SUPPLEMENTARY MATERIAL

Paradoxical suppression of small RNA activity at high Hfq concentrations due to random-order binding

Shiori Sagawa[1, 2], Jung-Eun Shin[1, 2], Razika Hussein[1] and Han N. Lim[1, 3]

[1] Department of Integrative Biology, University of California Berkeley

[2] Contributed equally

[3] Corresponding author: hanlim@berkeley.edu

Supplementary Methods

Mathematical model

The differential equations for the system, which are shown below, include the association and dissociation of the sRNAs (S), target mRNAs (T), Hfq (H), sRNA-Hfq (SH), mRNA–Hfq (TH), sRNA-mRNA-Hfq (SHT), duplex (D) and target protein (P).

$$\dot{S} = \alpha_S + k_{-S}SH + k_{-STH}SHT - k_S S \cdot H - k_{STH} S \cdot TH - \beta_S S, \quad [1]$$

$$\dot{T} = \alpha_T + k_{-T}TH + k_{-TSH}SHT - k_T T \cdot H - k_{TSH} T \cdot SH - \beta_T T, \quad [2]$$

$$\dot{H} = \alpha_H + k_{-S}SH + k_{-T}TH + k_{Dup}SHT + \beta_{SH}SH + \beta_{TH}TH + \beta_{SHT}SHT - k_S S \cdot H - k_T T \cdot H - \beta_H H, \quad [3]$$

$$\dot{SH} = k_S S \cdot H + k_{-TSH}SHT - k_{-S}SH - k_{TSH} T \cdot SH - \beta_{SH}SH - \beta_H SH, \quad [4]$$

$$\dot{TH} = k_T T \cdot H + k_{-STH}SHT - k_{-T}TH - k_{STH} S \cdot TH - \beta_{TH}TH - \beta_H TH, \quad [5]$$

$$\dot{STH} = k_{STH} S \cdot TH + k_{TSH} T \cdot SH - (k_{-STH} + k_{-TSH} + k_{Dup} + \beta_{SHT} + \beta_H)SHT, \quad [6]$$

$\dot{D} = k_{Dup}SHT - \beta_D D$, and [7]

$$\dot{P} = \alpha_P T - \beta_P P, \quad [8a]$$

or $\dot{P} = \alpha_P(T + TH + SHT) - \beta_P P$ [8b]

or $\dot{P} = \alpha_P D - \beta_P P$. [8c]

Eq. 8a, **8b** or **8c** were used when the free mRNA, free and bound mRNA, or duplex was translated.

Where possible the simulations used biologically relevant values obtained from experiments as previously reported (1). α_S, α_T and α_H are the production rates for the sRNA, mRNA and Hfq protein (respective values are 1, 1 and 0.3 nM·min^{-1}). α_P is the protein production rate (value = 5 nM·(mRNA nM)$^{-1}$·min^{-1}). β_S, β_T, β_{SH}, β_{TH}, β_{SHT}, and β_D are the degradation rate constants for the sRNA, mRNA, sRNA in SH, mRNA in TH, sRNA and mRNA in SHT, and duplex respectively (all values = 0.14 min^{-1}). β_H and β_P are the

degradation rate constants for Hfq (free and bound forms) and target protein (both values = 0.03 min^{-1}). k_S, k_T, k_{SH}, k_{TH}, k_{STH} and k_{TSH} are the RNA binding rate constants (all values = 1 $nM^{-1} \cdot min^{-1}$). k_{-S}, k_{-T}, k_{-SH}, k_{-TH}, k_{-STH} and k_{-TSH} are the RNA dissociation rate constants (all values = 0.1 min^{-1}). k_{Dup} is the rate constant for duplex formation and release from the SHT complex (value = 1 min^{-1}).

Supplementary References

1. Hussein, R. and Lim, H.N. (2012) Direct comparison of small RNA and transcription factor signaling. *Nucleic Acids Res*, **40**, 7269-7279.
2. Hussein, R. and Lim, H.N. (2011) Disruption of small RNA signaling caused by competition for Hfq. *Proc Natl Acad Sci U S A*, **108**, 1110-1115.
3. Lutz, R. and Bujard, H. (1997) Independent and tight regulation of transcriptional units in Escherichia coli via the LacR/O, the TetR/O and AraC/I1-I2 regulatory elements. *Nucleic Acids Res*, **25**, 1203-1210.
4. Adamson, D.N. and Lim, H.N. (2013) Rapid and robust signaling in the CsrA cascade via RNA-protein interactions and feedback regulation. *Proc Natl Acad Sci U S A*, **110**, 13120-13125.
5. Franchini, A.G. and Egli, T. (2006) Global gene expression in Escherichia coli K-12 during short-term and long-term adaptation to glucose-limited continuous culture conditions. *Microbiology*, **152**, 2111-2127.
6. Urban, J.H. and Vogel, J. (2008) Two seemingly homologous noncoding RNAs act hierarchically to activate glmS mRNA translation. *PLoS Biol*, **6**, e64.
7. Urban, J.H. and Vogel, J. (2009) A green fluorescent protein (GFP)-based plasmid system to study post-transcriptional control of gene expression in vivo. *Methods Mol Biol*, **540**, 301-319.

Supplementary Figure Legends

Figure S1. Plasmid maps.

Figure S2. Relative strengths of the different promoters and ribosome binding sequences (RBS). Measurements were obtained by placing *gfp* under the control of different promoters and RBS and measuring GFP fluorescence by flow cytometry. Note the measurements were performed on different dates to the measurements in **Figs. S3-S5** and should not be directly compared to them. (**A**) Relative expression of PCon and PConM8 (-35 site is mutated from 5' TTGACA 3' to 5' TTTACA 3' and the -10 site is mutated from 5' TATAAT 3' to 5' TATGGT 3'). Mean expression for PCon and PConM8 are 4.24 ± 0.41 a.u. and 44.83 ± 3.13 a.u. Errors are the SEM. Each promoter was measured in triplicate. Strains: HL2516 (PCon) and HL2916 (PConM8). (**B**) Relative expression of PLtetO-1 and PLtetO-1M9 (-10 site is mutated from 5' GATACT 3' to 5' GAGACT 3'). Mean expression for PLtetO-1 and PLtetO-1M9 are 3.90 ± 0.20 a.u. and 40.57 ± 1.32 a.u. Each promoter was measured in duplicate. Details of the PLtetO-1 and PLtetO-1M9 promoters are reported elsewhere (2,3). Strains: HL1085 (PLtetO-1) and HL1672 (PLtetO-1M9). (**C**) Relative expression of GFP with the st7 and T710 RBS. The *gfp* was transcribed from the PLlacO-1 promoter at maximum induction (1 mM IPTG). Mean expression with the st7 and T710 RBS are 220.46 ± 5.87 a.u. and 409.19 ± 15.09 a.u. All errors are the SEM. Each RBS was measured in triplicate. Strains: HL6442 (st7 RBS) and HL5189 (T710 RBS).

Figure S3. GFP expression with the st7 and T710 RBS. Measurements were obtained by placing *gfp* under the control of the PLlacO-1 promoter and measuring GFP fluorescence by flow cytometry at different concentrations of IPTG. Error bars are the SEM of triplicate measurements. Total $n = 48$. Strains: HL5189 (PLlacO-1:T710::*gfp*) and HL6442 (PLlacO-1:st7::*gfp*). Note the measurements were performed on different dates to the measurements in **Figs. S2, S4 and S5** and should not be directly compared to them.

Figure S4. GFP expression and relative induction of PLlacO-1 at different IPTG concentrations measured in parallel with the samples in Fig 4*B*. Measurements were obtained by placing the *gfp* gene under the control of the PLlacO-1 promoter and measuring GFP fluorescence by flow cytometry at different concentrations of IPTG. Error bars are the SEM. Note the measurements were performed on different dates to the measurements in **Figs. S2, S3 and S5** and should not be directly compared to them. (**A**) GFP expression with the st7 RBS in strain HL6582, which has both chr. *hfq* and *ryhB* deleted, as a function of IPTG concentration. Measurements were performed in triplicate at each concentration. Total n = 21. The line is a guide to the eye of the mean GFP expression at each IPTG concentration. (**B**) Relative induction as a function of IPTG concentration. The relative induction was calculated by subtracting the mean GFP fluorescence value at 0 μM from the mean values obtained at all IPTG concentrations, and then dividing by the mean GFP fluorescence at 1000 μM to normalize the values.

Figure S5. Relative strengths of the PLtetO-1, PLlacO-1 and PCon promoters. Measurements were obtained by placing *gfp* with the same RBS from ompC under the control of different promoters and measuring GFP fluorescence by flow cytometry. Strains: HL3167 (PLtetO-1:*ompC*::*gfp*), HL3186 (PLlacO-1:*ompC*::*gfp*) and HL3268 (PLtetO-1Con:*ompC*::gfp). Each strain was measured in duplicate biological replicates. Total n = 6. Note the measurements were performed on different dates to the measurements in **Figs. S2-S4** and should not be directly compared to them.

Figure S6. Additional experiments with varying sRNA and mRNA production for the RyhB and MicC silencing sRNAs. Error bars are the SEM of duplicate measurements (panels **A**, **B**, and **G**) or root mean square error calculated from the SEM (panels **C**, **D** and **H**). (**A**, **B**) GFP expression as a function *sodB* and *ompC* transcription in strains with chromosomal *hfq* deleted. These delta chr. *hfq* measurements were performed identically and with the same plasmids as the measurements with chromosomal *hfq* (**Fig. 5**). Strains: HL3333 (no RyhB, *sodB*), HL2413 (RyhB, *sodB*), HL2411 (MicC, *ompC*) and HL3610 (no MicC, *ompC*). (**C**, **D**) RyhB and MicC activity as a function of relative mRNA production in the absence of Hfq. sRNA activity was calculated by subtracting the average GFP fluorescence value with the sRNA from the average value without the sRNA at each level of mRNA

induction. Relative mRNA production at different IPTG concentrations was determined using previously reported data (2). (**E**) Simulation of GFP expression for a target mRNA that is only translated in the free state as a function of sRNA production. (**F**) Genetic circuit used to investigate the effect of varying sRNA production on sRNA activity with constant Hfq and mRNA production. sRNA production was varied by placing the gene under the inducible PLlacO-1 promoter. (**G**, **H**) GFP expression and sRNA activity as a function of sRNA production. Total n = 20 per strain with duplicate measurements for each IPTG concentration. Strains: HL2625 (RyhB, *sodB*) and HL839 (MicC, *ompC*).

Supplementary Table Legends

Table S1. Plasmids and strains.

†Note: For brevity the absence of the Hind **III** restriction enzyme site in the PCon and PConM8 promoters was not stated in **Fig. S5** and the main text. That is, PConNoHind = PCon and PConNoHindM8 = PConM8 except where otherwise indicated (*e.g.* pHL533 and pHL671). * Reported in (2). # Reported in (1). ‡ Reported in (4). † Reported in (5) as K1 fw or K1 rev.

Table S2. Oligonucleotides.

* Reported in (2). ‡ Reported in (4). ◊ Reported in (6). § Reported in (7). qRT-PCR = quantitative reverse transcriptase polymerase chain reaction.

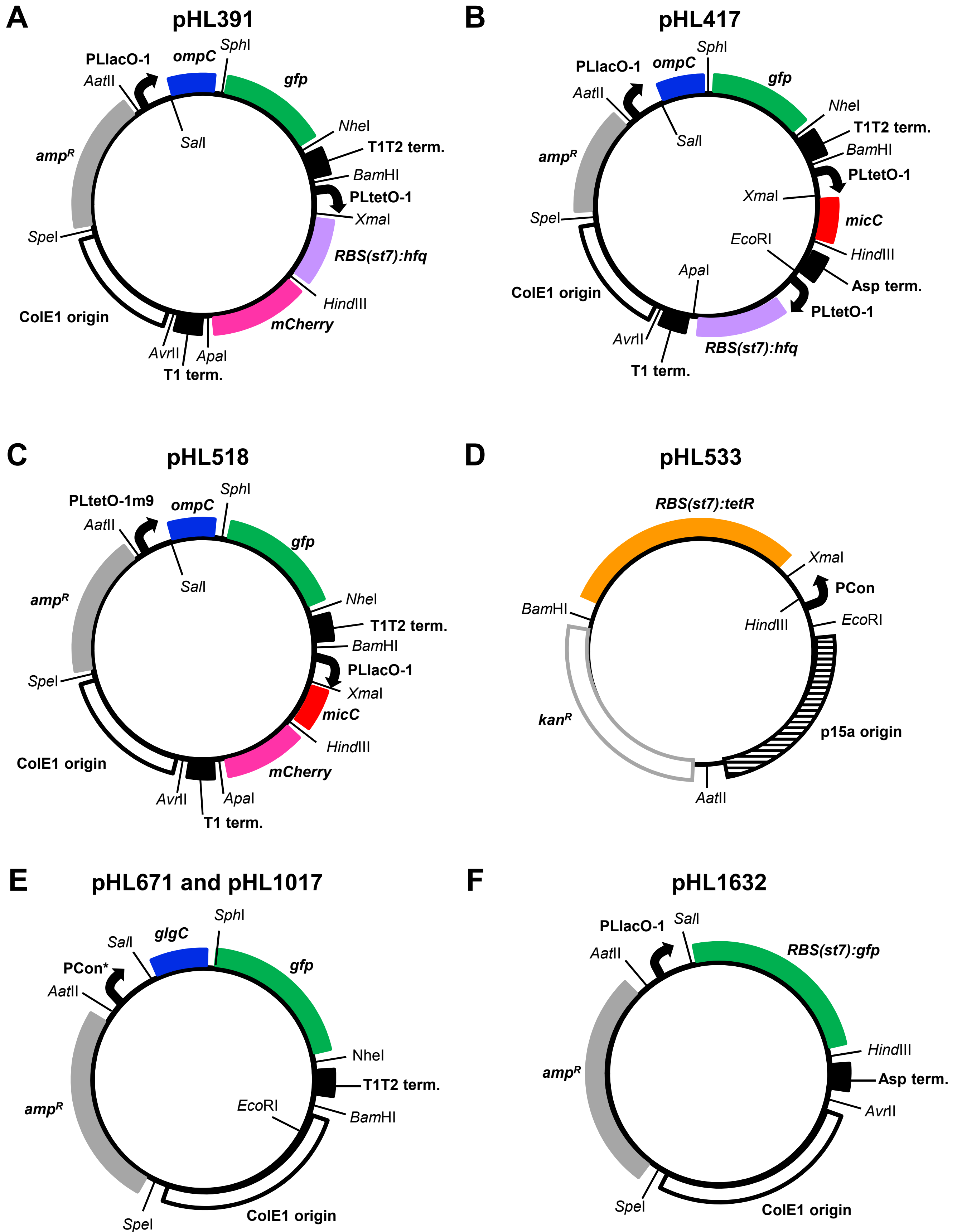

Figure S1. Plasmid maps.

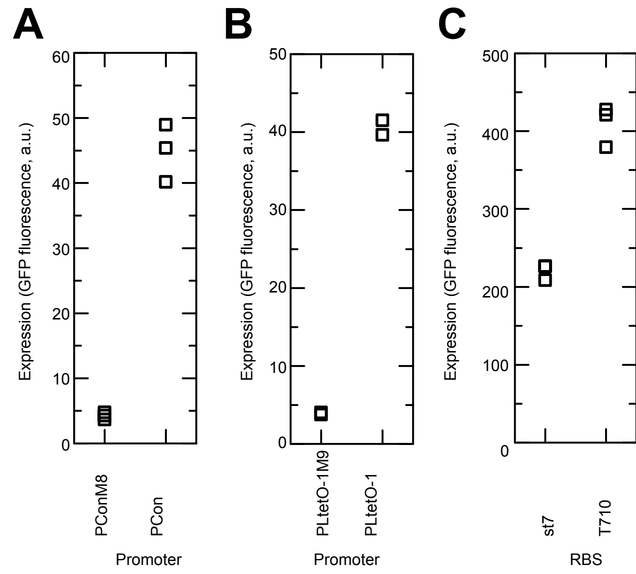

Figure S2. Relative strengths of the different promoters and ribosome binding sequences (RBS).

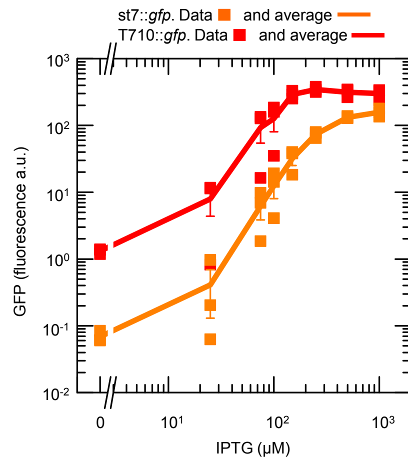

Figure S3. GFP expression with the st7 and T710 RBS.

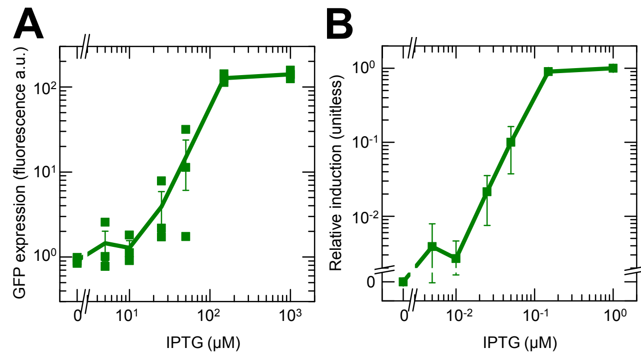

Figure S4. GFP expression and relative induction of PLlacO-1 at different IPTG concentrations measured in parallel with the samples in Fig 4B.

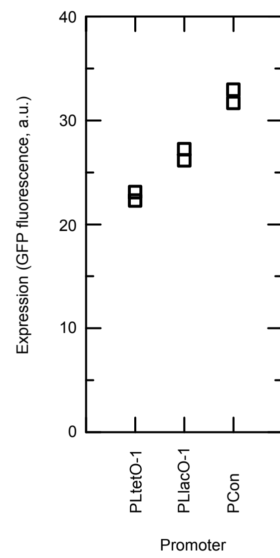

Figure S5. Relative strengths of the PLtetO-1, PLlacO-1 and PCon promoters.

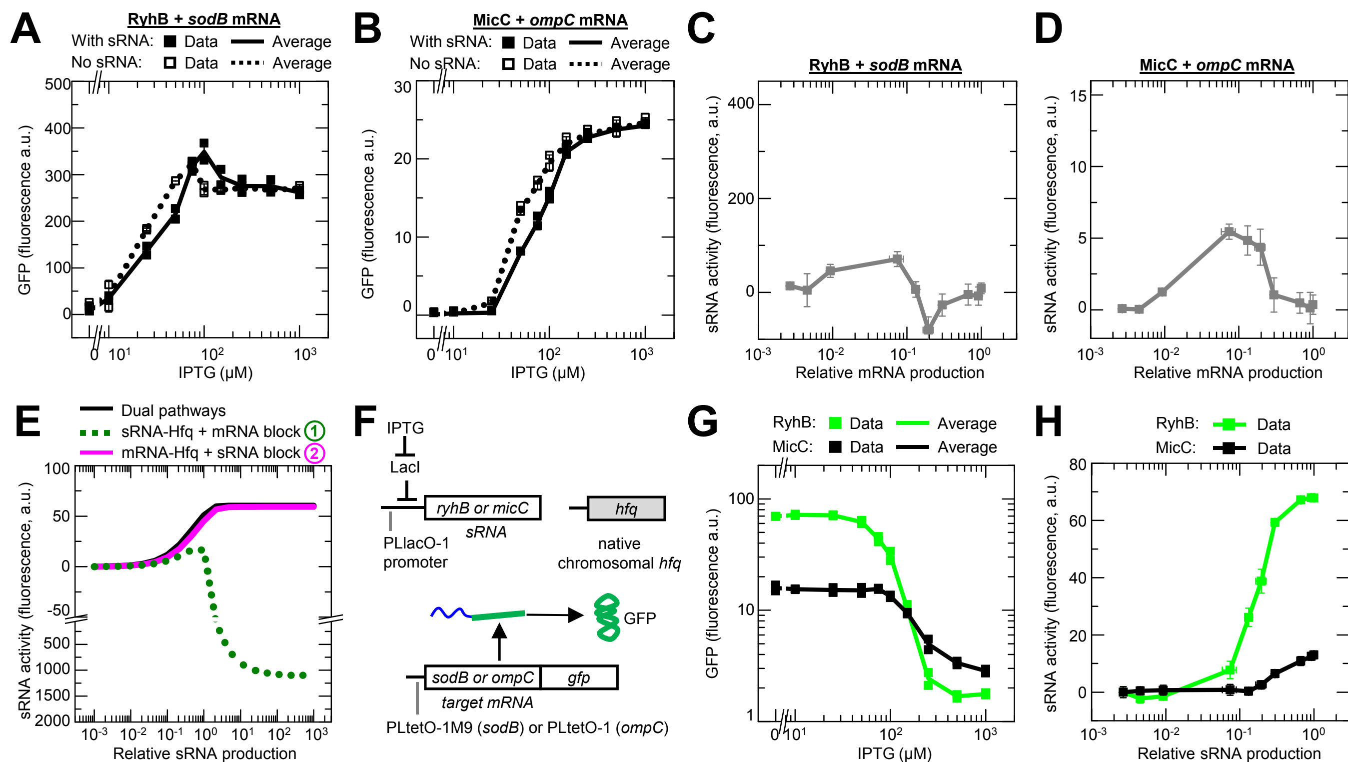

Figure S6. Additional experiments with varying sRNA and mRNA production for the RyhB and MicC silencing sRNAs.

Table S1. Plasmids and strains.

Plasmid (pHL)/ Strain (HL)	Description†	Antibiotic Resistance
*pHL108**	AmpR + PLlacO-1::*micC*::*mCherry*::T1 terminator + PLtetO-1::*ompC*::*gfp*::T1T2 terminator + ColE1	amp
*pHL269**	AmpR + PLtetO-1::*micC*::*mCherry*::T1 terminator + PLlacO-1::*ompC*::*gfp*::T1T2 terminator + ColE1	amp
*pHL282**	AmpR + PLtetO-1::*mCherry*::T1 terminator + PLlacO-1::*ompC*::*gfp*::T1T2 terminator + ColE1	amp
pHL391	AmpR + PLtetO-1::RBS (st7) *hfq*::*mCherry*::T1 terminator + PLlacO-1::*ompC*::*gfp*::T1T2 terminator + ColE1	amp
pHL417	AmpR + PLtetO-1::*micC*::Asp terminator + PLlacO-1::*ompC*::*gfp*::T1T2 terminator + PLtetO-1::RBS (st7) *hfq*::T1 terminator + ColE1	amp
pHL518	AmpR + PLlacO-1::*micC*::*mCherry*::T1 terminator + PLtetO-1m9::*ompC*::*gfp*::T1T2 terminator + ColE1	amp
pHL533	KanR + PCon::RBS(st7) *tetR* + p15a. Used to integrate PCon::RBS *(st7) tetR* into the chromosome at *galK*.	kan
pHL600‡	KanR + PLtetO-1::*csrB*::T1 terminator + PLlacO-1::RBS (st7) *csrA*::Asp terminator + p15a	kan
pHL671	AmpR + PCon::*glgC*::*gfp*::T1T2 terminator + ColE1	amp
*pHL720**	AmpR + PLtetO-1::*mCherry*::T1 terminator + PLlacO-1::*sodB*::*gfp*::T1T2 terminator + ColE1	amp
*pHL745**	AmpR + PLtetO-1::*ryhB*::*mCherry*::T1 terminator + PLlacO-1::*sodB*::*gfp*::T1T2 terminator + ColE1	amp
*pHL841**	AmpR + PLtetO-1::*ryhB*::*mCherry*::T1 terminator + PLlacO-1::*ompC*::*gfp*::T1T2 terminator + ColE1	amp
*pHL908**	AmpR + PLlacO-1::*ryhB*::*mCherry*::T1 terminator + PLtetO-1m9::*sodB*::*gfp*::T1T2 terminator + ColE1	amp
*pHL1009**	AmpR + PLtetO-1::RBS (st7) *gfp*::T1T2 terminator + PLlacO-1::*micC* + ColE1	amp
*pHL1011**	AmpR + PLtetO-1m9::RBS (st7) *gfp*::T1T2 terminator + PLlacO-1::*ryhB* + ColE1	amp
pHL1017	AmpR + PConNoHindM8::*glgC*::*gfp*::T1T2 terminator + ColE1	amp
*pHL1228**	AmpR + PLtetO-1::*dsrA*::*mCherry*::T1 terminator + PLlacO-1::*ompC*::*gfp*::T1T2 terminator + ColE1	amp
pHL1632	AmpR + PLlacO-1::RBS (st7) *gfp*::Asp terminator + ColE1	amp
pHL1633	AmpR + PLlacO-1::T710 *gfp*::Asp terminator + ColE1	amp
pHL1884	AmpR + PLlacO-1::*glmS*::*gfp*::T1T2 terminator + Asp terminator + ColE1	amp
pHL1954	AmpR + PConNoHindM8::*ryhB*::Asp terminator + PLlacO-1::RBS (st7) *hfq*::T1 terminator + PLtetO-1m9::*sodB*::*gfp*::T1T2 terminator + ColE1	amp
pHL1969	AmpR + PConNoHindM8::*ryhB*::Asp terminator + PLlacO-1::T710 *hfq*::T1 terminator + PLtetO-1m9::*sodB*::*gfp*::T1T2 terminator + ColE1	amp
pHL1979	AmpR + PCon::*glmZ*::T1T2 terminator + ColE1	amp
pHL2021	AmpR + PLlacO-1::T710 *hfq*::T1 terminator + PLtetO-1m9::*sodB*::*gfp*::T1T2 terminator + ColE1	amp
pHL2026	AmpR + PLlacO-1::T710 *hfq*::T1 terminator + PLtetO-1m9::*glmS*::*gfp*::T1T2 terminator + ColE1	amp
pHL2027	AmpR + PLlacO-1::T710 *hfq*::T1terminator + PLtetO-1::*glmS*::*gfp*::T1T2 terminator + ColE1	amp
pHL2053	AmpR + PLlacO-1::T710::*hfq*::T1 termintor + PLtetO-1m9::*ompC*::*gfp*::T1T2 terminator + ColE1	amp
pHL2073	AmpR + PConShortNoHind::*glmZ*::Asp terminator + PLlacO-1::T710 *hfq*::T1 terminator + PLtetO-1m9::*glmS*::*gfp*::T1T2 terminator + ColE1	amp
pHL2075	AmpR + PConShortNoHind::*glmZ*::Asp terminator + PLlacO-1::T710 *hfq*::T1 terminator + PLtetO-1::*glmS*::*gfp*::T1T2 terminator + ColE1	amp
pHL2084	AmpR + PConShortNoHindM8::*glmZ*::Asp terminator + PLlacO-1::T710 *hfq*::T1 terminator + PLtetO-1m9::*glmS*::*gfp*::T1T2 terminator + ColE1	amp
pHL2085	AmpR + PConShortNoHindM8::*glmZ*::Asp terminator + PLlacO-1::T710 *hfq*::T1 terminator + PLtetO-1::*glmS*::*gfp*::T1T2 terminator + ColE1	amp
pHL2107	AmpR + PConNoHindM8::*micC*::Asp terminator + PLlacO-1::T710::*hfq*::T1 terminator + PLtetO-1m9::*ompC*::*gfp*::T1T2 terminator + ColE1	amp
pHL2117	AmpR + PConNoHindM8::*ryhB*::Asp terminator + T1 terminator + PLtetO-1m9::*sodB*::*gfp*::T1T2 terminator + ColE1	amp
HL716*	MG1655 + pKD46 + *lacIq* inserted into the chromosome at *intS*	none
HL770*	HL716 + Δ*hfq*	none

HL839*	HL716 + pHL108	amp
HL862*	HL716 + Δ*micC*	none
HL1085#	HL862 + pHL108	amp
HL1128*	HL862 + Δ*hfq*	none
HL1178*	HL862 + pHL282	amp
HL1672	HL862 + pHL518	amp
HL1706	HL716 + Δ*csrB*	none
HL1752	HL1706 + Δ*csrC*	none
HL1933	HL1752 + PCon::*tetR* inserted into the chromosome at *galK*	none
HL2300*	HL716 + pHL745	amp
HL2304*	HL716 + pHL269	amp
HL2325	HL716 + pHL417	amp
HL2378	HL1933 + Δ*glgCAP*	none
HL2411	HL770 + pHL269	amp
HL2413	HL770 + pHL745	amp
HL2483	HL2378 + Δ*csrD*	none
HL2516	HL2483 + pHL600 + pHL671	amp, kan
HL2625*	HL716 + pHL908	amp
HL2752*	HL716 + Δ*ryhB*	none
HL2817*	HL716 + pHL720	amp
HL2839*	HL2752 + pHL720	amp
HL2916	HL2483 + pHL600 + pHL1017	amp, kan
HL3186*	HL716 + pHL282	amp
HL3333	HL770 + pHL720	amp
HL3338*	HL770 + Δ*ryhB*	none
HL3373*	HL2752 + pHL1011	amp
HL3395*	HL862 + pHL1009	amp
HL3610	HL770 + pHL282	amp
HL3611	HL716 + pHL391	amp
HL3612*	HL716 + pHL841	amp
HL3619*	HL716 + pHL1228	amp
HL5189	HL716 + pHL1633	amp
HL5226	HL716 + Δ*glmZ*	none
HL5410	HL5226 + Δ*hfq*	none
HL5963	HL5390 + pHL1884	amp
HL6199	HL2752 + pHL1954	amp
HL6200	HL2752 + pHL1969	amp
HL6257	HL5390 + pHL1979	amp
HL6357	HL3338 + pHL2021	amp
HL6361	HL3338 + pHL1969	amp
HL6370	HL3338 + pHL1954	amp
HL6379	HL5410 + pHL2026	amp
HL6380	HL5410 + pHL2027	amp
HL6442	HL716 + pHL1632	amp
HL6476	HL5410 + pHL2073	amp
HL6477	HL5410 + pHL2075	amp
HL6526	HL5410 + pHL2084	amp
HL6527	HL5410 + pHL2085	amp
HL6555	HL1128 + pHL2053	amp
HL6566	HL1128 + pHL2107	amp
HL6581	HL2752 + pHL2117	amp
HL6582	HL3338 + pHL1632	amp

Table S2. Oligonucleotides.

Oligonucleotide	Description	Sequence (5' to 3')
AsptermAvrR	PCR synthesis of Asp terminator from pLEX	agacctaggactgctcacaagaaaaaaggcacg
AsptermEcoRIR*	PCR synthesis of Asp terminator from pLEX	ccggaattcactgctcacaagaaaaaaggcacg
AsptermHindIIIF*	PCR synthesis of Asp terminator from pLEX	ggccaagctttaatcgtacagggtagtacaaata
ColEApaIF*	Amplify Ampicillin resistance, ColE origin with T1 terminator from pZE21	gacgggcccatggtacgcgtgctagagg
csrBKOpKD1F‡	For deletion of *csrB* using pKD13 as template	agcgccttgtaagacttcgcgaaaaagacgattctatc ttcgtcgacagggtgtaggctggagctgcttc
csrBKOpKD4R‡	For deletion of *csrB* using pKD13 as template	gtggtcataaagcaacctcaataagaaaaactgccg cgaaggatagcaggattccggggatccgtcgacc
csrCKOpKD1F‡	For deletion of *csrC* using pKD13 as template	actgatggcggttgattgtttgtttaaagcaaaggcgta aagtagcacccgtgtaggctggagctgcttc
csrCKOpKD4R‡	For deletion of *csrC* using pKD13 as template	gccgttttattcagtatagatttgcggcggaatctaacag aaagcaagcaattccggggatccgtcgacc
csrDpKD1F‡	For deletion of *csrD* using pKD12 as template	atctgatttgctagtatgcccgcttcctcactatcggagtt aacacaagggtgtaggctggagctgcttc
csrDpKD4R‡	For deletion of *csrD* using pKD12 as template	catgagacgcagcgcgcattattctacgtgaaaacgg attaaacggcaggattccggggatccgtcgacc
gfpRBSSalF*	PCR *gfp* with RBS7 and *Sal*I site from pTAK 102	cctgtcgactaaggaggaaaaaaaatgcgtaaagga gaagaacttttc
gfpRBSSphF	PCR *gfp* with RBS7 and *Sph*I site from pTAK 102	tacgcatgctaaggaggaaaaaaaatgcgtaaagg agaagaacttttc
glgCleadSalF‡	PCR 5′UTR of *glgC* leader for fusion to *gfp*	cctgtcgactctggcagggacctgcacacggattg
glgCleadSphR‡	PCR 5′UTR of *glgC* leader for fusion to *gfp*	tacgcatgctaaccatgactaactcctttttatcatctctg g
glgCpKD1F‡	For deletion of *glgCAP* using pKD13 as template	cctgcacacggattgtgtgtgttccagagatgataaaa aaggagttagtcgtgtaggctggagctgcttc
glgPpKD4R‡	For deletion of *glgCAP* using pKD13 as template	ttacaatctcaccggatcgatatgccagatatgatcggc gtactctttgaattccggggatccgtcgacc
glmSstartSphR§	PCR *glmS* with *Sal*I site added at the end of *glmU* to fuse to *gfp* and qRT-PCR	ttacgcatgctcgcgccaacaattccacacat
glmSUpSalF	qRT-PCR	ttagtcgaccccggtcacatgggatgaggagat
glmUendSalF§	PCR *glmS* with *Sal*I site added at the end of *glmU* to fuse to *gfp*	ttagtcgaccgtgtgccgcagactcagaaagaa
glmZ1stMutR	qRT-PCR	acgcctgctcttattacggagcaggcgttaaaacagct ctgtatgagaacaagtgggtgc
glmZHindR◊	PCR *glmZ* with native terminator	ggccaagcttgggccttcctgatacataaaaaaacgc
glmZKOpKD1F	For deletion of *glmZ* using pKD13 as template	tagttccttctcacccggaggcaagcacctccggggcc ttcctgatacatgtgtaggctggagctgcttc
glmZKOpKD4R	For deletion of *glmZ* using pKD13 as template	acaagtgttaagggatgttatttcccgattctctgtggcat aataaacgaattccggggatccgtcgacc
glmZXmaF◊	PCR *glmZ* and qRT-PCR	cctcccggggtagatgctcattccatctcttat
HfqApaIR*	PCR *hfq* with RBS (st7)	gacgggcccttattcggtttcttcgctgtcctg
HfqHindR*	PCR *hfq* with RBS (st7)	ggccaagcttattcggtttcttcgctgtcctg
HfqnoRBSBamF	PCR *hfq* without RBS	cgcggatccatggctaaggggcaatctttacaag
HfqRBSXmaIF*	PCR *hfq* with RBS (st7)	cctcccgggtaaggaggaaaaaaaatggctaaggg gcaatctttacaag
mCherryApaIR*	PCR *mCherry*	catgggcccttacttgtacagctcgtccatgcc
mCherryRBSBsiWIHindIIIF*	PCR *mCherry*	tcttaaaagcttattaaagaggagaaacgtacgatggt gagcaagggcgaggagg
MicC1XmaIF*	PCR *micC* and qRT-PCR	tcctcccgggttatatgcctttattgtcacaga
MicC2HindIIIR*	PCR *micC* with native terminator	ggccaagcttctggataaggattatccaattcta
MicC3NoTermHindIIIR*	qRT-PCR	ggccaagcttgttcgggcttgtctttttatatgt
PConEcoRF*	PCR synthesis of PCon promoter	ccggaattctcgagcaccgtcgttgttgacatttttaagct tggcggttataat
PConGalKR	For integration of PCon::*tetR* at *galK*	gtttgcgcgcagtcagcgatatccattttcgcgaatccg gagtgtaagaatcgagcaccgtcgttgttgac
PConM8NoHindAatF‡	PCR synthesis of PConM8 promoter with no *Hind*III site	cgcgacgtctcgagcaccgtcgttgtttacatttttatgctt ggcggttatggt

PConM8NoHindBamHF	PCR synthesis of PConM8 promoter with no *Hind*III site	cgcggatcctcgagcaccgtcgttgtttacatttttatgcttggcggttatggt
PConM8NoHindSalR	PCR synthesis of PConM8 promoter with no *Hind*III site	ttagtcgacgaatccaccataaccgccaagcataaaaatgtaaacaac
PConM8NoHindXmaR	PCR synthesis of PConM8 promoter with no *Hind*III site	cctcccggggaatccaccataaccgccaagcataaaaatgtaaacaac
PConM8ShortNoHindXmaR	PCR synthesis of PConM8 promoter with no *Hind*III site with deletion of 12 b.p. from 5' end of oligo	cctcccgggaccataaccgccaagcataaaaatgtaaacaac
PConAatF	PCR synthesis of PCon promoter	cgcgacgtctcgagcaccgtcgttgttgacatttttaagcttggcggttataat
PConNoHindBamHF*	PCR synthesis of PCon promoter with no *Hind*III site	cgcggatcctcgagcaccgtcgttgttgacatttttatgcttggcggttataat
PConSalR	PCR synthesis of PCon promoter	ttagtcgacctgtgtggaatccattataaccgccaagcttaaaaatgtcaacaac
PConNoHindXmaR*	PCR synthesis of PCon promoter with no *Hind*III site	cctcccgggtgtgtggaatccattataaccgccaagcataaaaatgtcaacaac
PConShortNoHindXmaR2	PCR synthesis of PCon promoter with no *Hind*III site with deletion of 12 b.p. from 5' end of oligo	cctcccgggattataaccgccaagcataaaaatgtcaacaac
PConXmaR*	PCR synthesis of PCon promoter	cctcccgggtgtgtggaatccattataaccgccaagcttaaaaatgtcaacaac
pKD1FAatII	PCR *kanR* with FRT sites	ctagacgtcgtgtaggctggagctgcttc
pKD1FGalKF	For integration of PCon::*tetR* at GalK	ttcatattgttcagcgacagcttgctgtacggcaggaccagctcttccggtgtaggctggagctgcttc
pKD4RKpnI	PCR kanR with FRT sites	tgatggtaccattccggggatccgtcgacc
pLacEcoRF	PCR synthesis of PLlacO-1 promoter with *Eco*RI site	ccggaattcgatccataaatgtgagcggataacattgacattg
pLacNotIF*	PCR synthesis of PLlacO-1 promoter with *Not*I site	tcctgcggccgcgatccataaatgtgagcggataacattgacattg
pLacO1BamHIF	PCR synthesis of PLlacO-1 promoter with BamHI site	cgcggatccataaatgtgagcggataacattgacattg
pLacpHNSAatSalF	Fuses PLlacO-1 directly to target mRNA::*gfp*, with *Aat*II and *Sal*I sites	ctagacgtcataaatgtgagcggataacattgacattgtgagcggataacaagatactgtcgacaacaaaccacccc
pLacXmaBamHT1T2R*	PCR synthesis of PLlacO-1 promoter with *Bam*HI and *Xma*I sites	tcctcccgggagtatcttgttatccgctcacaatgtcaatgttatccgctcacatttatggatcccctaggtcta
PLtetO1m9SalR*	PCR synthesis of PLtetO-1m9 promoter with *Sal*I site	caagtcgacagtctctctatcactgatagggatgtcaatct
pOmpC1SalIF*	PCR *ompC* sequence to fuse to *gfp* and qRT-PCR	tactgtcgacttgccgactgattaatgagggtta
pOmpC2SphR*	PCR *ompC* sequence to fuse to *gfp* and qRT-PCR	tacgcatgctagctgggaccaggagggacagtac
pTAKtermBamHIR	PCR *gfp* with T1T2 term with *Bam*HI site from pTAK102	cgcggatcctgagcggatacatatttgaatgta
pTAKtermNotR	PCR *gfp* with T1T2 term with *Not*I site from pTAK102	tcctgcggccgctgagcggatacatatttgaatgta
pTetO-1SalIR*	PCR synthesis of PLtetO-1 promoter with *Sal*I	tactgtcgacagtatctctatcactgatagggatgtcaatctctatcactgatagga
pTetOBamHIF*	PCR synthesis of PLtetO-1 promoter with *Bam*HI	cgcggatcctccctatcagtgatagagattgacatccctatcagtgatag
pTetOEcoRIF	PCR synthesis of PLtetO-1 promoter with *Eco*RI	ccggaattctccctatcagtgatagagattgacatccctatcagtgatag
pTetOXmaIR*	PCR synthesis of PLtetO-1 promoter with *Xma*I	cctcccgggagtatctctatcactgatagggatgtcaatctctatcactg
RhyBHindR*	PCR *ryhB* with native terminator	ggccaagcttgtggataaattgagaacgaaagat
RyhBnotermR*	qRT-PCR	ctaagtaatactggaagcaatgtg
rrnB1361F†	qRT-PCR 16S loading control	gaatgccacggtgaatacgtt
rrnB1475R†	qRT-PCR 16S loading control	acccactcccatggtgtga
RhyBXmaF*	PCR *ryhB* and qRT-PCR	cctcccggggcgatcaggaagaccctcgcggag
SodBSalIF*	PCR *sodB* sequence to fuse to *gfp* and qRT-PCR	caagtcgaccatacgcacaataaggctattgtacg
SodBSphR*	PCR *sodB* sequence to fuse to *gfp* and qRT-PCR	tacgcatgctcgcggtacctttaatcaggttgtt
T710RBSKpnF	PCR T710 RBS to fuse to *gfp* and *hfq*	cttaggtacctaagaaggagatatacatatg
T7codingRBS7BamR	PCR T710 RBS to fuse to *gfp* and *hfq*	catggatccgcgacccatttgctgtccaccagtcatgctagccattttttttcctcctta