Supplementary Data for:

Multiple *in vivo* roles for the C-terminal domain of the RNA chaperone Hfq

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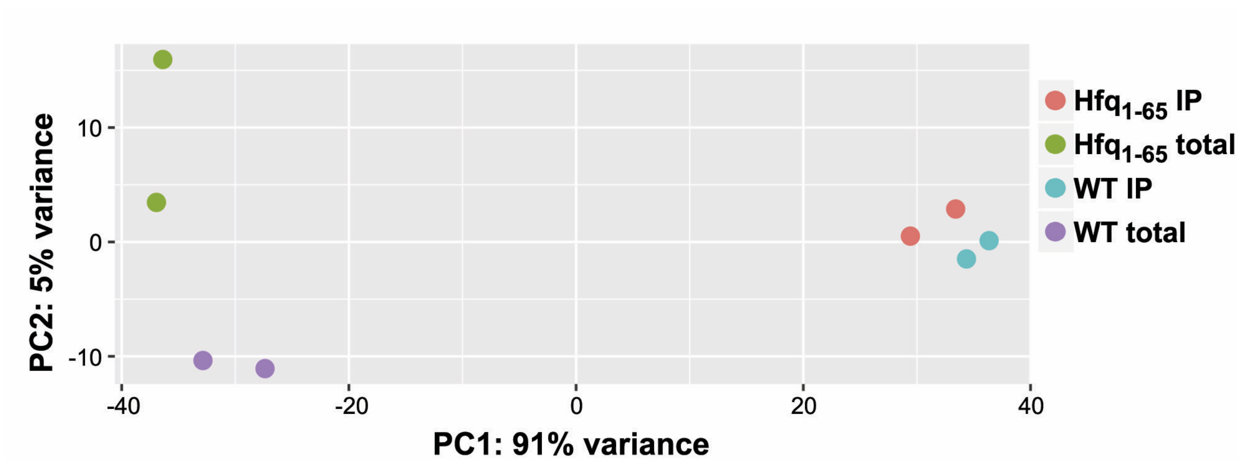

Figure S1. Reproducibility of RNA-Seq data for full length Hfq and $Hfq_{1\text{-}65}$. PCA plot for WT Hfq (KK2440) and $Hfq_{1\text{-}65}$ (KK2448) total and co-immunoprecipitation (co-IP) with Hfq RNA with biological replicates clustering together.

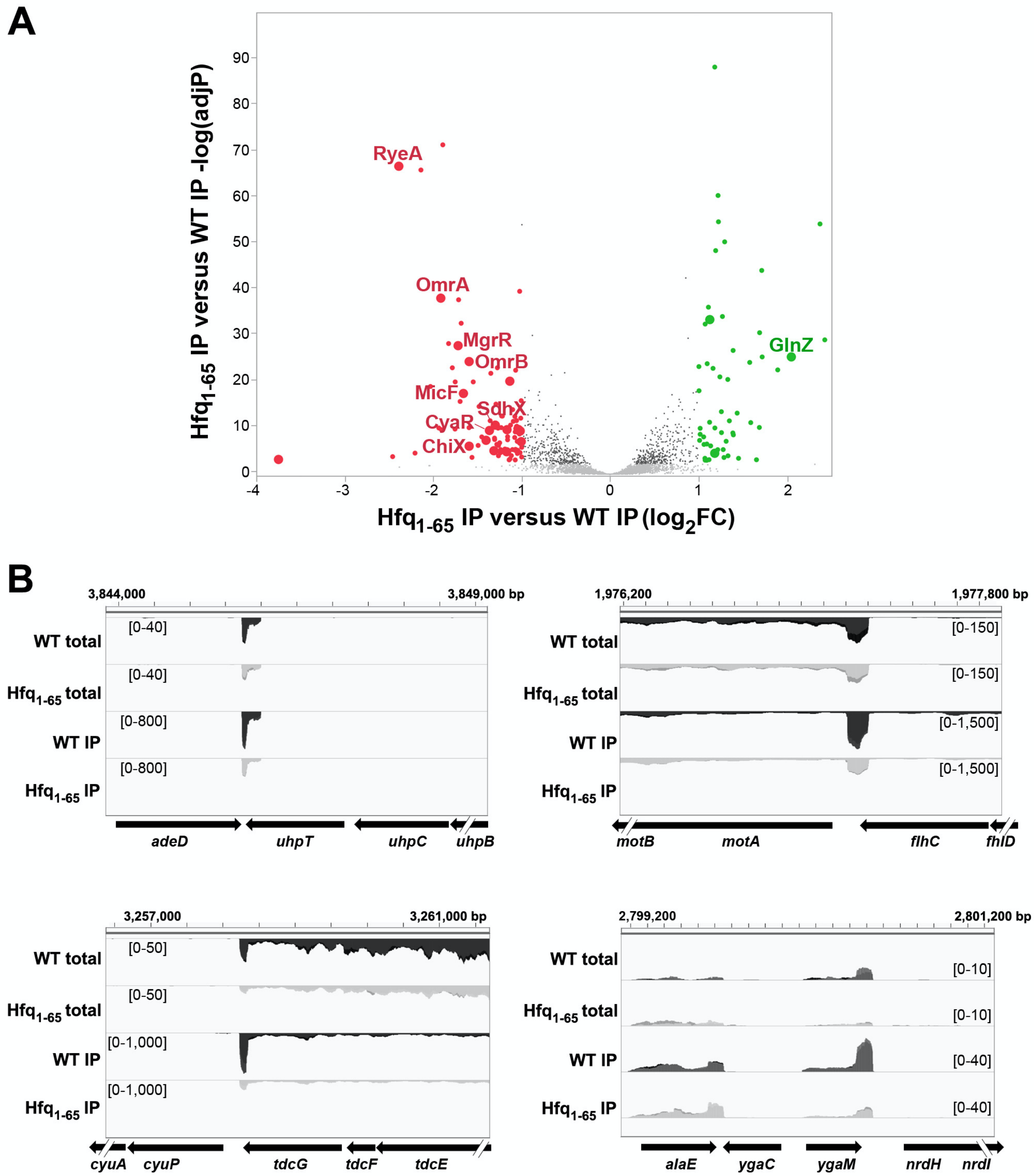

Figure S2. Volcano plot for co-IP of RNAs with anti-Hfq from WT and Hfq_{1-65} and genome browser for selected RNAs. (**A**) Volcano plot comparing co-IP RNA from Hfq_{1-65} (KK2448) to Hfq WT(KK2440). All genes with <2X change are shown in black or grey (signals too low to be significant). The red and green dots represent RNAs down and up regulated more than two-fold, respectively. A subset of sRNAs are labeled (larger red dots). (**B**) Genome browser images of data from total and Hfq co-IP RNA-Seq, showing patterns for two recently identified new sRNAs (UhpU, at the 3' UTR of *uhpT*, and MotR, at the 5' UTR of *motA* (1); unpublished, Storz lab), as well as two regions with patterns suggesting that they may also encode sRNAs (enrichment in co-IP signals at the 3' region of the gene, shown here for *tdcG* and *ygaM*).

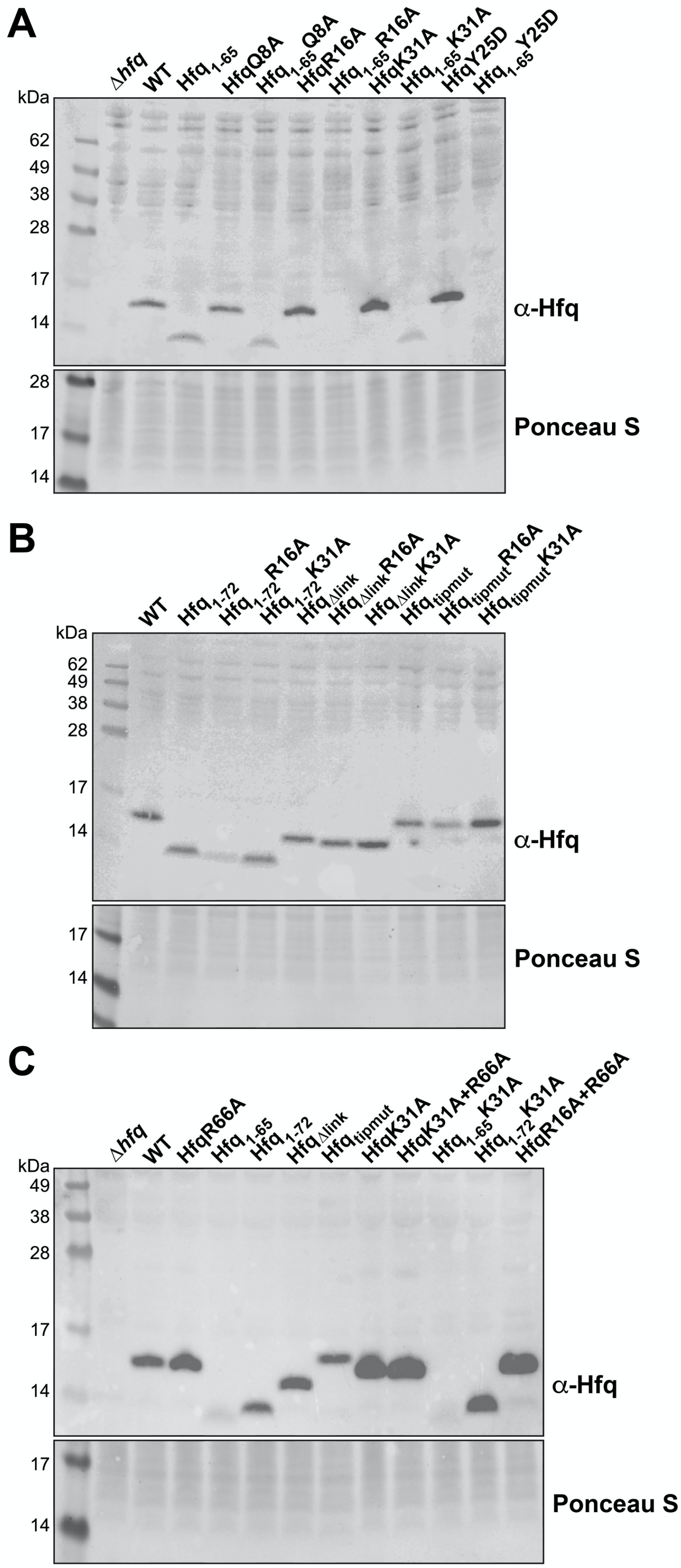
A
Δhfq
WT
Hfq1-65
HfqQ8A
Hfq1-65Q8A
HfqR16A
Hfq1-65R16A
HfqK31A
Hfq1-65K31A
HfqY25D
Hfq1-65Y25D
kDa
62
49
38
28
17
14
α-Hfq
28
17
14
Ponceau S
B
WT
Hfq1-72
Hfq1-72R16A
Hfq1-72K31A
HfqΔlink
HfqΔlinkR16A
HfqΔlinkK31A
Hfqtipmut
HfqtipmutR16A
HfqtipmutK31A
kDa
62
49
38
28
17
14
α-Hfq
17
14
Ponceau S
C
Δhfq
WT
HfqR66A
Hfq1-65
Hfq1-72
HfqΔlink
Hfqtipmut
HfqK31A
HfqK31A+R66A
Hfq1-65K31A
Hfq1-72K31A
HfqR16A+R66A
kDa
49
38
28
17
14
α-Hfq
17
14
Ponceau S

Figure S3. Levels of Hfq single and double mutants characterized in study.

Western blot of isogenic Hfq mutants. The strains are those used to measure *chiP-lacZ* activity in Figures 2B, 3B, 3E and 3F. (**A**) Δ*hfq* (DJS2689), WT (DJS2690), $hfq_{1\text{-}65}$ (KK01), *hfqQ8A* (DJS2691), $hfq_{1\text{-}65}$ *Q8A* (KK2414), *hfqR16A* (DJS2693), $hfq_{1\text{-}65}$ *R16A* (KK02), *hfqK31A* (DJS2695), $hfq_{1\text{-}65}$ *K31A* (KK04), *hfqY25D* (DJS2694), $hfq_{1\text{-}65}$*Y25D* (KK03), (**B**) WT (DJS2690), $hfq_{1\text{-}72}$ (KK2438), $hfq_{1\text{-}72}$*R16A* (KK2657), $hfq_{1\text{-}72}$*K31A* (KK2658), $hfq_{\Delta link}$ (KK2558) $hfq_{\Delta link}$ *R16A* (KK2654), $hfq_{\Delta link}$ *K31A* (KK2655), hfq_{tipmut} (KK2570), hfq_{tipmut} *R16A* (KK2651), hfq_{tipmut} *K31A* (KK2652) and (**C**) Δ*hfq* (DJS2689), WT (DJS2690), *hfqR66A* (KK2725), $hfq_{1\text{-}65}$ (KK01), $hfq_{1\text{-}72}$ (KK2438), $hfq_{\Delta\text{link}}$ (KK2558), hfq_{tipmut} (KK2570), *hfq*K31A (DJS2695), *hfqK31A R66A (*KK2727*), hfq*$_{1\text{-}65}$ *K31A* (KK04), $hfq_{1\text{-}72}$ *K31A* (DD2658), *hfqR16A R66A* (KK2726). The anti-Hfq antibody was used (upper panel) and Ponceau S as loading control (lower panel). Total protein from 1 ml of culture at $OD_{600} = 1$ was assayed. The sample preparation and antibody used are as reported previously (1). Our earlier report (2) suggests that Hfq antibody does not recognize the $Hfq_{1\text{-}65}$ monomer well, possibly due to loss of critical epitopes affecting the interpretation of the relative amounts of Hfq.

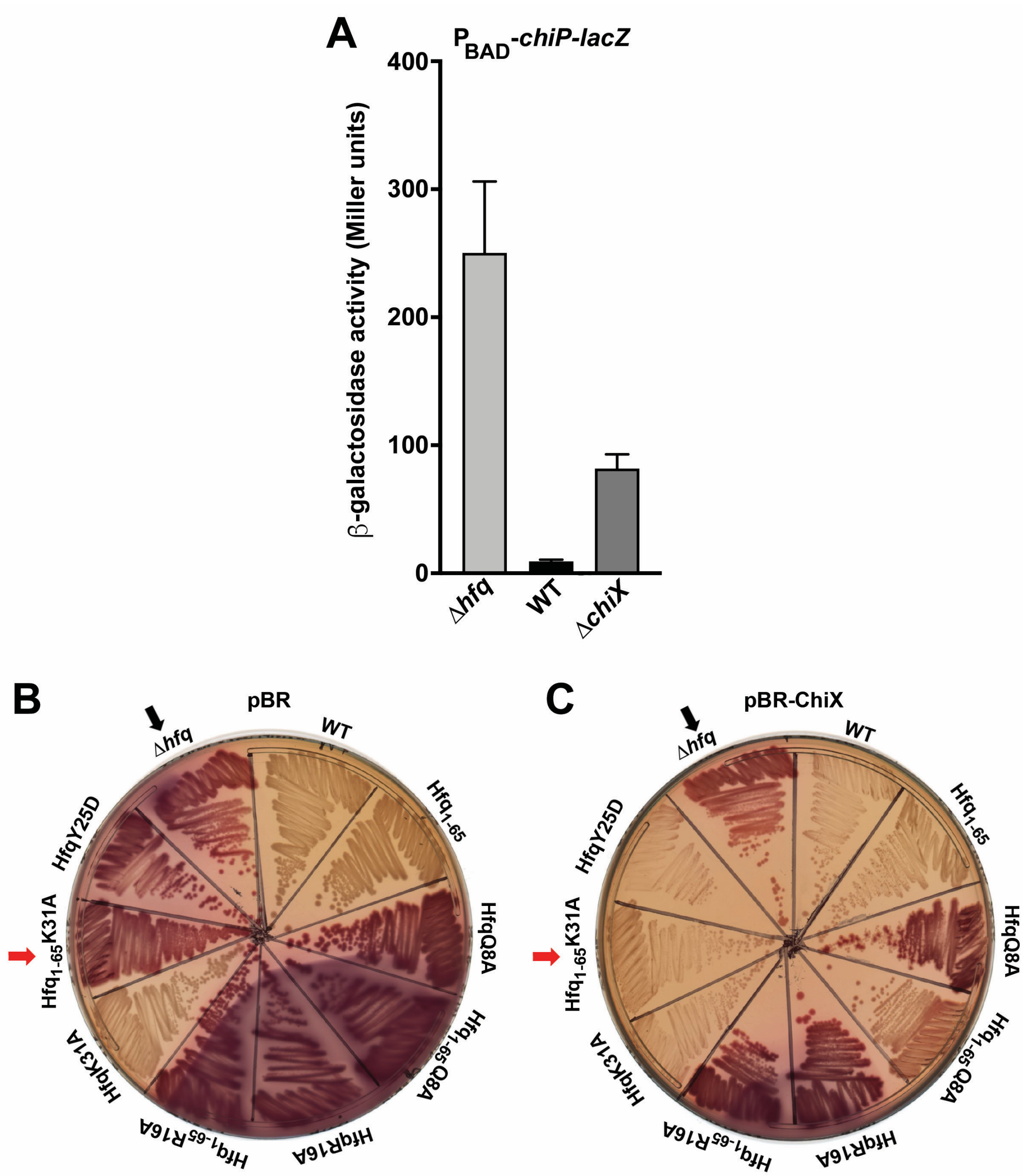

Figure S4. Absence of ChiX relieves repression and multicopy ChiX can suppress the regulatory defect for ChiX regulation of *chiP-lacZ* in a $Hfq_{1\text{-}65}$ K31A background. (A) P_{BAD}-*chiP-lacZ* expression in absence of *hfq* or *chiX* compared to WT strain. Assay conditions as in Fig. 2B. Strains used: Δ*hfq* (DJS2689), WT (DJS2690), Δ*chiX* (DJS2680). B. Regulation of P_{BAD}-*chiP-lacZ* [Δ*hfq* (DJS2689), WT (DJS2690), $hfq_{1\text{-}65}$ (KK01), *hfqQ8A* (DJS2691), $hfq_{1\text{-}65}$ *Q8A* (KK2414), *hfqR16A* (DJS2693), $hfq_{1\text{-}65}$ *R16A* (KK02), *hfqK31A* (DJS2695), $hfq_{1\text{-}65}$ *K31A* (KK04), *hfqY25D* (DJS2694)] in the presence of ChiX expressed from the chromosome with vector (pBR-plac) plasmid (**B**), or from a plasmid (**C**) (pBR-ChiX). The assay was performed on

MacConkey plates with 0.0005% arabinose and 50 µg ampicillin incubated at 37˚C for 16 h. All Hfq mutants were expressed from the native *hfq* locus.

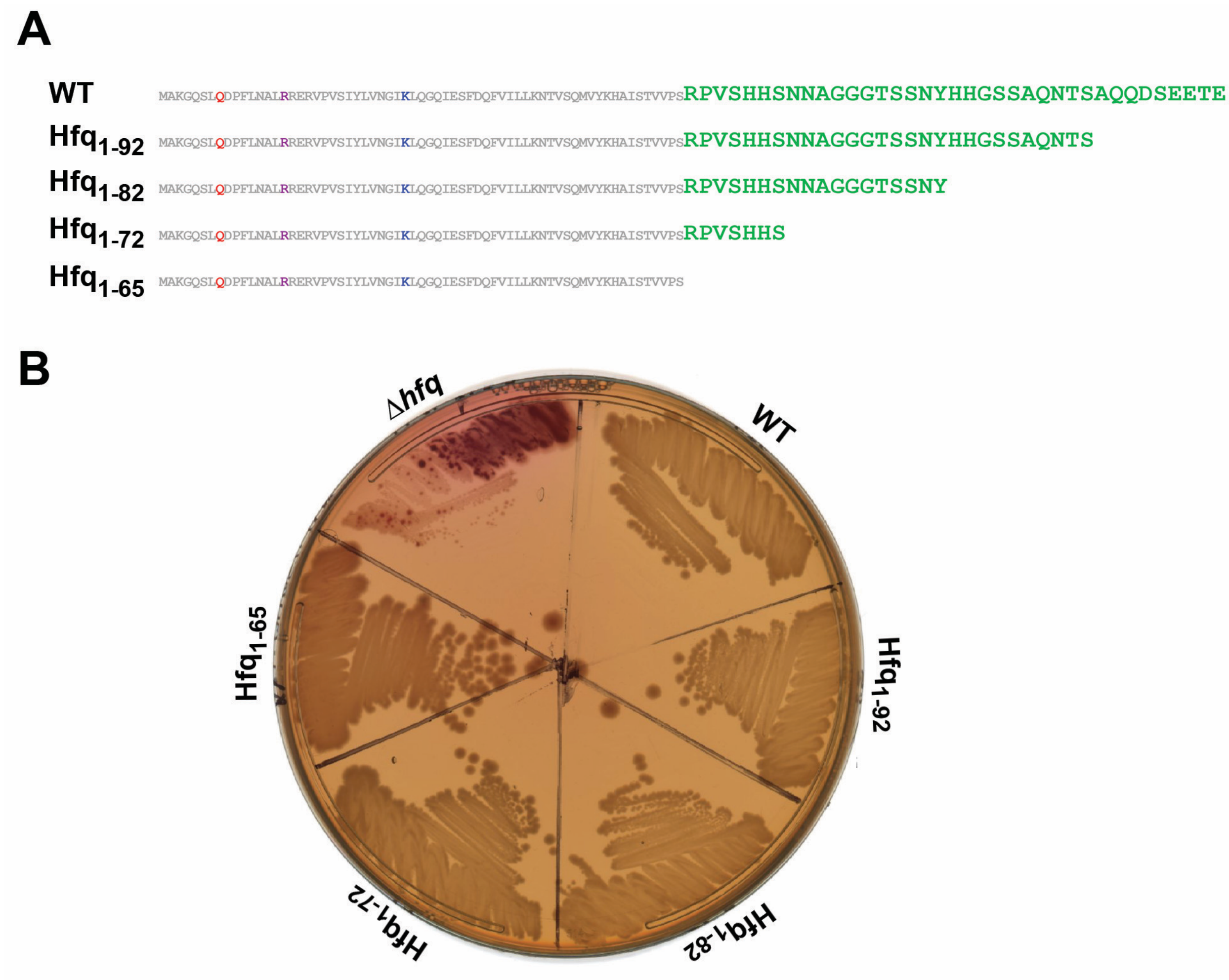

Figure S5. Effect of Hfq CTD truncations on ChiX-based regulation of *chiP-lacZ*. (**A**) Schematic of CTD truncations (**B**) Truncated HfqCTD mutant derivatives of PM1205 (*lacI'::*P_{BAD}*-chiP-lacZ*), expressing ChiX from the chromosome, were constructed and the activity of P_{BAD}-*chiP-lacZ* measured on MacConkey lactose plates containing 0.0005% arabinose incubated at 37˚C for 16 h. Strains used: Δ*hfq* (DJS2689), *WT* (DJS2690), $hfq_{1\text{-}65}$ (KK01), $hfq_{1\text{-}72}$ (KK2438), $hfq_{1\text{-}82}$ (KK2437), $hfq_{1\text{-}92}$ (KK2436).

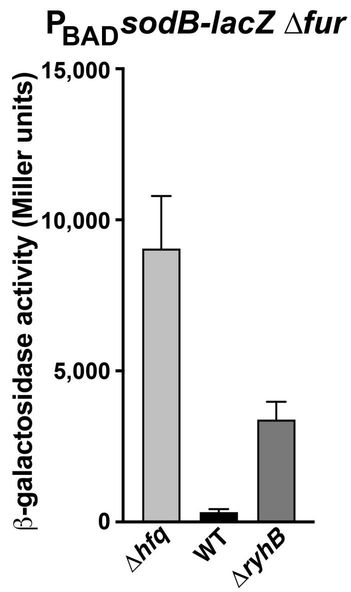

Figure S6. Relief of P_{sodB} repression by deletion of *ryhB*. Strains, all containing P_{BAD}--*sodB-lacZ, Δfur::zeo,* were grown and assayed as for Figure 4A. Shown are Δ*hfq* (KK2706), WT (KK2693), and Δ*ryhB* (KK2734).

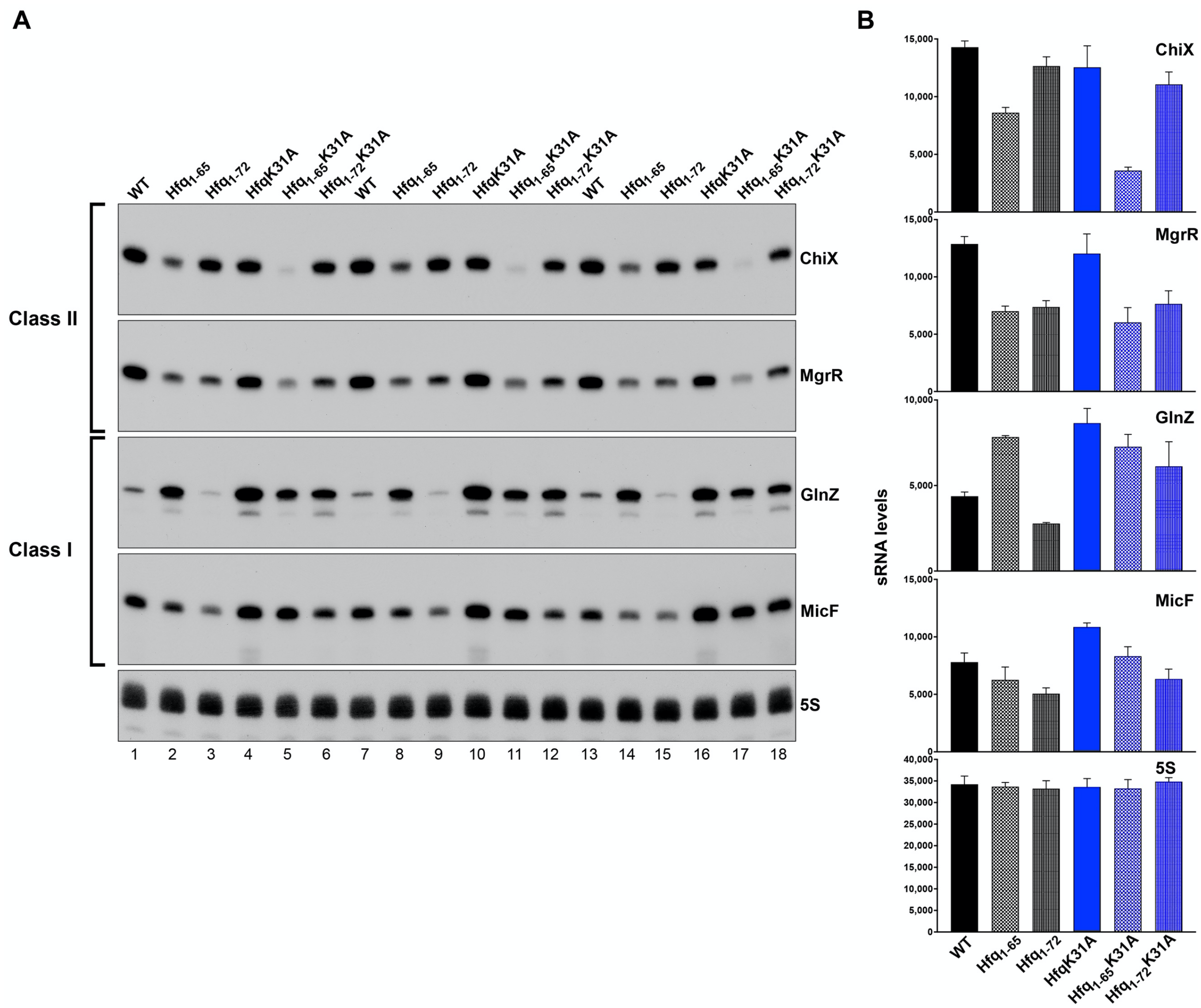

Figure S7. sRNA levels in different Hfq mutant backgrounds. (**A**) To address some variability in samples grown at different times, a subset of the strains in Figure 6 were grown in three independent cultures as for Figure 6 and RNA extracted, run on a gel and probed for ChiX, MgrR, GlnZ and MicF and 5S RNA. (**B**) Northern blots were quantified using Image J and results graphed. Strains used: WT (*hfq*$^+$; DJS2690), *hfq*$_{1-65}$ (KK01), *hfq*$_{1-72}$ (KK2438), *hfqK31A* (DJS2695), *hfq*$_{1-65}$*K31A* (KK04), *hfq*$_{1-72}$*K31A* (KK2658).

Table S1. Strains used in this study.

Strains	Description	Reference or Source
AZ237	MC4100 *hfq*+	(3)
AZ238	MC4100 *hfq*Q8A	(3)
AZ241	MC4100 *hfq*K31A	(3)
C600	F^- *tonA21 thi-1 thr-1 leuB6 lacY1 glnV44 rfbC1 fhuA1* λ^-	Lab collection
DJS2255	PM1205 *lacI'::PBAD-ompX-lacZ Δhfq::trpAterm-kan-*P_{BAD}*-ccdB miniλ::tet*	(3)
DJS2286	*Δhfq::cat-sacB ΔpurA::kan*	(3)
DJS2604	MG1655*Δhfq::cat-sacB ΔpurA::kan*	MG1655 + P1(DJS2286)
DJS2609	MG1655 *hfq*$^+$	DJS2604 + P1(AZ237)
DJS2676	PM1205 *lacI'::pBAD-sodB-lacZ, Δhfq:: cat-sacB::ΔpurA::kan*	(3)
DJS2677	PM1205 *lacI'::*P_{BAD} *-chiP-lacZ Δhfq::cat-sacB ΔpurA::kan*	(3)
DJS2680	PM1205 *lacI'::pBAD-sodB-lacZ* Δ*chiX*	KM329 + P1(KM359)
DJS2682	PM1205 *lacI'::pBAD-sodB-lacZ, Δhfq:: cat-sacB*	(3)
DJS2683	PM1205 *lacI'::pBAD-sodB-lacZ, hfq*$^+$	(3)
DJS2684	PM1205 *lacI'::pBAD-sodB-lacZ, hfqQ8A*	(3)
DJS2686	PM1205 *lacI'::pBAD-sodB-lacZ, hfqR16A*	(3)
DJS2687	PM1205 *lacI'::pBAD-sodB-lacZ, hfqY25D*	(3)
DJS2688	PM1205 *lacI'::pBAD-sodB-lacZ, hfqK31A*	(3)
DJS2689	PM1205 *lacI'::*P_{BAD}*-chiP-lacZ Δhfq::cat-sacB*	(3)
DJS2690	PM1205 *lacI'::*P_{BAD}*-chiP-lacZ hfq*$^+$(WT)	(3)
DJS2691	PM1205 *lacI'::*P_{BAD}*-chiP-lacZ hfqQ8A*	(3)
DJS2693	PM1205 *lacI'::*P_{BAD}*-chiP-lacZ hfqR16A*	(3)
DJS2694	PM1205 *lacI'::*P_{BAD}*-chiP-lacZ hfqY25D*	(3)
DJS2695	PM1205 *lacI'::*P_{BAD}*-chiP-lacZ hfqK31A*	(3)
DJS2814	MG1655 *ΔlacX74. mal::lacIq ΔaraBAD*	(4)

	Δhfq::trpAterm-kan-P$_{BAD}$-ccdB miniλ::tet	
DJS2985	PM1205 *lacI'::P$_{BAD}$-chiPsodBbplacZ*	(4)
DJS2925	C600 *Δhfq:: cat-sacBΔpurA::kan*	C600 + P1(DJS2286)
DJS3007	PM1205 *lacI'::p$_{BAD}$-chiPsodB-lacZ Δhfq:: cat-sacB*	(4)
DJS3008	PM1205 *lacI'::p$_{BAD}$-chiPsodB-lacZ, Δhfq:: cat-sacBΔpurA::kan*	(4)
DJS3009	PM1205 *lacI'::p$_{BAD}$-chiPsodB-lacZ, hfq^{+}*	(4)
DJS3010	PM1205 *lacI'::p$_{BAD}$-chiPsodB-lacZ, hfqR16A*	(4)
DJS3011	PM1205 *lacI'::p$_{BAD}$-chiPsodB-lacZ, hfqY25D*	(4)
EM1238	MG1655 ΔX74l*ac* Δ*ryhB::cat*	(5)
JC1060	*MG1655 mal::lacIQ ΔaraBAD araC+ lacI'::kan-Cp12b-mutS-lacZ*	(6)
JC1316	MG1655 *lacI^{Q} Δfur::zeo*	(7)
KK01	PM1205 *lacI'::P$_{BAD}$-chiP-lacZ hfq*$_{1-65}$	(2)
KK02	PM1205 *lacI'::P$_{BAD}$-chiP-lacZ hfq*$_{1-65}$ *R16A*	DJS2677+P1(RAF1049)
KK03	PM1205 *lacI'::P$_{BAD}$-chiP-lacZ hfq*$_{1-65}$ *Y25D*	DJS2677+P1(RAF1050)
KK04	PM1205 *lacI'::P$_{BAD}$-chiP-lacZ hfq*$_{1-65}$ *K31A*	DJS2677 + P1(RAF1047)
KK05	MG1655 *Δhfq:: cat-sacBΔpurA::kan*	MG1655 + P1(DJS2925)
KK2414	PM1205 *lacI'::P$_{BAD}$-chiP-lacZ hfq*$_{1-65}$ *Q8A*	DJS2677+P1(RAF1048)
KK2436	PM1205 *lacI'::P$_{BAD}$-chiP-lacZ, hfq*$_{1-92}$	DJS2677+P1(RAF1002)
KK2437	PM1205 *lacI'::P$_{BAD}$-chiP-lacZ, hfq*$_{1-82}$	DJS2677+P1(RAF1001)
KK2438	PM1205 *lacI'::P$_{BAD}$-chiP-lacZ, hfq*$_{1-72}$	DJS2677+P1(RAF1000)
KK2440	MG1655 *hfq^{+}*	KK05 + P1(DJS2609)
KK2446	C600 *hfq*$_{1-65}$	DJS2925 + P1(RAF1042)
KK2448	MG1655 *hfq*$_{1-65}$	KK05 + P1(KK2446)
KK2455	PM1205 *lacI'::kan-Cp12b-mutS-lacZ, Δhfq:: cat-sacB*	DJS2689+P1(JC1060)
KK2456	PM1205 *lacI'::kan-Cp12b-mutS-lacZ, hfq^{+}*	DJS2690+P1(JC1060)
KK2457	PM1205 *lacI'::kan-Cp12b-mutS-lacZ, hfq*$_{1-65}$	KK01+P1(JC1060)
KK2460	PM1205 *lacI'::kan-Cp12b-mutS-lacZ, hfqQ8A*	DJS2691+P1(JC1060)
KK2461	PM1205 *lacI'::kan-Cp12b-mutS-lacZ, hfq*$_{1-65}$ *Q8A*	KK2441+P1(JC1060)
KK2462	PM1205 *lacI'::kan-Cp12b-mutS-lacZ, hfqR16A*	DJS2693+P1(JC1060)

KK2463	PM1205 *lacI'::kan-Cp12b-mutS-lacZ, hfq*$_{1-65}$ *R16A*	KK02+ P1(JC1060)
KK2464	PM1205 *lacI'::kan-Cp12b-mutS-lacZ, hfqY25D*	DJS2694+P1(JC1060)
KK2466	PM1205 *lacI'::kan-Cp12b-mutS-lacZ, hfqK31A*	DJS2695+P1(JC1060)
KK2467	PM1205 *lacI'::kan-Cp12b-mutS-lacZ, hfq*$_{1-65}$ *K31A*	KK04+ P1(JC1060)
KK2540	MG1655 *ΔlacX74 mal::lacIq ΔaraBAD hfq* $_{\Delta link}$	DJS2814 + *hfq*$_{\Delta link}$ gblock, recombineering[1]
KK2541	MG1655 *ΔlacX74 mal::lacIq ΔaraBAD hfq*$_{TipMut}$	DJS2814+*TipMut*-CTD gblock, recombineering[1]
KK2558	PM1205 *lacI'::P*$_{BAD}$*-chiP-lacZ hfq*$_{\Delta link}$	DJS2677+P1(KK2540)
KK2570	PM1205 *lacI'::P*$_{BAD}$*-chiP-lacZ hfq*$_{TipMut}$	DJS2677+P1(KK2541)
KK2611	PM1205 *lacI'::pBAD-sodB-lacZ,hfq*$_{1-65}$	DJS2676+P1(KK01)
KK2613	PM1205 *lacI'::pBAD-sodB-lacZ,hfq*$_{1-65}$ *Q8A*	DJS2676+P1(RAF1048)
KK2613r*	PM1205 *lacI'::pBAD-sodB-lacZ,hfq*$_{1-65}$ *Q8A*	Reconstructed, as for KK2613
KK2615	PM1205 *lacI'::pBAD-sodB-lacZ, hfq*$_{1-65}$ *R16A*	DJS2676+P1(RAF1049)
KK2619	PM1205 *lacI'::pBAD-sodB-lacZ, hfq*$_{1-65}$ *K31A*	DJS2676+P1(RAF1047)
KK2622	PM1205 *lacI'::pBAD-chiPsodB-lacZ, hfq*$_{1-65}$	DJS3008+P1(KK01)
KK2623	PM1205 *lacI'::pBAD-chiPsodB-lacZ, hfqQ8A*	DJS3008 + P1(AZ238)
KK2624	PM1205 *lacI'::pBAD-chiPsodB-lacZ, hfq*$_{1-65}$ *Q8A*	DJS3008+P1(RAF1048)
KK2624r*	PM1205 *lacI'::pBAD-chiPsodB-lacZ, hfq*$_{1-65}$ *Q8A*	Reconstructed, as for KK2624
KK2626	PM1205 *lacI'::pBAD-chiPsodB-lacZ, hfq*$_{1-65}$ *R16A*	DJS3008+P1(RAF1049)
KK2629	PM1205 *lacI'::pBAD-chiPsodB-lacZ, hfqK31A*	DJS3008+P1(AZ241)
KK2630	PM1205 *lacI'::pBAD-chiPsodB-lacZ, hfq*$_{1-65}$ *K31A*	DJS3008+P1(RAF1047)
KK2646	MG1655 *ΔlacX74 mal::lacIq ΔaraBAD hfq*$_{TipMut}$*R16A*	DJS2814 + *hfq*$_{TipMut}$*R16A* gblock, recombineering[1]
KK2647	MG1655 *ΔlacX74 mal::lacIq ΔaraBAD hfq*$_{TipMut}$*K31A*	DJS2814 + *hfq*$_{TipMut}$*K31A* gblock recombineering[1]
KK2649	MG1655 *ΔlacX74 mal::lacIq ΔaraBAD hfq*$_{\Delta link}$ R16A	DJS2814 + *hfq*$_{\Delta link}$*R16A* gblock, recombineering[1]
KK2650	MG1655 *ΔlacX74 mal::lacIq ΔaraBAD hfq*$_{\Delta link}$*K31A*	DJS2814 + *hfqK31A*$_{\Delta link}$ gblock, recombineering[1]
KK2651	PM1205 *lacI'::P*$_{BAD}$*-chiP-lacZ hfq*$_{TipMut}$*R16A*	DJS2677+P1(KK2646)

KK2652	PM1205 *lacI'::*P_{BAD}*-chiP-lacZ* *hfq*$_{TipMut}$*K31A*	DJS2677+P1(KK2647)
KK2654	PM1205 *lacI'::*P_{BAD}*-chiP-lacZ* *hfq*$_{\Delta link}$*R16A*	DJS2677+P1(KK2649)
KK2655	PM1205 *lacI'::*P_{BAD}*-chiP-lacZ* *hfq*$_{\Delta link}$*K31A*	DJS2677+P1(KK2650)
KK2657	PM1205 *lacI'::*P_{BAD}*-chiP-lacZ, hfq*$_{1-72}$*R16A*	DJS2677+P1(RAF1053)
KK2658	PM1205 *lacI'::*P_{BAD}*-chiP-lacZ, hfq*$_{1-72}$*K31A*	DJS2677+P1(RAF1051)
KK2659	PM1205 *lacI'::pBAD-sodB-lacZ, hfq*$_{TipMut}$	DJS2676+P1(KK2541)
KK2660	PM1205 *lacI'::pBAD-sodB-lacZ, hfq*$_{\Delta link}$	DJS2676+P1(KK2540)
KK2661	PM1205 *lacI'::pBAD-sodB-lacZ, hfq*$_{TipMut}$*R16A*	DJS2676+P1(KK2646)
KK2662	PM1205 *lacI'::pBAD-sodB-lacZ, hfq*$_{TipMut}$*K31A*	DJS2676+P1(KK2647)
KK2664	PM1205 *lacI'::pBAD-sodB-lacZ, hfq*$_{\Delta link}$*R16A*	DJS2676+P1(KK2649)
KK2665	PM1205 *lacI'::pBAD-sodB-lacZ, hfq*$_{\Delta link}$*K31A*	DJS2676+P1(KK2650)
KK2667	PM1205 *lacI'::pBAD-sodB-lacZ, Hfq*$_{1-72}$*R16A*	DJS2676+P1(RAF1053)
KK2668	PM1205 *lacI'::pBAD-sodB-lacZ* *hfq*$_{1-72}$*K31A*	DJS2676+P1(RAF1051)
KK2674	MG1655 *ΔlacX74 mal::lacIq ΔaraBAD hfq*$_{1-72}$*R66AK31A*	DJS2814+*hfq*$_{1-72}$R66AK31A gblock, recombineering[1]
KK2675	MG1655 *ΔlacX74 mal::lacIq ΔaraBAD hfq*$_{1-72}$*P67AK31A*	DJS2814+*hfq*$_{1-72}$P67AK31A gblock, recombineering[1]
KK2676	MG1655 *ΔlacX74 mal::lacIq ΔaraBAD hfq*$_{1-72}$*H70AK31A*	DJS2814+*hfq*$_{1-72}$H70AK31A gblock, recombineering[1]
KK2677	MG1655 *ΔlacX74 mal::lacIq ΔaraBAD hfq*$_{1-72}$*H71AK31A*	DJS2814+*hfq*$_{1-72}$H71AK31A gblock, recombineering[1]
KK2678	MG1655 *ΔlacX74 mal::lacIq ΔaraBAD hfq*$_{1-72}$*S72AK31A*	DJS2814+*hfq*$_{1-72}$S72AK31A gblock, recombineering[1]
KK2680	PM1205 *lacI'::*P_{BAD}*-chiP-lacZ, hfq*$_{1-72}$*R66AK31A*	DJS2677+P1(KK2674)
KK2681	PM1205 *lacI'::*P_{BAD}*-chiP-lacZ, hfq*$_{1-72}$*P67AK31A*	DJS2677+P1(KK2675)
KK2682	PM1205 *lacI'::*P_{BAD}*-chiP-lacZ, hfq*$_{1-72}$*H70AK31A*	DJS2677+P1(KK2676)
KK2683	PM1205 *lacI'::*P_{BAD}*-chiP-lacZ, hfq*$_{1-72}$*H71AK31A*	DJS2677+P1(KK2677)
KK2684	PM1205 *lacI'::*P_{BAD}*-chiP-lacZ, hfq*$_{1-72}$*S72AK31A*	DJS2677+P1(KK2678)

KK2685	MG1655 *ΔlacX74 mal::lacIq ΔaraBAD hfq*$_{1-72}$*V68AK31A*	DJS2814+*hfq*$_{1-72}$V68AK31A gblock, recombineering[1]
KK2686	MG1655 *ΔlacX74 mal::lacIq ΔaraBAD hfq*$_{1-72}$*S69AK31A*	DJS2814+*hfq*$_{1-72}$S69AK31A gblock, recombineering[1]
KK2687	PM1205 *lacI'::P*$_{BAD}$*-chiP-lacZ, hfq*$_{1-72}$*V68AK31A*	DJS2677+P1(KK2685)
KK2688	PM1205 *lacI'::P*$_{BAD}$*-chiP-lacZ, hfq*$_{1-72}$*S69AK31A*	DJS2677+P1(KK2686)
KK2689	PM1205 *lacI'::kan-Cp12b-mutS-lacZ, hfq*$_{1-72}$	KK2438+ P1(JC1060)
KK2691	PM1205 *lacI'::kan-Cp12b-mutS-lacZ, hfq*$_{1-72}$*R16A*	KK2657+P1(JC1060)
KK2692	PM1205 *lacI'::kan-Cp12b-mutS-lacZ, hfq*$_{1-72}$*K31A*	KK2658+ P1(JC1060)
KK2693	PM1205 *lacI'::pBAD-sodB-lacZ, Δfur::zeo, hfq*$^{+}$*/WT*	DJS2683+ P1(JC1316)
KK2694	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, hfq*$_{1-65}$	KK2611+ P1(JC1316)
KK2695	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, hfqQ8A*	DJS2684+ P1(JC1316)
KK2696	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, hfqR16A*	DJS2686+ P1(JC1316)
KK2697	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, hfq*$_{Δlink}$*R16A*	KK2664+ P1(JC1316)
KK2698	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, hfq*$_{tipmut}$*R16A*	KK2661+ P1(JC1316)
KK2699	PM1205 *lacI'::pBAD-sodB-lacZ, Δfur::zeo, hfq*$_{Δlink}$	KK2660+P1(JC1316)
KK2700	PM1205 *lacI'::pBAD-sodB-lacZ, Δfur::zeo, hfq*$_{tipmut}$	KK2659+P1(JC1316)
KK2701	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, hfqK31A*	DJS2688+ P1(JC1316)
KK2702	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, hfq*$_{1-65}$ *K31A*	KK2619+ P1(JC1316)
KK2704	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, hfq*$_{1-72}$*R16A*	KK2667+P1(JC1316)
KK2705	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, hfq*$_{1-72}$*K31A*	KK2668+P1(JC1316)
KK2706	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, Δhfq:: cat-sacB*	DJS2682+P1(JC1316)
KK2707	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, hfq*$_{1-65}$ *R16A*	KK2615+ P1(JC1316)
KK2708	PM1205 *lacI'::pBAD-sodB-lacZ, hfq*$_{1-72}$	DJS2676+P1(RAF1000)
KK2714	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, hfq*$_{tipmut}$*K31A*	KK2662+ P1(JC1316)

KK2715	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo,hfq$_{\Delta link}$K31A*	KK2665+ P1(JC1316)
KK2717	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, hfq*$_{1-65}$ *Q8A*	KK2613+ P1(JC1316)
KK2717r*	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, hfq*$_{1-65}$ *Q8A*	Reconstructed, as for KK2717
KK2718	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, hfq$_{1-72}$*	KK2708+P1(JC1316)
KK2719	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, hfqY25D*	DJS2687+ P1(JC1316)
KK2722	MG1655 *ΔlacX74 mal::lacIq ΔaraBAD hfqR66A*	DJS2814 + R66A gblock, recombineering[1]
KK2723	MG1655 *ΔlacX74mal::lacIq ΔaraBAD hfqR66AR16A*	DJS2814 + R66AR16A gblock, recombineering[1]
KK2724	MG1655 *ΔlacX74 mal::lacIq ΔaraBAD hfqR66AK31A*	DJS2814 + R66AK31A gblock, recombineering[1]
KK2725	PM1205 *lacI'::pBAD-chiP-lacZ, hfq*R66A	DJS2677+P1(KK2722)
KK2726	PM1205 *lacI'::pBAD-chiP-lacZ, hfq*R66AR16A	DJS2677+P1(KK2723)
KK2727	PM1205 *lacI'::pBAD-chiP-lacZ, hfq*R66AK31A	DJS2677+P1(KK2724)
KK2728	PM1205 *lacI'::pBAD-sodB-lacZ , hfqR66A*	DJS2676+P1(KK2722)
KK2729	PM1205 *lacI'::pBAD-sodB-lacZ, hfqR66AR16A*	DJS2676+P1(KK2723)
KK2730	PM1205 *lacI'::pBAD-sodB-lacZ, hfqR66AK31A*	DJS2676+P1(KK2724)
KK2731	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, hfqR66A*	KK2728+ P1(JC1316)
KK2732	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, hfqR66AR16A*	KK2729+ P1(JC1316)
KK2732r*	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, hfqR66AR16A*	Reconstructed as for KK2732
KK2733	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, hfqR66AK31A*	KK2730+ P1(JC1316)
KK2733r*	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, hfqR66AK31A*	Reconstructed as for KK2733
KK2734	PM1205 *lacI'::pBAD-sodB-lacZ Δfur::zeo, ΔryhB::cat*	KK2693 + P1(EM1238)
KM329	PM1205 *lacI'::P$_{BAD}$ -chiP-lacZ hfq$^+$*	(8)
KM359	*lacI'*::P$_{BAD}$*-lacZ-lacZ* Δ*chiX*::*kan*	(8)
PM1205	*mal::lacI^q*, *araC$^+$*, *P$_{BAD}$-cat-sacB-lacZ*, mini λ tet^R	(9)
RAF1000	PM1205 *lacI'::P$_{BAD}$-ompX-lacZ hfq*$_{1-72}$	Hfq72 PCR fragment amplified from pNRD414

		with Aztunc1000 forward and Hfq72 reverse primers + DJS2255, recombineering. [1]
RAF1001	PM1205 *lacI'*::P_{BAD}-*ompX-lacZ* $hfq_{1\text{-}82}$	Hfq82 PCR fragment amplified from pNRD414 with Aztunc1000 forward and Hfq82 reverse primers + DJS2255, recombineering. [1]
RAF1002	PM1205 *lacI'*::P_{BAD}-*ompX-lacZ* $hfq_{1\text{-}92}$	Hfq 92 PCR fragment amplified from pNRD414 with Aztunc1000 forward and Hfq92 reverse primers + DJS2255, recombineering. [1]
RAF1042	PM1205 *lacI'*::P_{BAD}-*ompX-lacZ* $hfq_{1\text{-}65}$	Santiago-Frangos et al 2016
RAF1047	PM1205 *lacI'*::P_{BAD}-*ompX-lacZ* $hfq_{1\text{-}65}$ *K31A*	Hfq 65K31A PCR fragment amplified from DJS2695 with Aztunc1000 forward and Hfq65 reverse primers + DJS2255, recombineering. [1]
RAF1048	PM1205 *lacI'*::P_{BAD}-*ompX-lacZ* $hfq_{1\text{-}65}$ *Q8A*	Hfq 65Q8A PCR fragment amplified from DJS2691 with Aztunc1000 forward and Hfq65 reverse primers + DJS2255, recombineering. [1]
RAF1049	PM1205 *lacI'*::P_{BAD}-*ompX-lacZ* $hfq_{1\text{-}65}$ *R16A*	Hfq 65R16A PCR fragment amplified from DJS2693 with Aztunc1000 forward and Hfq65 reverse primers + DJS2255, recombineering. [1]
RAF1050	PM1205 *lacI'*::P_{BAD}-*ompX-lacZ* $hfq_{1\text{-}65}$ *Y25D*	Hfq 65Y25D PCR fragment amplified from DJS2694 with Aztunc1000 forward and Hfq65 reverse primers + DJS2255 recombineering. [1]
RAF1051	PM1205 *lacI'*::P_{BAD}-*ompX-lacZ* $hfq_{1\text{-}72}$*K31A*	Hfq72K31APCR fragment amplified from DJS2695 with Aztunc1000 forward and Hfq72 reverse primers + DJS2255 recombineering. [1]
RAF1053	PM1205 *lacI'*::P_{BAD}-*ompX-lacZ* $hfq_{1\text{-}72}$*R16A*	Hfq 72R16A PCR fragment amplified from DJS2693 with Aztunc1000 forward and Hfq72reverse primers + DJS2255, recombineering. [1]

[1]Using lambda red recombineering, as described in Materials and Methods, DNA fragments (gblocks or PCR fragments) with flanking homology to *hfq* were introduced into the bacterial chromosome at the native *hfq* locus. The recipient strains carry counterelectable markers in the *hfq* locus.

Table S2. Oligonucleotides used in this study.

Name	Oligonucleotide Sequence
gBlocks	**Sequence (5' to 3')**
hfqTipMut	AAGGTTCAAAGTACAAATAAGCATATAAGGAAAAGAGAGAATGG CTAAGGGGCAATCTTTACAAGATCCGTTCCTGAACGCACTGCGTCG GGAACGTGTTCCAGTTTCTATTTATTTGGTGAATGGTATTAAGCTG CAAGGGCAAATCGAGTCTTTTGATCAGTTCGTGATCCTGTTGAAAA ACACGGTCAGCCAGATGGTTTACAAGCACGCGATTTCTACTGTTGT CCCGTCTCGCCCGGTTTCTCATCACAGTAACAACGCCGGTGGCGGT ACCAGCAGTAACTACCATCATGGTAGCAGCGCGCAGAATACTTCC GCGCAACAGCGTAGCAACAAAACCAACTAAGGTTTCGGGCTGTTT TTTTACACGGGGAGCCAGCGATCCT
$hfq_{\Delta link}$	AAGGTTCAAAGTACAAATAAGCATATAAGGAAAAGAGAGAATGG CTAAGGGGCAATCTTTACAAGATCCGTTCCTGAACGCACTGCGTCG GGAACGTGTTCCAGTTTCTATTTATTTGGTGAATGGTATTAAGCTG CAAGGGCAAATCGAGTCTTTTGATCAGTTCGTGATCCTGTTGAAAA ACACGGTCAGCCAGATGGTTTACAAGCACGCGATTTCTACTGTTGT CCCGTCTCGCCCGGTTTCTCATCACAGTAACAACGCCGGTGGCGGT ACCAGCAGTAACTACCATCATGACAGCGAAGAAACCGAATAAGGT TTCGGGCTGTTTTTTACACGGGGAGCCAGCGATCCT
$hfq_{1\text{-}72}R66AK31A$	AAGGTTCAAAGTACAAATAAGCATATAAGGAAAAGAGAGAatgGCT AAGGGGCAATCTTTACAAGATCCGTTCCTGAACGCACTGCGTCGG GAACGTGTTCCAGTTTCTATTTATTTGGTGAATGGTATTGCGCTGC AAGGGCAAATCGAGTCTTTTGATCAGTTCGTGATCCTGTTGAAAAA CACGGTCAGCCAGATGGTTTACAAGCACGCGATTTCTACTGTTGTC CCGTCTGCCCCGGTTTCTCATCACAGTtaaGGTTTCGGGCTGTTTTTT TACACGGGGAGCCAGCGATCCT
$hfq_{1\text{-}72}P67AK31A$	AAGGTTCAAAGTACAAATAAGCATATAAGGAAAAGAGAGAATGG CTAAGGGGCAATCTTTACAAGATCCGTTCCTGAACGCACTGCGTCG GGAACGTGTTCCAGTTTCTATTTATTTGGTGAATGGTATTGCGCTG CAAGGGCAAATCGAGTCTTTTGATCAGTTCGTGATCCTGTTGAAAA ACACGGTCAGCCAGATGGTTTACAAGCACGCGATTTCTACTGTTGT CCCGTCTCGCGCGGTTTCTCATCACAGTTAAGGTTTCGGGCTGTTT TTTTACACGGGGAGCCAGCGATCCT
$hfq_{1\text{-}72}V68AK31A$	AAGGTTCAAAGTACAAATAAGCATATAAGGAAAAGAGAGAATGG CTAAGGGGCAATCTTTACAAGATCCGTTCCTGAACGCACTGCGTCG GGAACGTGTTCCAGTTTCTATTTATTTGGTGAATGGTATTGCGCTG CAAGGGCAAATCGAGTCTTTTGATCAGTTCGTGATCCTGTTGAAAA ACACGGTCAGCCAGATGGTTTACAAGCACGCGATTTCTACTGTTGT CCCGTCTCGCCCGGCTTCTCATCACAGTTAAGGTTTCGGGCTGTTT TTTTACACGGGGAGCCAGCGATCCT

hfq$_{1-72}$*S69AK31A*

AAGGTTCAAAGTACAAATAAGCATATAAGGAAAAGAGAGAATGG
CTAAGGGGCAATCTTTACAAGATCCGTTCCTGAACGCACTGCGTCG
GGAACGTGTTCCAGTTTCTATTTATTTGGTGAATGGTATTGCGCTG
CAAGGGCAAATCGAGTCTTTTGATCAGTTCGTGATCCTGTTGAAAA
ACACGGTCAGCCAGATGGTTTACAAGCACGCGATTTCTACTGTTGT
CCCGTCTCGCCCGGTTGCTCATCACAGTTAAGGTTTCGGGCTGTTT
TTTTACACGGGGAGCCAGCGATCCT

hfq$_{1-72}$*H70AK31A*

AAGGTTCAAAGTACAAATAAGCATATAAGGAAAAGAGAGAATGG
CTAAGGGGCAATCTTTACAAGATCCGTTCCTGAACGCACTGCGTCG
GGAACGTGTTCCAGTTTCTATTTATTTGGTGAATGGTATTGCGCTG
CAAGGGCAAATCGAGTCTTTTGATCAGTTCGTGATCCTGTTGAAAA
ACACGGTCAGCCAGATGGTTTACAAGCACGCGATTTCTACTGTTGT
CCCGTCTCGCCCGGTTTCTGCTCACAGTTAAGGTTTCGGGCTGTTTT
TTTACACGGGGAGCCAGCGATCCT

hfq$_{1-72}$*H71AK31A*

AAGGTTCAAAGTACAAATAAGCATATAAGGAAAAGAGAGAATGG
CTAAGGGGCAATCTTTACAAGATCCGTTCCTGAACGCACTGCGTCG
GGAACGTGTTCCAGTTTCTATTTATTTGGTGAATGGTATTGCGCTG
CAAGGGCAAATCGAGTCTTTTGATCAGTTCGTGATCCTGTTGAAAA
ACACGGTCAGCCAGATGGTTTACAAGCACGCGATTTCTACTGTTGT
CCCGTCTCGCCCGGTTTCTCATGCCAGTTAAGGTTTCGGGCTGTTTT
TTTACACGGGGAGCCAGCGATCCT

hfq$_{1-72}$*S72AK31A*

AAGGTTCAAAGTACAAATAAGCATATAAGGAAAAGAGAGAATGG
CTAAGGGGCAATCTTTACAAGATCCGTTCCTGAACGCACTGCGTCG
GGAACGTGTTCCAGTTTCTATTTATTTGGTGAATGGTATTGCGCTG
CAAGGGCAAATCGAGTCTTTTGATCAGTTCGTGATCCTGTTGAAAA
ACACGGTCAGCCAGATGGTTTACAAGCACGCGATTTCTACTGTTGT
CCCGTCTCGCCCGGTTTCTCATCACGCTTAAGGTTTCGGGCTGTTTT
TTTACACGGGGAGCCAGCGATCCT

hfqR66A

AAGGTTCAAAGTACAAATAAGCATATAAGGAAAAGAGAGAAtgGC
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GGAACGTGTTCCAGTTTCTATTTATTTGGTGAATGGTATTAAGCTG
CAAGGGCAAATCGAGTCTTTTGATCAGTTCGTGATCCTGTTGAAAA
ACACGGTCAGCCAGATGGTTTACAAGCACGCGATTTCTACTGTTGT
CCCGTCTGCCCCGGTTTCTCATCACAGTAACAACGCCGGTGGCGGT
ACCAGCAGTAACTACCATCATGGTAGCAGCGCGCAGAATACTTCC
GCGCAACAGGACAGCGAAGAAACCGAAtaaGGTTTCGGGCTGTTTT
TTTACACGGGGAGCCAGCGATCCT

hfqR66AR16A

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TAAGGGGCAATCTTTACAAGATCCGTTCCTGAACGCACTGGCTCG
GGAACGTGTTCCAGTTTCTATTTATTTGGTGAATGGTATTAAGCTG
CAAGGGCAAATCGAGTCTTTTGATCAGTTCGTGATCCTGTTGAAAA
ACACGGTCAGCCAGATGGTTTACAAGCACGCGATTTCTACTGTTGT
CCCGTCTGCCCCGGTTTCTCATCACAGTAACAACGCCGGTGGCGGT
ACCAGCAGTAACTACCATCATGGTAGCAGCGCGCAGAATACTTCC

GCGCAACAGGACAGCGAAGAAACCGAAtaaGGTTTCGGGCTGTTTT
TTTACACGGGGAGCCAGCGATCCT

hfqR66AK31A

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GGAACGTGTTCCAGTTTCTATTTATTTGGTGAATGGTATTGCGCTG
CAAGGGCAAATCGAGTCTTTTGATCAGTTCGTGATCCTGTTGAAAA
ACACGGTCAGCCAGATGGTTTACAAGCACGCGATTTCTACTGTTGT
CCCGTCTGCCCCGGTTTCTCATCACAGTAACAACGCCGGTGGCGGT
ACCAGCAGTAACTACCATCATGGTAGCAGCGCGCAGAATACTTCC
GCGCAACAGGACAGCGAAGAAACCGAAtaaGGTTTCGGGCTGTTTT
TTTACACGGGGAGCCAGCGATCCT

hfqQ8ATipMut

AAGGTTCAAAGTACAAATAAGCATATAAGGAAAAGAGAGAATGG
CTAAGGGGCAATCTTTAGCAGATCCGTTCCTGAACGCACTGCGTCG
GGAACGTGTTCCAGTTTCTATTTATTTGGTGAATGGTATTAAGCTG
CAAGGGCAAATCGAGTCTTTTGATCAGTTCGTGATCCTGTTGAAAA
ACACGGTCAGCCAGATGGTTTACAAGCACGCGATTTCTACTGTTGT
CCCGTCTCGCCCGGTTTCTCATCACAGTAACAACGCCGGTGGCGGT
ACCAGCAGTAACTACCATCATGGTAGCAGCGCGCAGAATACTTCC
GCGCAACAGCGTAGCAACAAAACCAACTAAGGTTTCGGGCTGTTT
TTTTACACGGGGAGCCAGCGATCCT

hfqR16ATipMut

AAGGTTCAAAGTACAAATAAGCATATAAGGAAAAGAGAGAATGG
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GGAACGTGTTCCAGTTTCTATTTATTTGGTGAATGGTATTAAGCTG
CAAGGGCAAATCGAGTCTTTTGATCAGTTCGTGATCCTGTTGAAAA
ACACGGTCAGCCAGATGGTTTACAAGCACGCGATTTCTACTGTTGT
CCCGTCTCGCCCGGTTTCTCATCACAGTAACAACGCCGGTGGCGGT
ACCAGCAGTAACTACCATCATGGTAGCAGCGCGCAGAATACTTCC
GCGCAACAGCGTAGCAACAAAACCAACTAAGGTTTCGGGCTGTTT
TTTTACACGGGGAGCCAGCGATCCT

hfqK31ATipMut

AAGGTTCAAAGTACAAATAAGCATATAAGGAAAAGAGAGAATGG
CTAAGGGGCAATCTTTACAAGATCCGTTCCTGAACGCACTGCGTCG
GGAACGTGTTCCAGTTTCTATTTATTTGGTGAATGGTATTGCGCTG
CAAGGGCAAATCGAGTCTTTTGATCAGTTCGTGATCCTGTTGAAAA
ACACGGTCAGCCAGATGGTTTACAAGCACGCGATTTCTACTGTTGT
CCCGTCTCGCCCGGTTTCTCATCACAGTAACAACGCCGGTGGCGGT
ACCAGCAGTAACTACCATCATGGTAGCAGCGCGCAGAATACTTCC
GCGCAACAGCGTAGCAACAAAACCAACTAAGGTTTCGGGCTGTTT
TTTTACACGGGGAGCCAGCGATCCT

hfq$_{\Delta link}$*Q8A*

AAGGTTCAAAGTACAAATAAGCATATAAGGAAAAGAGAGAATGG
CTAAGGGGCAATCTTTAGCAGATCCGTTCCTGAACGCACTGCGTCG
GGAACGTGTTCCAGTTTCTATTTATTTGGTGAATGGTATTAAGCTG
CAAGGGCAAATCGAGTCTTTTGATCAGTTCGTGATCCTGTTGAAAA
ACACGGTCAGCCAGATGGTTTACAAGCACGCGATTTCTACTGTTGT
CCCGTCTCGCCCGGTTTCTCATCACAGTAACAACGCCGGTGGCGGT

	ACCAGCAGTAACTACCATCATGACAGCGAAGAAACCGAATAAGGT TTCGGGCTGTTTTTTACACGGGGAGCCAGCGATCCT
hfq$_{\Delta link}$*R16A*	AAGGTTCAAAGTACAAATAAGCATATAAGGAAAAGAGAGAATGG CTAAGGGGCAATCTTTACAAGATCCGTTCCTGAACGCACTGGCTCG GGAACGTGTTCCAGTTTCTATTTATTTGGTGAATGGTATTAAGCTG CAAGGGCAAATCGAGTCTTTTGATCAGTTCGTGATCCTGTTGAAAA ACACGGTCAGCCAGATGGTTTACAAGCACGCGATTTCTACTGTTGT CCCGTCTCGCCCGGTTTCTCATCACAGTAACAACGCCGGTGGCGGT ACCAGCAGTAACTACCATCATGACAGCGAAGAAACCGAATAAGGT TTCGGGCTGTTTTTTACACGGGGAGCCAGCGATCCT
hfq$_{\Delta link}$*K31A*	AAGGTTCAAAGTACAAATAAGCATATAAGGAAAAGAGAGAATGG CTAAGGGGCAATCTTTACAAGATCCGTTCCTGAACGCACTGCGTCG GGAACGTGTTCCAGTTTCTATTTATTTGGTGAATGGTATTGCGCTG CAAGGGCAAATCGAGTCTTTTGATCAGTTCGTGATCCTGTTGAAAA ACACGGTCAGCCAGATGGTTTACAAGCACGCGATTTCTACTGTTGT CCCGTCTCGCCCGGTTTCTCATCACAGTAACAACGCCGGTGGCGGT ACCAGCAGTAACTACCATCATGACAGCGAAGAAACCGAATAAGGT TTCGGGCTGTTTTTTACACGGGGAGCCAGCGATCCT
hfq$_{1\text{-}65}$	AAGGTTCAAAGTACAAATAAGCATATAAGGAAAAGAGAGAATGG CTAAGGGGCAATCTTTACAAGATCCGTTCCTGAACGCACTGCGTCG GGAACGTGTTCCAGTTTCTATTTATTTGGTGAATGGTATTAAGCTG CAAGGGCAAATCGAGTCTTTTGATCAGTTCGTGATCCTGTTGAAAA ACACGGTCAGCCAGATGGTTTACAAGCACGCGATTTCTACTGTTGT CCCGTCTTAAGGTTTCGGGCTGTTTTTTACACGGGGAGCCAGCGA TCCT
hfq102	AAGGTTCAAAGTACAAATAAGCATATAAGGAAAAGAGAGAATGG CTAAGGGGCAATCTTTACAAGATCCGTTCCTGAACGCACTGCGTCG GGAACGTGTTCCAGTTTCTATTTATTTGGTGAATGGTATTAAGCTG CAAGGGCAAATCGAGTCTTTTGATCAGTTCGTGATCCTGTTGAAAA ACACGGTCAGCCAGATGGTTTACAAGCACGCGATTTCTACTGTTGT CCCGTCTCGCCCGGTTTCTCATCACAGTAACAACGCCGGTGGCGGT ACCAGCAGTAACTACCATCATGGTAGCAGCGCGCAGAATACTTCC GCGCAACAGGACAGCGAAGAAACCGAATAAGGTTTCGGGCTGTTT TTTTACACGGGGAGCCAGCGATCCT
Primers for strain construction	**Sequences (5’ to 3’)**
AZ1000TrunF	AAGGTTCAAAGTACAAATAAGCATATAAGGAAAAGAGAGAATGGC TAAGGGGCAATCTTT
Hfq Trunc 72R	AGGATCGCTGGCTCCCCGTGTAAAAAACAGCCCGAAACCTTA ACTGTGATGAGAAACCGGGC
Hfq Trunc 82R	AGGATCGCTGGCTCCCCGTGTAAAAAACAGCCCGAAACCTTA GTTACTGCTGGTACCGCCAC

Hfq Trunc 92R	AGGATCGCTGGCTCCCCGTGTAAAAAAACAGCCCGAAACCTTAAGT ATTCTGCGCGCTGCTAC
Primers used for cDNA library preparation (RNA-Seq), no. of nucleotides	**3’ Barcode adapter sequences**
AZ1331, BC1, 30	5'P-AACATTATTAGATCGGAAGAGCGTCGTGTA-3'SpC
AZ1332, BC2, 30	5'P-AAAGTGTTGAGATCGGAAGAGCGTCGTGTA-3'SpC
AZ1333, BC3, 30	5'P-AAGAATTATAGATCGGAAGAGCGTCGTGTA-3'SpC
AZ1334, BC4, 30	5'P-AATATGGACAGATCGGAAGAGCGTCGTGTA-3'SpC
AZ1335, BC5, 30	5'P-AATCACTTGAGATCGGAAGAGCGTCGTGTA-3'SpC
AZ1336, BC6, 30	5'P-ACCAAGTCGAGATCGGAAGAGCGTCGTGTA-3'SpC
AZ1337, BC7, 30	5'P-ACAACTCGCAGATCGGAAGAGCGTCGTGTA-3'SpC
AZ1338, BC8, 30	5'P-ACCCGTCTTAGATCGGAAGAGCGTCGTGTA-3'SpC
AZ1339, BC9, 30	5'P-ACCCTACAGAGATCGGAAGAGCGTCGTGTA-3'SpC
AZ1340, BC10, 30	5'P-ACCCTCGGCAGATCGGAAGAGCGTCGTGTA-3'SpC
AZ1341, BC11, 30	5'P-ACCGGTACCAGATCGGAAGAGCGTCGTGTA-3'SpC
AZ1342, BC12, 30	5'P-ACGGAGGGCAGATCGGAAGAGCGTCGTGTA-3'SpC
AZ1343, 3Tr3, 22	5'P-AGA TCG GAA GAG CAC ACG TCT G-3'SpC
AZ1344, AR2, 19	5'P-TACACGACGCTCTTCCGAT
Primers used for northern analysis	
AZ1200, ChiX	GCTATTGGCCCGTCAAAGAG
AZ1371, MgrR	GCGGTGAATGCTTGCATGGATAGA
PA006, CyaR	GGGAGATTACACAGGCTAAGGAGGTGGTTCCTGGTACAGC
AK280, GlnZ	ATGGGCTACAGATAGCTGACAAACTTCACG

AZ1318, MicF	GCGAGGCATCCGGTTGAAATAGGGGTAAACAGACATTCAG
AZ1455, OmrB	CATCTGCGCAGGCTGGTGTAATTCATGTGCTCAAC
AZ1324, RyhB	ACTGGAAGCAATGTGAGCAATGTCGTGCT
PA027, 5S	CGGCGCTACGGCGTTTCACTTCTG

Table S3. RNA-Seq: Levels of all RNA species in total RNA and co-IP samples for WT and Hfq_{1-65}. Gene names in column A and "type" (CDS, sRNA, etc.) are as determined by genome version used for the analysis. For a small number of the genes of interest discussed here, recent studies and the profile of the browser images for Hfq Co-IP data suggest the existence of unannotated sRNAs. In this table, that information is shown in column AH.

Table S4. RNA-Seq: Total RNA results, two-fold change or better, Hfq_{1-65}/WT. This table includes all RNA species that are at least two-fold up (sheet S4A) or two-fold down (sheet S4B) for Hfq_{1-65}/WT total RNAs. All RNA species that are enriched more than two-fold for Hfq co-IP/total are highlighted in yellow (columns A and V). Novel sRNAs recently described in the literature or suggested by the work here, but not annotated as sRNAs in the original annotation file are indicated here as sRNA* in column B, and in the cases in which they have now been given gene names, that name is shown in column A.

Table S5. RNA-Seq: Hfq co-IP, two-fold change or better, Hfq_{1-65}/WT. This table includes all RNA species that are at least two-fold up (Sheet S5A) or two-fold down (sheet S5B) for Hfq_{1-65}/WT co-IP values. All RNA species that are enriched more than two-fold for Hfq co-IP/total are highlighted in yellow (columns A and V). Novel sRNAs recently described in the literature or suggested by the work here, but not annotated as sRNAs in the original annotation file are indicated here as sRNA* in column B, and in the cases in which they have now been given gene names, that name is shown in column A.

Table S6. Summary of significant changes in Hfq_{1-65} compared to WT RNA levels for RNA-binding RNAs. All genes showing the following characteristics (yellow highlights in Tables S4 and S5) are shown in this table: 1) two-fold enrichment upon Hfq IP compared to total RNA; 2) two-fold increase (green print) or two-fold decrease (red print) in Hfq1-65 vs. WT, for total RNAs (column I) or for IP (column G). The table also shows enrichment by Hfq IP over total for both WT (column C) and Hfq_{1-65} (column E). Novel sRNAs recently described in the literature or suggested by the work here, but not annotated as sRNAs in the original annotation file are indicated here as sRNA* in column B, and in the cases in which they have now been given gene names, that name is shown in column A.

Table S7. Summary of literature on *in vivo* roles of *E. coli* Hfq CTD

Reference	Year	In vivo conditions	Assays	Results	Comparison and comments	Near core/linker needed?
Tsui et al (10)	1994	*hfq1*::kmR disrupts; *hfq2*::kmR inserts after aa 78.	1) Growth, LB, low temperature; 2) high osmolarity sensitivity	Stationary phase phenotypes (RpoS dependent) not disrupted by *hfq2*.	Original assay of Hfq roles *in vivo*. Hfq2 considered wild-type control.	Not tested here.
Sonnleitner et al (11)	2004	R66 amber, from plac in pACYC compared to WT.	1) Qbeta growth 2) *ompA-lacZ* 3) DsrA stability	R66 stop functional for these assays.	Consistent; *ompA-lacZ* Class I regulation. DsrA consistent	Not distinguished.
Vecerek et al (12)	2008	Plac-hfq$_{1-65}$ in pACYC; reporters also plac induced and normalized to RNA levels. Levels of Hfq not compared to chromosome.	1) Long term survival, downshift 2) Growth on succinate + DIP 3) *hfq-lacZ* autoregulation 4) *sodB-lacZ* regulation 5) RpoS Western	Hfq$_{1-65}$ fully defective in all assays.	Inconsistent; Hfq$_{1-65}$ more likely to aggregate on overproduction ?	Not distinguished.
Olsen et al (13)	2010	Plac-Hfq$_{1-69}$, $_{1-72}$, $_{1-65}$, $_{1-66}$; low copy plasmid, measured as 2x chromosome. Overproduced sRNAs (pBADMicA, RybB)	1) RpoS Western 2) RyhB sRNA, *sodB* mRNA, after DIP. 3) *ompA* mRNA with pBAD-MicA 4) *ompC* mRNA, with pBAD-RybB 5) MicM (ChiX), *ybfM*	Hfq$_{1-69}$, $_{1-72}$ generally functional. Hfq$_{1-65}$, $_{1-66}$, function for rpoS and ChiX regulation. No quantitation	Consistent; Modestly lower MicA, RybB. Modestly lower ChiX in Hfq$_{1-65}$, Hfq$_{1-66}$, not Hfq$_{1-69;}$ stationary phase, consistent. Regulation normal (not quantitated).	ChiX levels dependent on near core region.
Beich-Frandsen et al (14)	2011	Hfq$_{1-65}$, $_{1-75}$, $_{1-85}$ from plac/pACYC derivatives	1) RpoS Western, 22°C.	RpoS absent in Hfq$_{1-65}$, $_{1-75}$; present in $_{1-85}$.	Inconsistent; region between aa 75-85 implicated in	Role for near-core and other parts of CTD for RpoS.

		(pAH65, 75, 85), as per Vecerek.			RpoS induction. If aggregate with overproduction, tip/linker helps protect.	
Salim et al (15)	2012	Hfq_{1-65}, Hfq_{1-72}, Hfq_{1-87} from P_{tac}-Kan (pSC101 plasmid, moderate copy number)	1) GlmS activation by GlmZ or GlmY	Hfq_{1-72}, Hfq_{1-87} like WT; Hfq_{1-65} reduced activation	Consistent; Distal face/activation defect with near-core only.	Near core needed for GlmS activation.
Caillet et al (16)	2014	Hfq_{1-65}, native promoter on pTX plasmid (Tsui et al)	1) Growth 2) *hfq-lacZ* autoregulation 3) *rpoS* activation, ArcZ, DsrA levels 4) *oppA* repression, GcvB 5) *ptsG* repression, SgrS levels	Partial defect seen for auto-regulation; Other assays show no significant defect.	Consistent; Autoregulation likely dependent on strong distal binding. Modest decrease in ArcZ (processed), DsrA processed differently.	Not distinguished.

Supplementary References

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