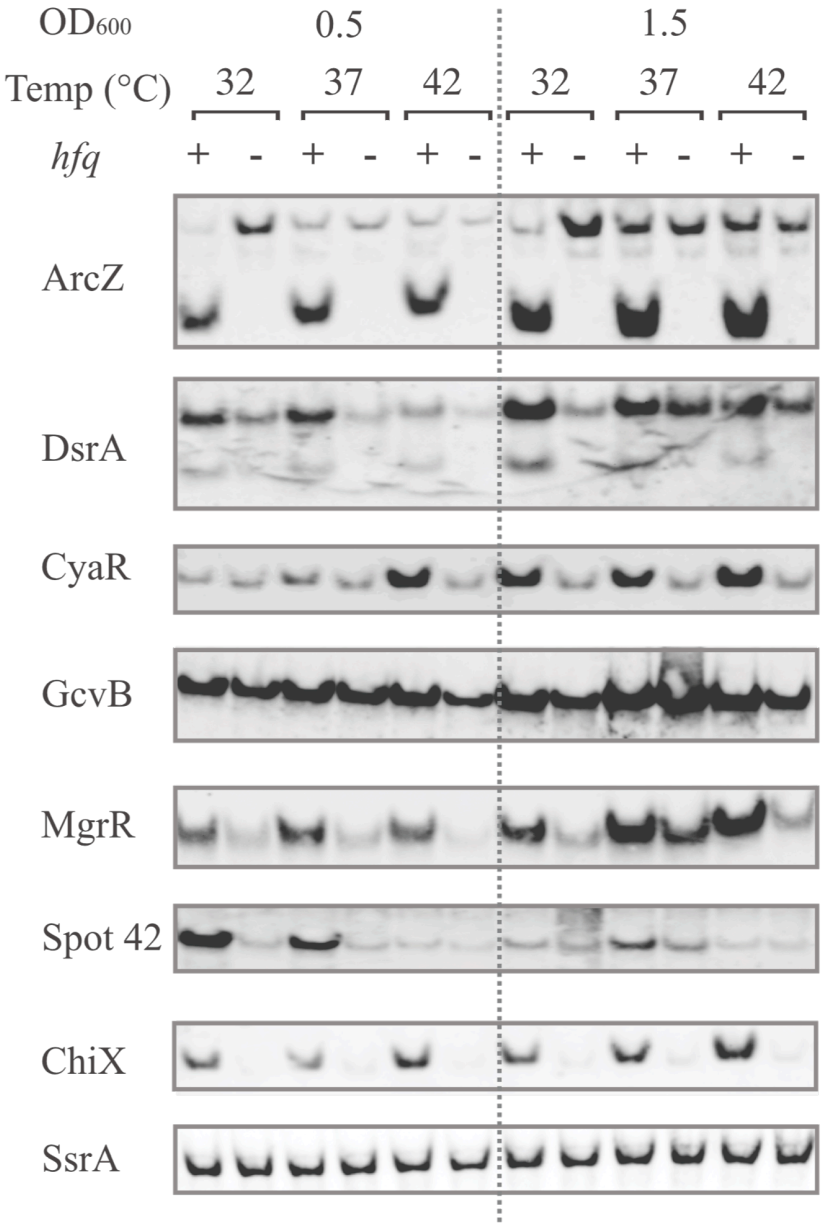
Table S3. Regulation of P_{BAD}-*ybfM-lacZ* in a WT (KM329) and *chiX* deletion mutant (KM333).

	WT	Δ*chiX*::*kan*
	Ave (+/- SD)	Ave (+/- SD)
plac	0 (+/- 0)	20.5 (+/- 5.2)
SgrS	0 (+/- 0)	18.7 (+/- 1.9)
ChiX	0 (+/- 0)	**0 (+/- 0)**
RybB	0 (+/- 0)	12.6 (+/- 2.3)
FnrS	0 (+/- 0)	22.8 (+/- 5.6)
MicC	**1.8 (+/- 0.6)**	23.2 (+/- 4.7)
RydC	**2.1 (+/- 1.6)**	18.5 (+/- 4.4)
MgrR	0 (+/- 0)	13.6 (+/- 1.8)
RprA	0 (+/- 0)	**1.5 (+/- 0.7)**
RyeB	0.5 (+/- 0.6)	13.8 (+/- 1.1)
CyaR	0.2 (+/- 0.3)	13.5 (+/- 2.2)
MicF	0 (+/- 0)	18.2 (+/- 4.3)
GlmY	0 (+/- 0)	16 (+/- 2.8)
MicA	0 (+/- 0)	13.9 (+/- 3.1)
GcvB	0 (+/- 0)	16.8 (+/- 2.8)
OmrA	**1.9 (+/- 0.5)**	14.6 (+/- 1.7)
OmrB	0.3 (+/- 0.3)	14.6 (+/- 1.9)
ArcZ	0.9 (+/- 1)	16.5 (+/- 4.6)
RyhB	0.8 (+/- 0.8)	16.7 (+/- 5.8)
GadY	0 (+/- 0)	13.3 (+/- 1.8)
GlmZ	0 (+/- 0)	13.9 (+/- 2)
OxyS	0 (+/- 0)	10.2 (+/- 1.2)
DsrA	0 (+/- 0)	16.2 (+/- 4.3)
Spot42	0.4 (+/- 0.3)	18.9 (+/- 4.8)
RseX	0 (+/- 0)	15 (+/- 2.7)

KM329 carrying a P_{BAD}-*ybfM-lacZ* construct, or its isogenic derivative deleted for *chiX* (KM333) were transformed with either the pBR-plac control plasmid or with plasmids containing the sRNAs. Transformed cells were grown in microtiter plates with LB containing 0.02% arabinose. Negative effects of the overexpression of each sRNA on the *yqaE-lacZ* fusion in the WT background were not measurable due to tight repression by chromosomally encoded ChiX; however, some sRNAs allowed some expression (bolded in the table), suggesting modest competition with ChiX. In the Δ*chiX* mutant, the basal level with a vector was significantly higher, and thus the negative effect of ChiX overexpression, as well as a negative effect of RprA overexpression was measurable (bold numbers in table).

Supplemental Figures

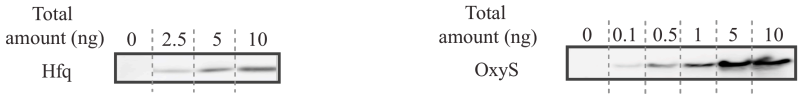
Fig. S1: Levels of sRNAs in the presence and absence of Hfq.



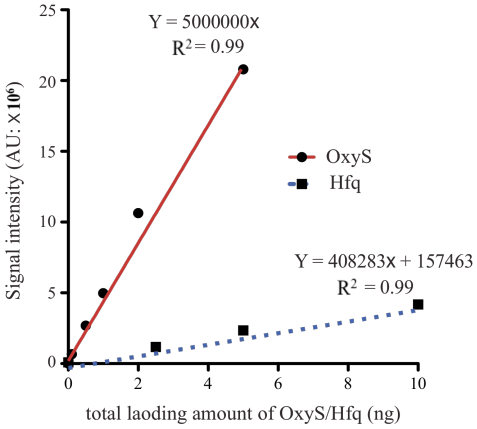
Wild type (NM22540) and *hfq::cat* (NM22562) cells were grown in LB at the three temperatures shown and samples taken at OD_{600}= 0.5 and OD_{600}=1.5 and processed as described in Materials and Methods. The Northern blots shown are representative of three experiments. Quantitation of these blots is given in Table 1 and was determined using SsrA as a loading control.

Fig. S2: Quantitation of Hfq and OxyS levels in the cell.

A.



B.



C. Hfq Quantification

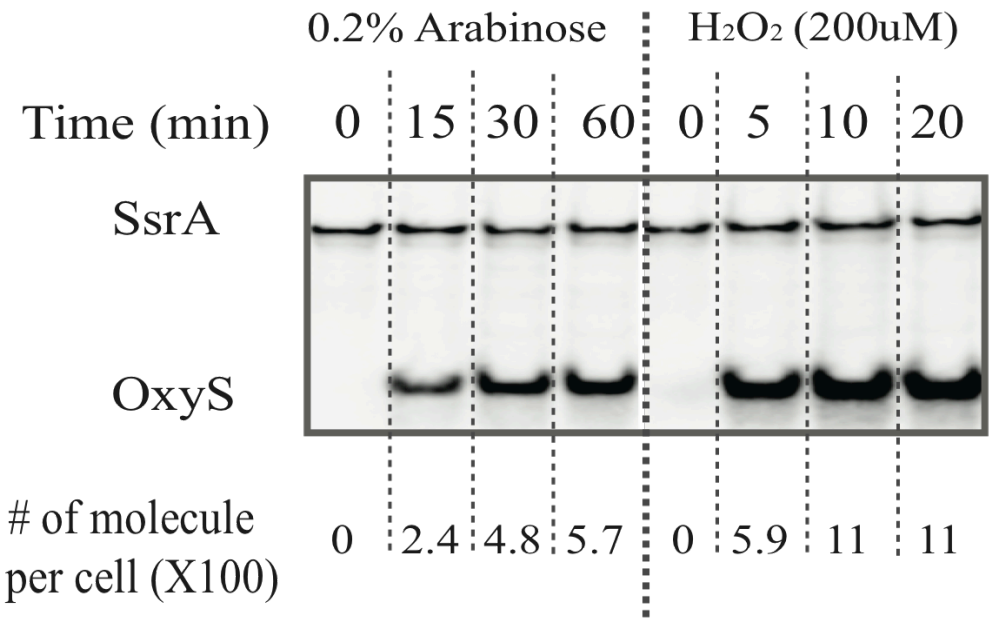
plasmids	Hfq amount (ng)		#of molecule/cell	hexamers
	Ave	SD		
-OxyS/-Hfq	54.5	9.2	29100	4850
-OxyS/+Hfq	77.8	13.9	41950	6992
+OxyS/-Hfq	55.8	8.5	30373	5062
+OxyS/+Hfq	82.9	4.8	44613	7436

OxyS Quantification

plasmids	OxyS amount (ng)		#of molecule/cell
	Ave	SD	
-OxyS/-Hfq	0	0	0
-OxyS/+Hfq	0.002	0	2
+OxyS/-Hfq	1.34	0.2	1733
+OxyS/+Hfq	2.1	0.5	2756

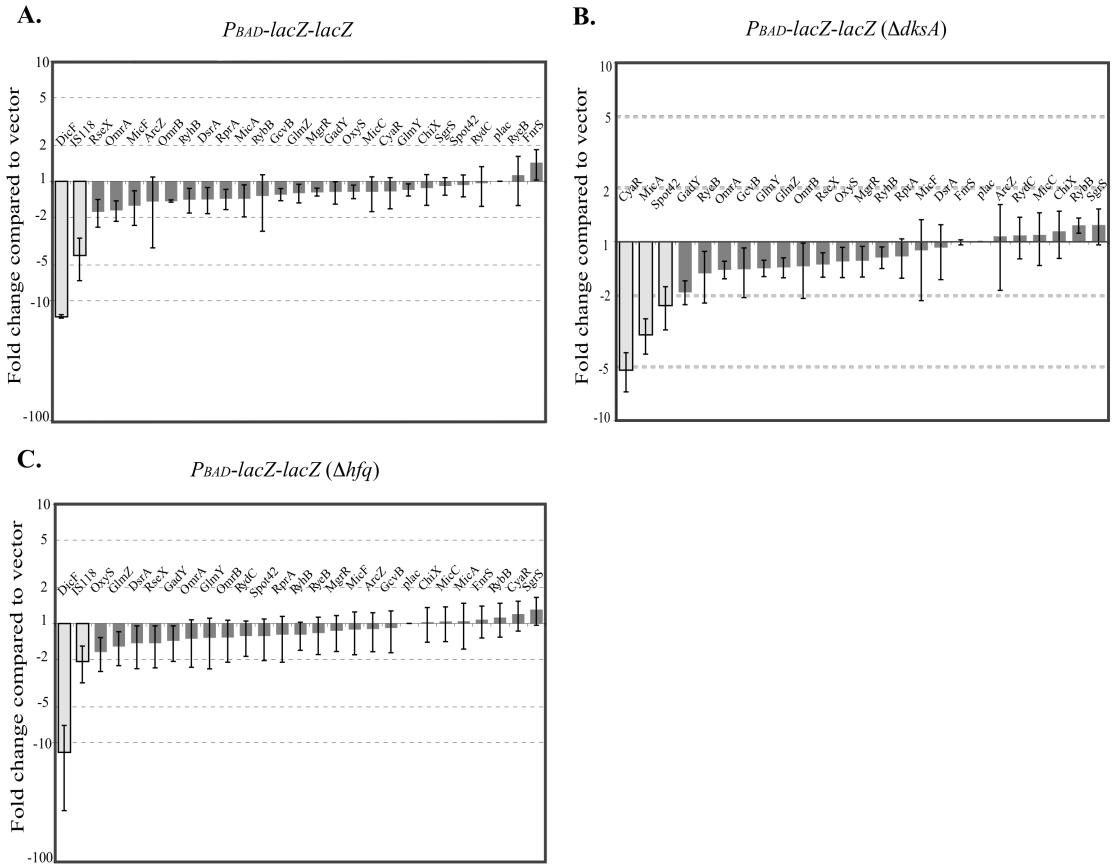
Strain KM153 (*hfq*$^{+}$ Δ*oxyS*) containing various vectors was used to express either Hfq or OxyS as described in the Figure 2 legend. A. Western blot for Hfq standard and Northern blot for OxyS standard. To quantify the amount of Hfq and OxyS in each sample, Western blot for Hfq and Northern blot for OxyS were performed with known amounts of purified Hfq protein (obtained from A. Zhang and G. Storz, NICHD) and OxyS from in vitro transcription (described in Materials and Methods). B. Standard curve from Western blot and Northern blot. After Western blot, each band was quantified by a Multi gauge program (Fuji). The Y axis shows signal intensity (AU) given by the program and the values were plotted with the known amount of Hfq protein. Equation and R values were obtained in Excel. C. The number of Hfq and OxyS molecules. Cells containing each plasmid were grown in LB containing ampicillin and chlorampenicol at 30°C. The cells were induced with 0.2% arabinose at OD_{600}=0.2-0.3 for 1 hour. Samples were taken at OD_{600}=0.5, and processed as previously described for the Western blots for Hfq and Northern blots for OxyS. The amounts of both Hfq protein and OxyS in cells were obtained using the standard curve obtained in (B) and presented as an average of triplicates; this value represents the number of molecules in 1 ml of cells. SD: standard deviation. The number of cells for each sample was determined by plating with serial dilutions and was approximately 10^8 per ml at OD_{600} = 0.5 under our conditions.

Fig. S3. Comparison of OxyS induction levels from a chromosomal P_{BAD} promoter and hydrogen peroxide treatment.



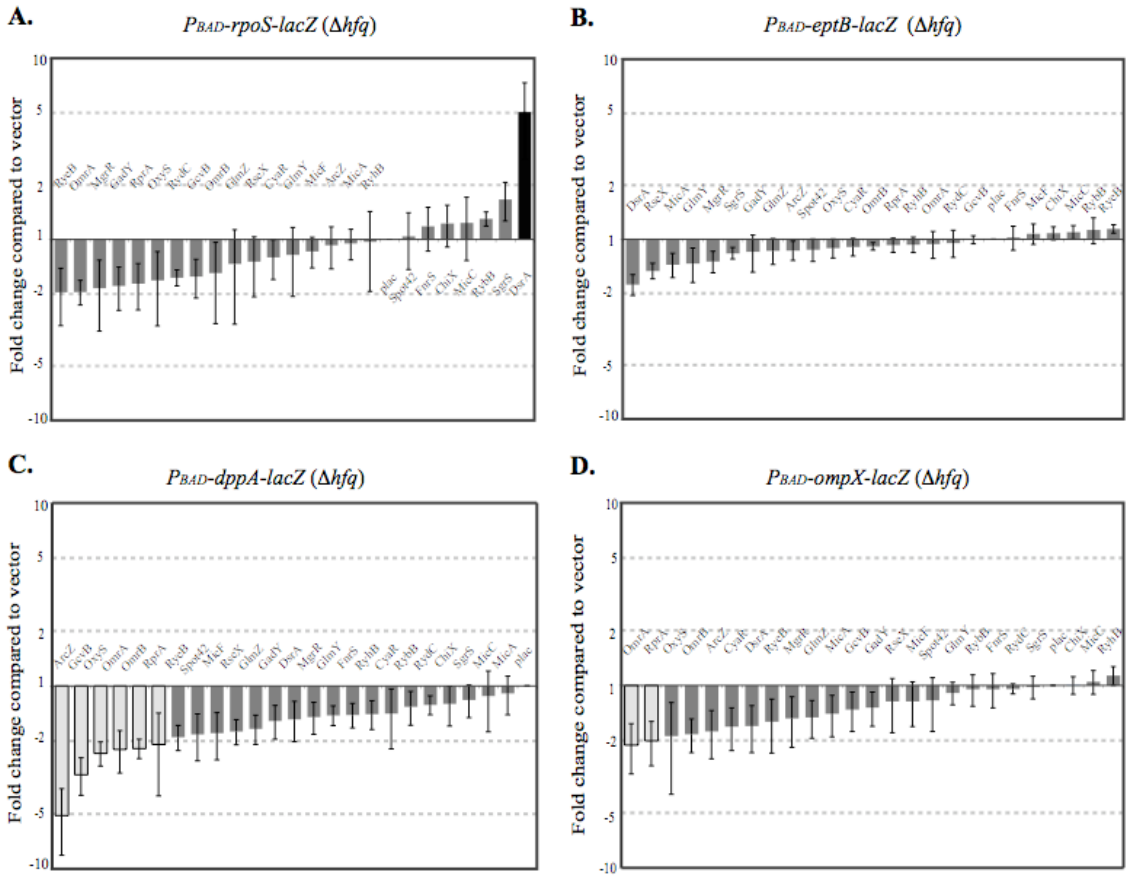
The strain harboring P_{BAD}-OxyS in the chromosome (KM94) grown at 30°C in LB was induced with 0.2% arabinose at OD=0.3 and WT (MG1655) was treated with a final concentration of 200uM of H_2O_2. RNA samples were collected at each time point for the northern blot as indicated. SsrA was an internal standard. The number of OxyS molecules was obtained from the standard curve (Fig. S2).

Fig. S4. Effect of the sRNA library on a control p_{BAD}-*lacZ* fusion



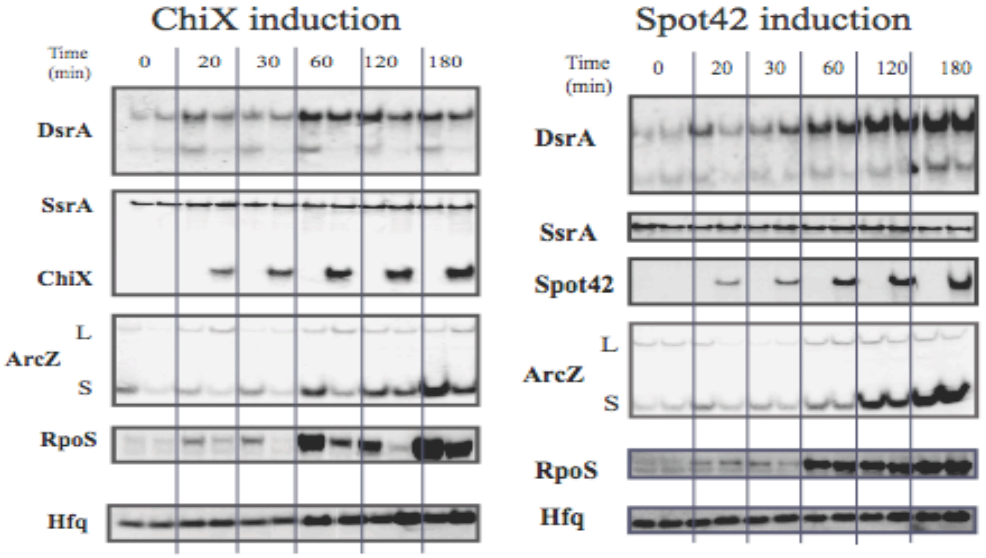
Strains carrying a P_{BAD}-*lacZ* fusion in various backgrounds were used; A. WT (PM1410); B. Δ*dksA* deletion mutant (KM368), and C. Δ*hfq* deletion mutant (KM344). The effect of the overexpression of each sRNA on the p_{BAD}-*lacZ* fusion was plotted as a function of the fold change it induced compared to the basal activity of strain PM1410 containing the pBR-plac control vector; shading is as for Fig. 4. Note that DicF and IS118 show an effect on the p_{BAD}-*lacZ-lacZ* fusion in both WT and Δ*hfq* deletion mutant and were therefore not used in other library screening experiments.

Fig. S5: Effects of sRNAs on fusions in the absence of Hfq.



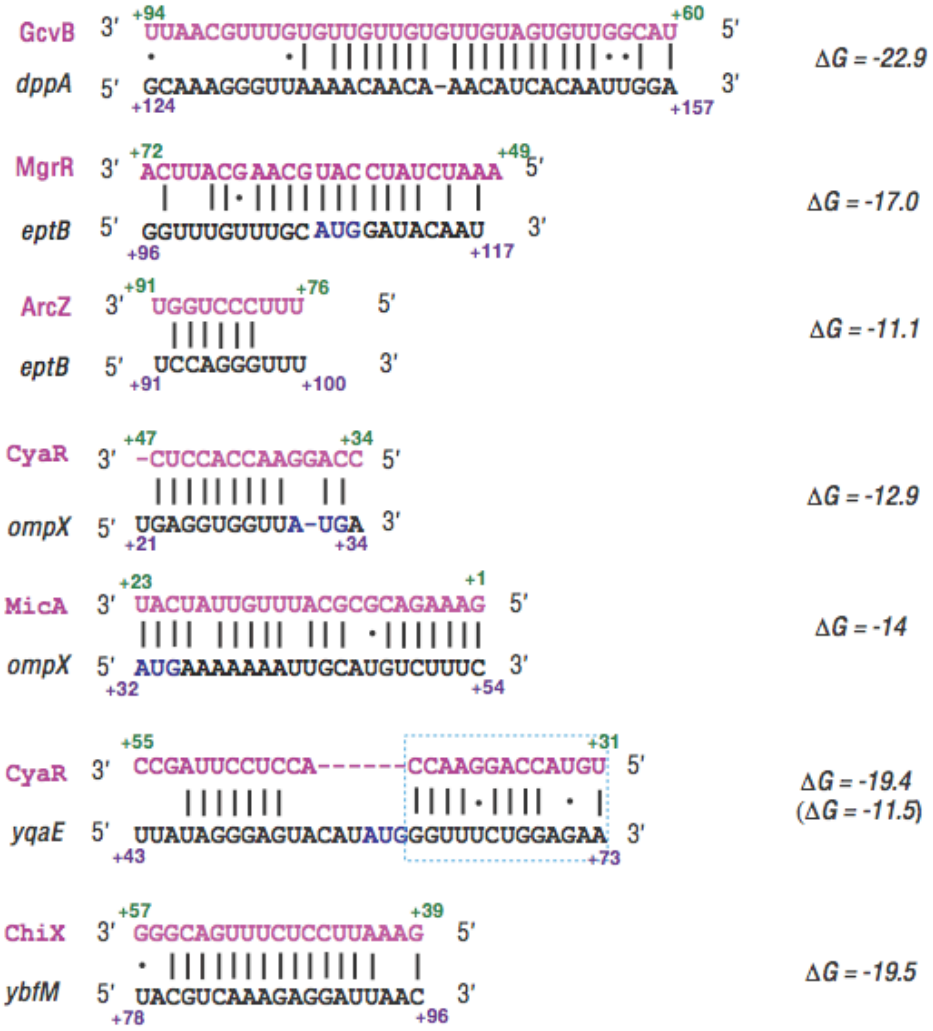
Isogenic derivatives of cells harboring different *lac* fusions in an Δ*hfq* background were grown in LB with arabinose (0.02% for both *rpoS-lacZ* and *eptB-lacZ* and 0.0005% for *dppA-lacZ* and *ompX-lacZ*) at 37°C and specific activity was obtained. A. P_{BAD}-*rpoS-lacZ* (PM1420) basal level is 20 units; B. P_{BAD}-*eptB-lacZ* (KM328) basal level is 1.8 units; C. P_{BAD}-*dppA-lacZ* (KM336) basal level is 154 units; D. P_{BAD}-*ompX-lacZ* (KM334); basal activity is 192 units.

Fig. S6: Effects on induction of ChiX and Spot42 on other sRNAs.



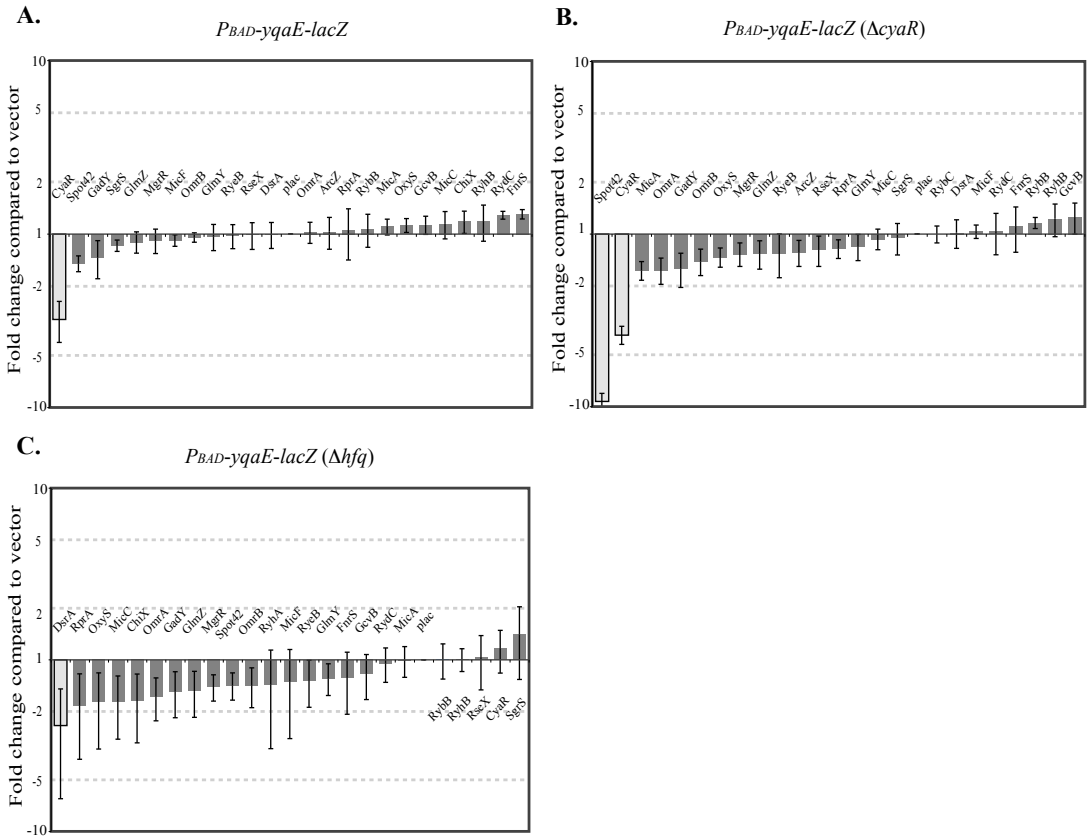
Cells carrying either vector (pNM12) or plasmids expressing ChiX (A) or Spot 42 (B) were growth in LB ampicillin at 30°C and induced with 0.2% arabinose at OD_{600}=0.3. Both RNA and protein samples for Northern blot and Western blot were taken at each time point. The OD_{600} at 30min and 60min induction were approximately 0.5 and 0.8, respectively. SsrA was used as an internal control for the Northern blot. Graphs of the results are in Fig. 5. A. Strain KM255 (Δ*chiX*) containing either a vector control (pNM12) or a plasmid expressing ChiX (pGFK1035). B. Strain KM349 (Δ*spf*) containing either a vector control (pNM12) or a plasmid expressing Spot42 (pGFK1034).

Fig. S7. Strength of the base pairing between sRNAs and their target genes.



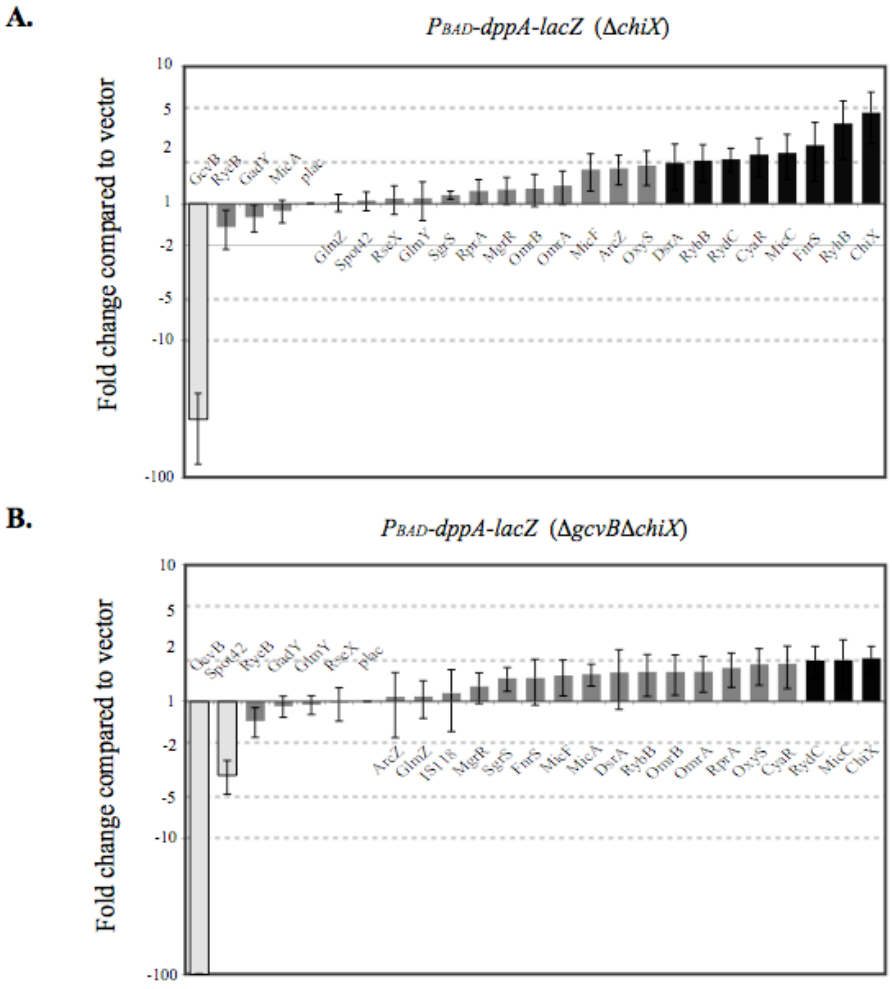
Predicted base-pairing of each sRNA and its target and free energy (ΔG) was obtained by mFold. Pairing has also been reported in the literature for most of these cases, although the extent to which pairing was tested by mutation varies greatly and in no case were all pairings tested. These have been examined in: GcvB:*dppA*: (Sharma *et al.*, 2007); MgrR: *eptB* (Moon & Gottesman, 2009); ArcZ:*eptB* (regulation demonstrated in this work, further tested in unpublished work, Moon, Lee and Gottesman; CyaR:*ompX* (Papenfort *et al.*, 2008, Johansen *et al.*, 2008, DeLay & Gottesman, 2009); MicA:*ompX* (Johansen et al., 2008); CyaR:*yqaE* (DeLay & Gottesman, 2009); ChiX:*ybfM* (Figueroa-Bossi *et al.*, 2009, Overgaard *et al.*, 2009). The base pairing in the blue dashed box for *yqaE* and CyaR was the originally reported base pairing (DeLay & Gottesman, 2009); its ΔG values is -11.5. Numbers above and below base-pairing indicate the location of nucleotides relative to the transcriptional start site. ChiX and *ybfM* has the longest single stretch of base pairing (14bp) among the tested library sets, although GcvB and *dppA* show longer base pairing divided into two stretches (10bp and 7bp).

Fig. S8. Effect of sRNA library on p_{BAD}-*yqaE-lacZ* constructs.



Isogenic strains all carrying a p_{BAD}-*yqaE-lacZ* fusion were transformed with either the pBR-plac control plasmid or with the sRNA plasmid library and assayed as described in Materials and Methods and the Fig. 4 legend. Transformed cells were grown with LB/Amp containing 0.0005% arabinose. A. WT (NRD397), basal activity is 19; B. Δ*cyaR* (NRD400), basal activity is 170; C. Δ*hfq* (KM335), basal activity is 7.

Fig. S9: Effects of sRNAs on *dppA-lacZ* fusion in absence of *chiX*.



A set of isogenic strains carrying a translational fusion of *dppA* beginning at the transcriptional start site under the control of the *araBAD* promoter was examined for the effect of the sRNA library in *chiX* deletion mutations. A. Δ*chiX* (KM352); basal activity is 61 units; B. Δ*gcvB* Δ*chiX* double mutant (KM353); basal activity is 71 units.

References

DeLay, N. & S. Gottesman, (2009) The Crp-activated small noncoding regulatory RNA CyaR (RyeE) links nutritional status to group behavior. *J. Bacteriol.* 191: 461-476.

Figueroa-Bossi, N., M. Valentini, L. Malleret & L. Bossi, (2009) Caught at its own game: regulatory small RNA inactivated by an inducible transcript mimicking its target. *Genes Dev.* 23: 1981-1985.

Guzman, L. M., D. Belin, M. J. Carson & J. Beckwith, (1995) Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. *J. Bacteriol.* 177: 4121-4130.

Hobbs, E. C., J. L. Astarita & G. Storz, (2010) Small RNAs and small proteins involved in resistance to cell envelope stress and acid shock in *Escherichia coli*: analysis of a bar-coded mutant collection. *J. Bacteriol.* 192: 59-67.

Johansen, J., M. Eriksen, B. Kallipolitis & P. Valentin-Hansen, (2008) Down-regulation of outer membrane proteins by noncoding RNAs: unraveling the cAMP-CRP- and σ^E-dependent CyaR-*ompX* regulatory case. *J. Mol. Biol.* 383: 1-9.

Majdalani, N., C. Cunning, D. Sledjeski, T. Elliott & S. Gottesman, (1998) DsrA RNA regulates translation of RpoS message by an anti-antisense mechanism, independent of its action as an antisilencer of transcription. *Proc. Natl. Acad. Sci. USA* 95: 12462-12467.

Mandin, P. & S. Gottesman, (2009) A genetic approach for finding small RNA regulators of genes of interest identifies RybC as regulating the DpiA/DpiB two-component system. *Mol. Microbiol.* 72: 551-565.

Mandin, P. & S. Gottesman, (2010) Integrating anaerobic/aerobic sensing and the general stress response through the ArcZ small RNA. *EMBO J.* 29: 3094-3107.

Moon, K. & S. Gottesman, (2009) A PhoQ/P-regulated small RNA regulates sensitivity of *Escherichia coli* to antimicrobial peptides. *Molec. Microbiol.* 74: 1314-1330.

Overgaard, M., J. Johansen, J. Moller-Jensen & P. Valentin-Hansen, (2009) Switching off small RNA regulation with trap-mRNA. *Mol. Microbiol.* 73: 790-800.

Papenfort, K., V. Pfeiffer, S. Lucchini, A. Sonawane, J. C. Hinton & J. Vogel, (2008) Systematic deletion of Salmonella small RNA genes identifies CyaR, a conserved CRP-dependent riboregulator of OmpX synthesis. *Mol Microbiol* 68: 890-906.

Sharma, C. M., F. Darfeuille, T. H. Plantinga & J. Vogel, (2007) A small RNA regulates multiple ABC transporter mRNAs by targeting C/A-rich elements inside and upstream of ribosome-binding sites. *Genes Dev* 21: 2804-2817.

Zhang, A., S. Altuvia, A. Tiwari, L. Argaman, R. Hengge-Aronis & G. Storz, (1998) The *oxyS* regulatory RNA represses *rpoS* translation by binding Hfq (HF-1) protein. *EMBO J.* 17: 6061-6068.

Zhang, A., K. M. Wassarman, J. Ortega, A. C. Steven & G. Storz, (2002) The Sm-like Hfq protein increases OxyS RNA interaction with target mRNAs. *Mol. Cell* 9: 11-22.