Supplementary information

Determinants of target prioritization and regulatory hierarchy for the bacterial small RNA SgrS

Maksym Bobrovskyy[1,2], Muhammad S. Azam[1], Jane K. Frandsen[3,4,5], Jichuan Zhang[6], Anustup Poddar[6], Xiangqian Ma[1], Tina M. Henkin[3], Taekjip Ha[6,7] and Carin K. Vanderpool[1]*

[1]Department of Microbiology, University of Illinois at Urbana-Champaign, 601 S. Goodwin Ave., Urbana, Illinois 61801
[2]Present address: Department of Microbiology, The University of Chicago, 920 E. 58th St., Chicago, Illinois 60637
[3]Department of Microbiology and Center for RNA Biology, The Ohio State University, Columbus, Ohio 43210
[4]Biochemistry Program, The Ohio State University, Columbus, OH 43210
[5]Present address: Department of Biochemistry and Molecular Biology, Pennsylvania State University, State College, Pennsylvania, USA 16802
[6]Department of Biophysics and Biophysical Chemistry, Johns Hopkins University, Baltimore, Maryland, USA 21205
[7]Howard Hughes Medical Institute, Baltimore, Maryland, USA 21205

*Corresponding author
Carin K. Vanderpool, Ph.D.
(t) 217-333-7033
(f) 217-244-6697
cvanderp@illinois.edu

Table S1. Strains and plasmids used in this study.

Plasmid	**Vector**	**Genotype**	**Source or Reference**
pLCV1	pHDB3	P_{lac}-*sgrS*	Vanderpool *et al.*, 2004
pHDB3	pBR322 derivative	vector control	Wadler *et al.*, 2009
pZAMB1	pZA31R	$P_{Ltet0-1}$-*sgrS*	Bobrovskyy et al., 2016
pZEMB8	pZE12S	$P_{Llac0-1}$-*ptsG-gfpsf*	Bobrovskyy et al., 2016
pZEMB10	pZEMB8	$P_{Llac0-1}$-*manX-gfpsf*	This study
pZEMB15	pZEMB8	$P_{Llac0-1}$-*yigL-gfpsf*	This study
pZEMB25	pZEMB8	$P_{Llac0-1}$-*purR-gfpsf*	Bobrovskyy et al., 2016
pZEMB26	pZEMB8	$P_{Llac0-1}$-*asdI-gfpsf*	This study
pZEMB27	pZEMB8	$P_{Llac0-1}$-*asdI-II-gfpsf*	This study
Strain	**Background**	**Genotype**	**Source or Reference**
BW14894	BL21 (DE3)	Δ*lac* X74, *Δphn33-30*	G. M. Yakovleva, et al., 1998
CV104	DJ480	Δ*lac* X74, *mal::lacI^q, ΔsgrS::kan*	C. K. Vanderpool, S. Gottesman, 2004
DJ480	MG1655	Δ*lac* X74	D. Jin, NCI
DJS2411		*hfq* Q8A	S. Gottesman, NCI
DJS2420		*hfq* Y25D	S. Gottesman, NCI
JH111	CS104	λ*attB::lacI^q*- PN25*tet^R-spec^R*	Wadler et al., 2007
MB170	PM1203	pBAD-*asdI-'lacZ*, λattB::*lacIq*-PN25*tet^R-spec^R*	This study
MB171	PM1203	pBAD-*asdI-II-'lacZ*, λattB::*lacIq*-PN25*tet^R-spec^R*	This study
MB183	PM1203	pBAD-*asdII-'lacZ*, λattB::*lacIq*-PN25*tet^R-spec^R*	This study
NM200	MG1655	Δ*lac* X74, mini λ::*cat*	N. Majdalani, NCI
PM1205	PM1203	*lacI'*:: P_{BAD}-*cat-sacB-lacZ*, miniλ*tet^R*, Δ*araBAD araC*+, *mal::lacI^q*	Mandin et al., 2009
SA1408	NM2000	*hfq::cat-sacB, mal::lacIq*	This study
SA1410	NM2000	*hfq* R16A	This study
TK310	MG1655	Δ*cyaA* Δ*cpdA* Δ*lacY*	T. Kuhlman, et al., 2007
XM102	JH111	Δ*sgrS, hfq::cat-sacB*, *mal::lacI^q*	This study
XM105	XM102	pSIM6	This study
XM106	JH111	*hfq* Q8A	This study
XM107	JH111	*hfq* Y25D	This study
XM108	JH111	*hfq* Q8A	This study

Table S2. Oligonucleotides used in this study.

Oligo	Description	Sequence 5'-3'
MBP84F	T7 forward primer for *in vitro* transcription *ptsG* (+1)	TAATACGACTCACTATAGGATAAATAAAGGGCGCTTAGATGCCCTG
MBP213R	reverse primer for *in vitro* transcription *ptsG* (+240)	TGCGATACAACGGCGGGC
O-JH218	T7 forward primer for *in vitro* transcription *manX* (+1)	TAATACGACTCACTATAGGGGATGAAGCAAGGGGGTGCCCCATG
MBP214R	reverse primer for *in vitro* transcription *manX* (+240)	GCATTTTCACCTGGAACGAAATCGATCC
MBP56F	T7 forward primer for *in vitro* transcription *asd* (+1)	TAATACGACTCACTATAGGATGTGCCAAGAGGAGACCGG
MBP215R	reverse primer for *in vitro* transcription *asdI-II* (+240)	AGATCAAAGGCATCCTGAAGTGTGC
MBP222R	reverse primer for *in vitro* transcription *asdI* (+110)	GAACGGAGCCGACCATACCG
MBP226F	T7 forward primer for *in vitro* transcription *asdII* (+51)	TAATACGACTCACTATAGGGTTGGTTTTATCGGCTGGCGCG
MBP226R	reverse primer for *in vitro* transcription *asdII* (+310)	TGGATAGATTTCGTTGGTATAATCGCCGCC
MBP225F	T7 forward primer for *in vitro* transcription *yigL* (-250 relative to *yigL* start codon)	TAATACGACTCACTATAGGCCAACGCTTCTCTTGCAGGC
MBP216R	reverse primer for *in vitro* transcription *yigL* (+50 relative to *yigL* start codon)	TCGGGAGAAAGTAACGTGCCATC
MBP65F	T7 forward primer for *in vitro* transcription *purR* (+1)	TAATACGACTCACTATAGGTACACTATTTGCGTACTGGCCATTGACC
MBP174R	reverse primer for *in vitro* transcription *purR* (+290)	TGTTTTGTTGATCACGTGTGACACAGTTGT
MBP16F2	forward primer for *yigL* cloning to make pZEMB15	CCCCGAATTCGAACGCGTGGTGGATAACCGCATG
MBP16R41	reverse primer for *yigL* cloning to make pZEMB15	CCCCGGTACCCGCAAACACAAAGTTGATGCCGCG
MBP2F	forward primer for *manX* cloning to make pZEMB10	CCCCGAATTCGCGAAACGCAGGGGTTTTTGGTTG
MBP2R34	reverse primer for *manX* cloning to make pZEMB10	CCCCGGTACCGATCCAGCCGACGTTTTCCTGCTC
MBP92F	forward primer for *asd* cloning to make pZEMB26 or pZEMB27	CCCCGAATTCATGTGCCAAGAGGAGACCGG
MBP92R1	reverse primer for *asdI* cloning to make pZEMB26	CCCCGGTACCCTGAGAAGTAGAAAAGAAGACAGGGCG
MBP92R2	reverse primer for *asdI-II* cloning to make pZEMB27	CCCCGGTACCCATACCGCGCCAGCCGATAAAAC
MBP151F	forward primer for cloning *asd* into PM1205	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATATGTGCCAAGAGGAGACCGG
MBP151R	reverse primer for cloning *asdI* into PM1205	TAACGCCAGGTTTTCCCAGTCACGACGTTGTAAAACGACCATAAGCGTTTTTTTCCTGCAAAGATGTGTGCTG
MBP193F	Forward primer for cloning *asdII* into PM1205	ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATCTTTGCAGGAAAAAAACGCT

MBP193R	Reverse primer for cloning *asdI-II* and *asdII* into PM1205	TAACGCCAGGTTTTCCCAGTCACGACGTTGTAAAAC GACGGCGTCGAAGTCGCGCTC
SgrS	SgrS probes for smFISH	GTGCTGATAAAACTGACGCA ACTTCGCTGTCGCGGTAAAA CTTAACCAACGCAACCAGCA CATGGTTAATCGTTGTGGGA ATCCCACTGCATCAGTCCTT GTCAACTTTCAGAATTGCGG TCAGTCACACATGATGCAGG GCGGGTGATTTTACACCAAT AACCAGCAGGTATAATCTGC
lacZ	*lacZ* probes for smFISH	GTGAATCCGTAATCATGGTC TATTACGCCAGCTGGCGAAA AGTATCGGCCTCAGGAAGAT AATGTGAGCGAGTAACAACC AGATGAAACGCCGAGTTAAC ATCTTCCAGATAACTGCCGT TTAAAGCGAGTGGCAACATG TTTCGACGTTCAGACGTAGT ACCATTTTCAATCCGCACCT TCTGCTCATCCATGACCTGA TGGTTCGGATAATGCGAACA ATCGGTCAGACGATTCATTG GATCGACAGATTTGATCCAG TATTCGCAAAGGATCAGCGG AAACGGGGATACTGACGAAA TCGGCGTATCGCCAAAATCA ACGGAACTGGAAAAACTGCT TTTACCTTGTGGAGCGACAT AGCGTCACACTGAGGTTTTC CGGTTAAATTGCCAACGCTT TACGCCAATGTCGTTATCCA GTAATCGCCATTTGACCACT ATAATTCAATTCGCGCGTCC ATTCAGCCATGTGCCTTCTT
1	*sgrS* qPCR forward primer	GCTTGAAGGACTGATGCAGTGGGATGACCGC
2	*sgrS* qPCR reverse primer	CACCAATACTCAGTCACACATGATGCAGGC
3	*ptsG-1* qPCR forward primer	GTATCCGTACTGCCTATCGCAGGTATTCTG
4	*ptsG-1* qPCR reverse primer	CGGATACGCCATCGTTATTGGTAAAGCC
5	*ptsG-2* qPCR forward primer	GTACTGCCTATCGCAGGTATTCTGCTG
6	*ptsG-2* qPCR reverse primer	CAGTGGCATGTTTGCAAAGACGGAAC
7	*ptsG-3* qPCR forward primer	TGGTTGCTGCATTTGGTGGT
8	*ptsG-3* qPCR reverse primer	GCCGGCCTGATCCACTTTAG
9	*yigL* qPCR forward primer	CTAACGTTTATCGCGACGACG
10	*yigL* qPCR reverse primer	CAGGCTCATACAGCGCATATT
11	*manXYZ* qPCR forward primer	CTCGACACCACTAAAGGCGTGCTG
12	*manXYZ* qPCR reverse primer	CGTTTCCACGAGCATTGGAATGTTAACG

13	*rrsA*qPCR forward primer	AGGCCTTCGGGTTGTAAAGT
14	*rrsA*qPCR reverse primer	ATTCCGATTAACGCTTGCAC
SA035	forward primer for amplification of *hfq* variants	AAGCGGCAGATAACCTGGCT
SA036	reverse primer for amplification of *hfq* variants	GTGTACCAGTACCGCCTGCT

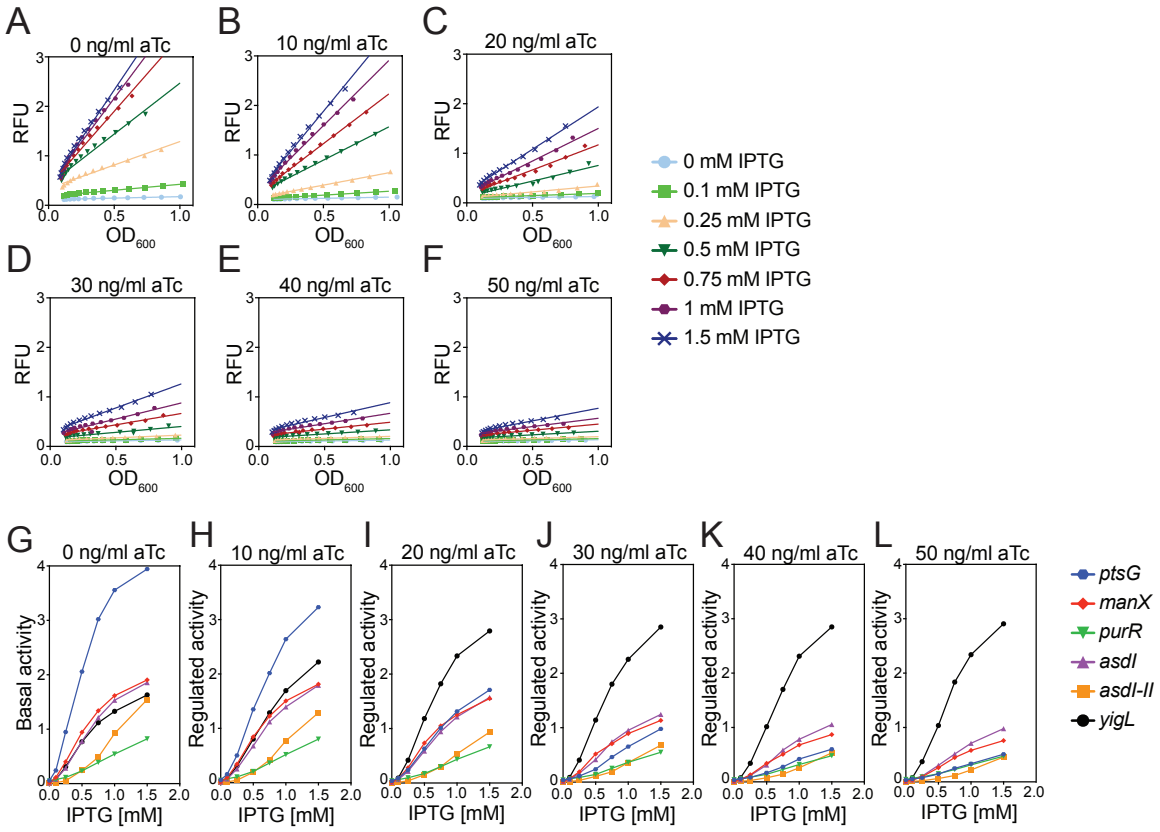
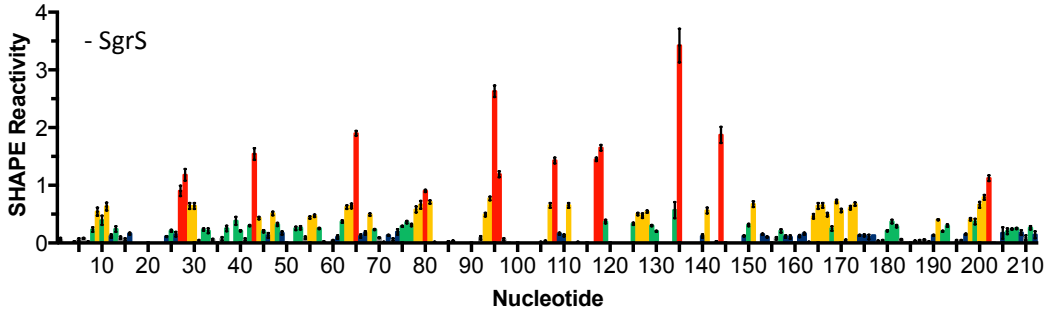
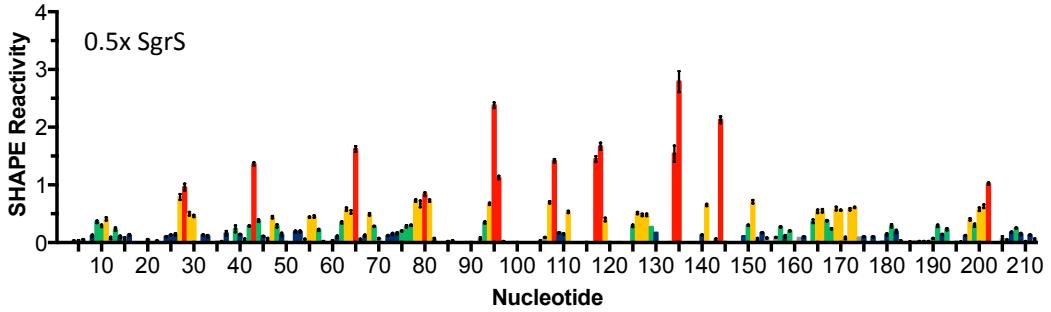


Figure S1. Inducer concentration-dependent activity and regulation of the target-sf*gfp* fusions. A) Plots of *ptsG-sfgfp* translational fusion activity (RFU) over optical density of the culture (OD_{600}) at various IPTG inducer concentrations at A) basal (0 ng/ml aTc) or B-F) increasing SgrS expression levels (10-50 ng/ml aTc). Slopes of the linear regression plots for each IPTG concentration were calculated to obtain “basal activity” (0 ng/ml aTc) and “regulated activity” (10-50 ng/ml aTc) values. G) Basal activity (0 ng/ml aTc) or H-L) regulated activity (10-50 ng/ml aTc) of *ptsG*, *manX*, *purR*, *asdI*, *asdI-II* and *yigL* fused to *sfgfp* reporter at varying IPTG concentrations (0-1.5 mM IPTG).

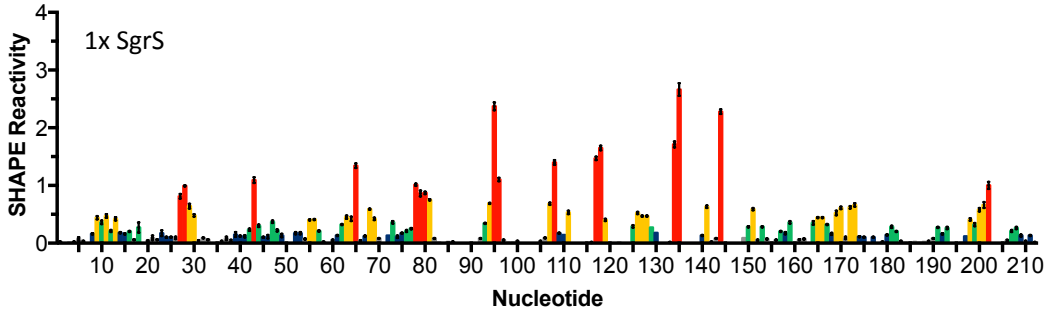
A


- SgrS
SHAPE Reactivity
0
1
2
3
4
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210
Nucleotide

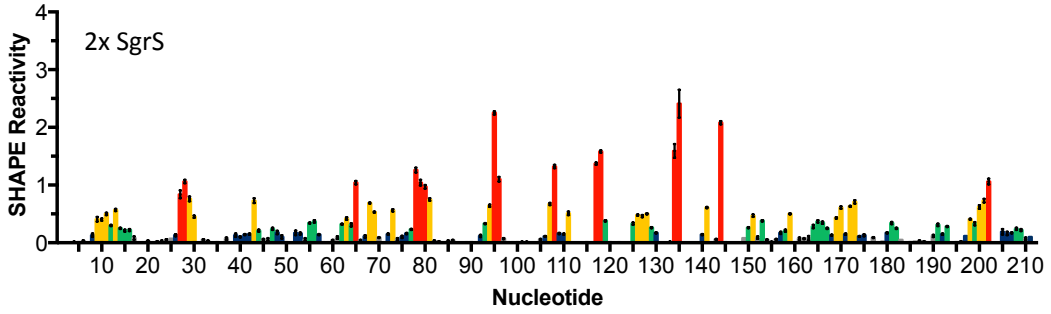
B


0.5x SgrS
SHAPE Reactivity
0
1
2
3
4
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210
Nucleotide

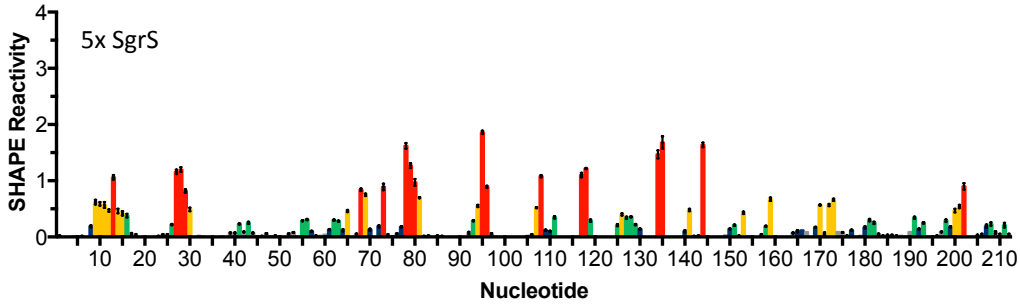
C


1x SgrS
SHAPE Reactivity
0
1
2
3
4
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210
Nucleotide

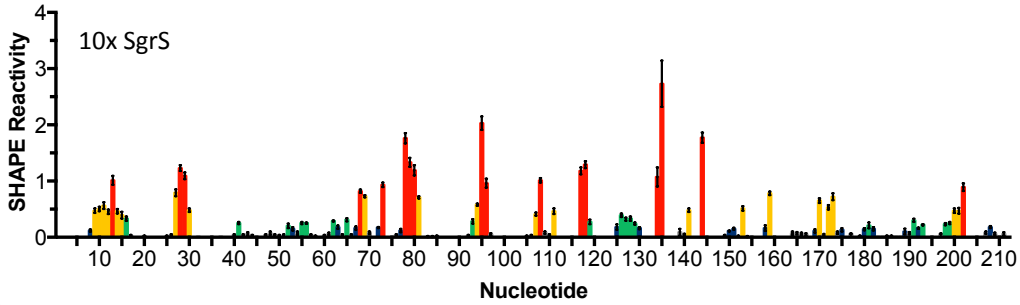
D


2x SgrS
SHAPE Reactivity
0
1
2
3
4
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210
Nucleotide

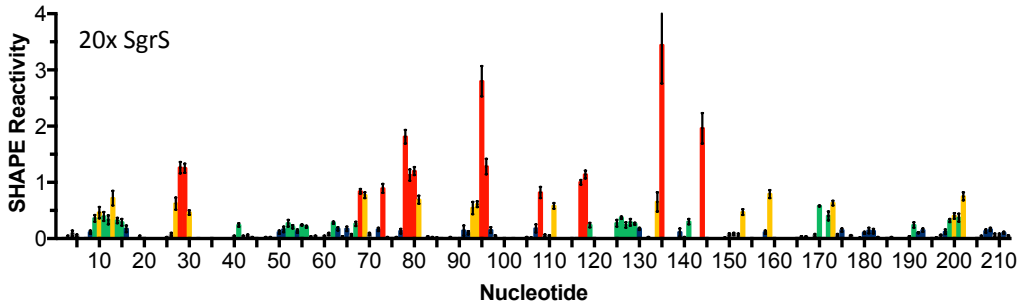
E


5x SgrS
SHAPE Reactivity
0
1
2
3
4
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210
Nucleotide

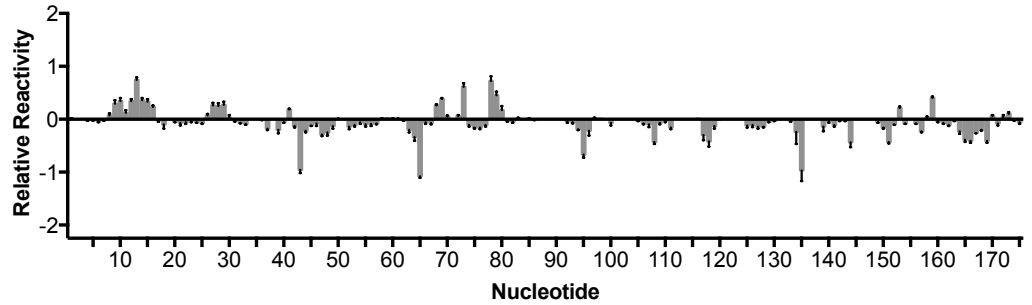
F


10x SgrS
SHAPE Reactivity
0
1
2
3
4
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210
Nucleotide

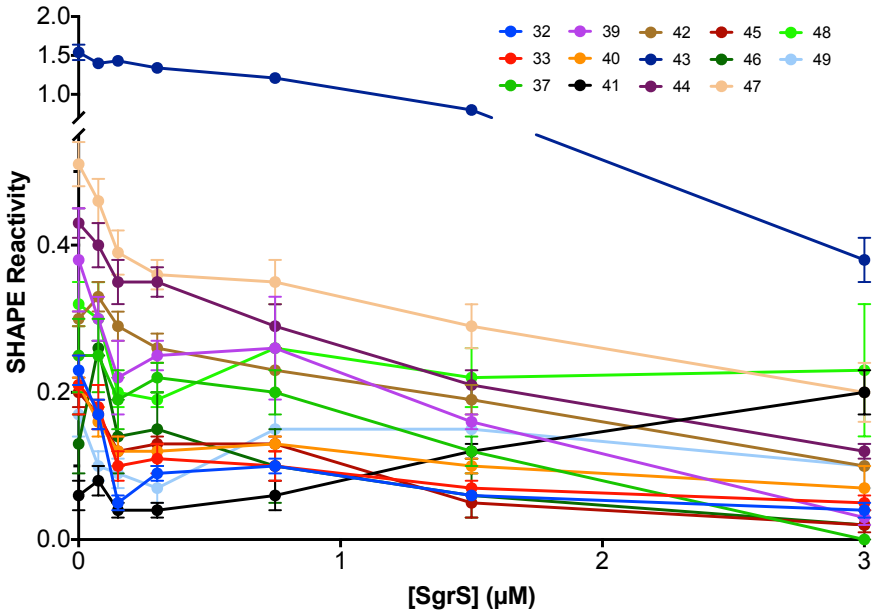
G


20x SgrS
SHAPE Reactivity
0
1
2
3
4
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210
Nucleotide

H


Relative Reactivity
-2
-1
0
1
2
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170
Nucleotide

I


32
33
37
39
40
41
42
43
44
45
46
47
48
49
SHAPE Reactivity
[SgrS] (μM)

J

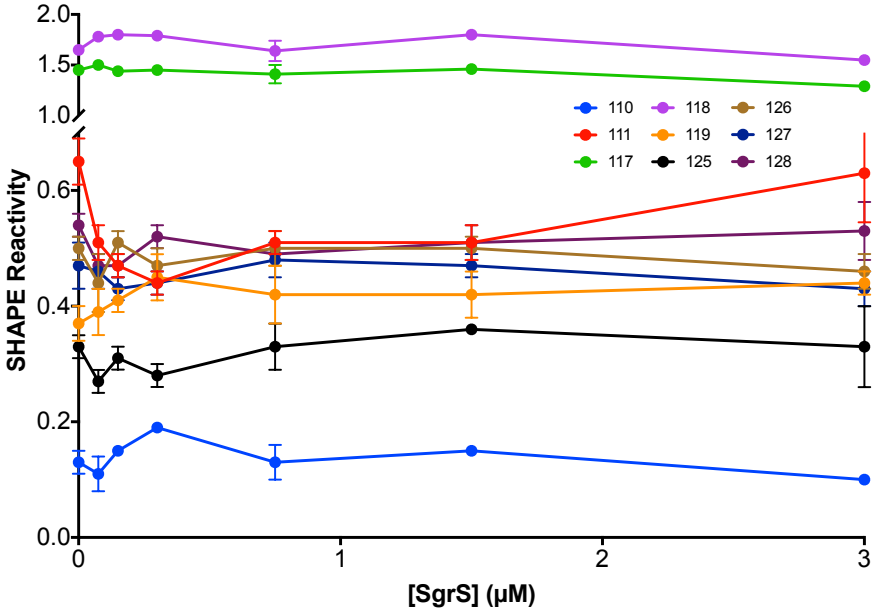

110
111
117
118
119
125
126
127
128
SHAPE Reactivity
[SgrS] (μM)

Figure S2. SHAPE analysis of *asdI-II* RNA. A-G) SHAPE reactivity of the *asdI-II* RNA alone and in complex with increasing concentrations of SgrS (0.5, 1, 2, 5, 10, or 20X). The structure of the *asdI-II* RNA in the absence and presence of SgrS was probed with NMIA and the modified RNA was analyzed by primer extension inhibition. SHAPE reactivity is the difference between the frequency of primer extension products at each nucleotide in +NMIA vs. -NMIA samples. Colors indicate SHAPE reactivity as following: red, highly reactive (≥ 0.8); gold, reactive (0.4-0.79); green, moderately reactive (0.2-0.39); blue, minimally reactive (0.1-0.19); grey, unreactive (< 0.01). H) Relative SHAPE reactivity of the *asdI-II* RNA in the presence of wild-type vs mutant SgrS. Relative reactivity is the difference in the SHAPE reactivity in the presence of wild-type and mutant SgrS. Error bars denote SEM, n = 9. The *asdI-II* RNA nucleotides are numbered below the X-axis and the SgrS binding sites are indicated. I-J) SHAPE reactivity as a function of mutant SgrS concentration for binding site I (I) and site II (J). The same nucleotides as in Fig. 5D are shown. Error bars denote SEM, n = 6.

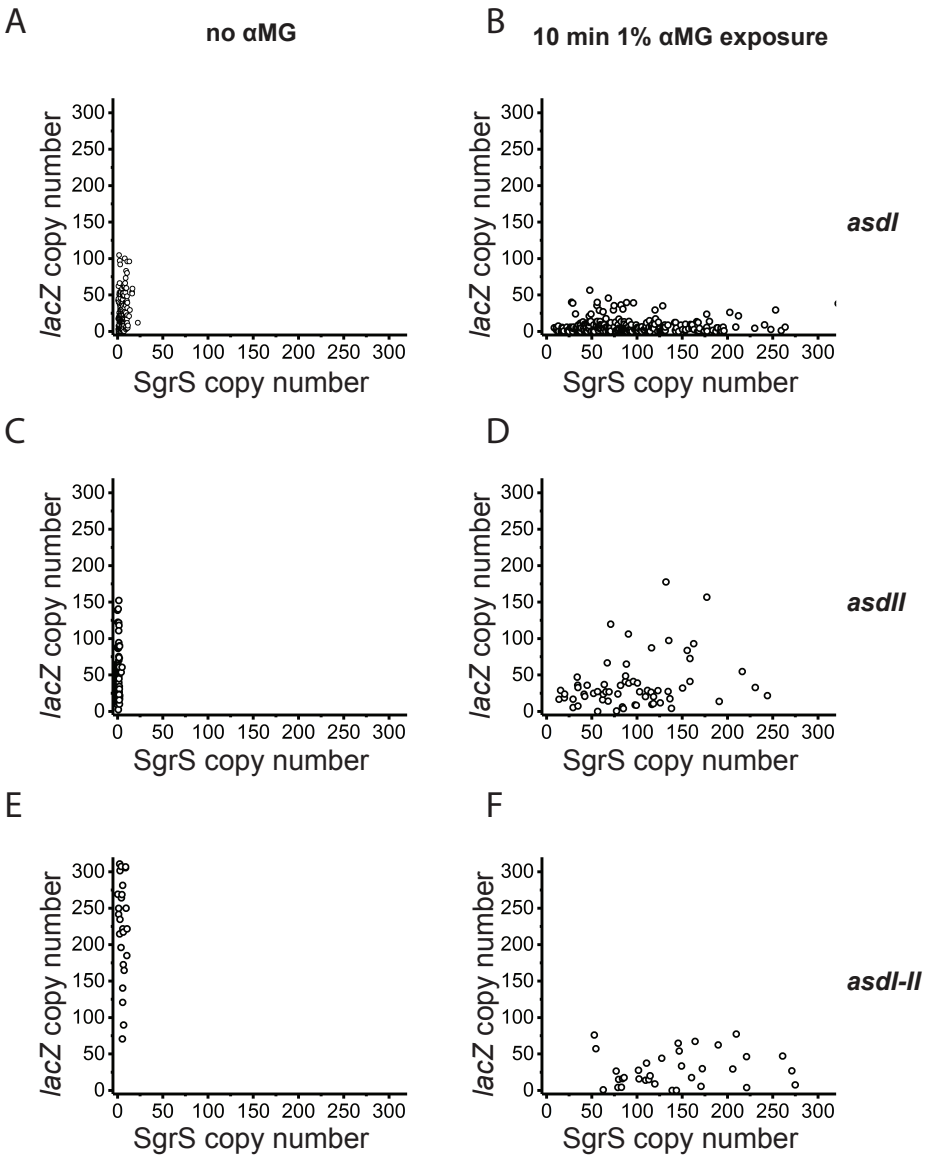


Figure S3. Quantification of SgrS and *asd* mRNA variants using STORM. Copy number of *lacZ* mRNA vs. SgrS sRNA in 30-250 individual cells for the different *asd-lacZ* variants, (A-B) *asdI-lacZ*, (C-D) *asdII-lacZ*, and (E-F) *asdI-II-lacZ*, before (A,C,E) and after (B,D,F) 10 min 1% αMG induction.

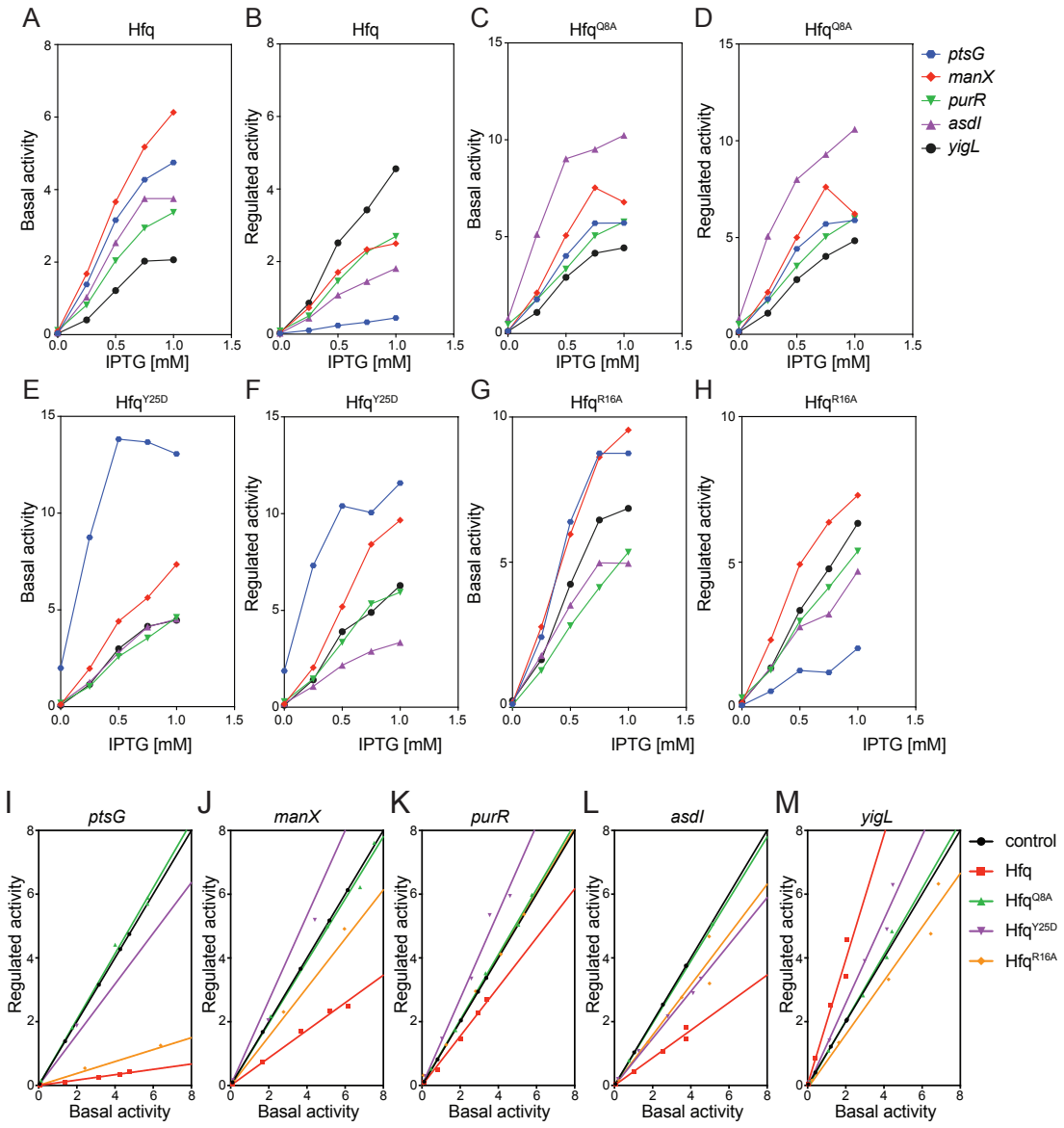


Figure S4. Inducer concentration-dependent activity of target-sf*gfp* fusions in the Hfq mutant strains. Basal activity (0 ng/ml aTc) or regulated activity (50 ng/ml aTc) of *ptsG*, *manX*, *purR*, *asdI*, and *yigL* fusions plotted as a function of IPTG concentrations (0, 0.1, 0.25, 0.5 and 1 mM IPTG) for A, B) wild-type, C, D) Hfq^{Q8A}, E, F) Hfq^{Y25D} and G, H) Hfq^{R16A} strains. Regulated activity plotted as a function of basal activity for I) *ptsG*, J) *manX*, K) *purR,* L) *asdI,* and M) *yigL* fused to *sfgfp* reporter gene in wild-type, Hfq^{Q8A}, Hfq^{Y25D} and Hfq^{R16A} strains. The plots with slopes =1 indicate lack of SgrS regulation, <1 indicate repression and >1 indicate activation by SgrS.

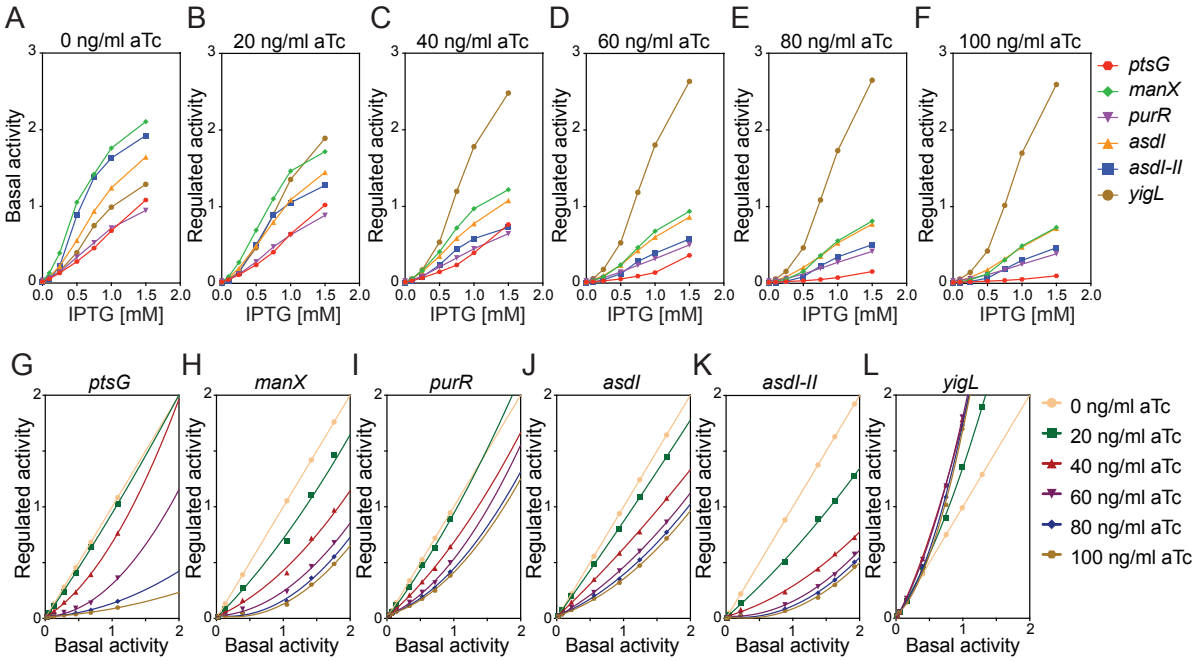


Figure S5. Inducer concentration-dependent activity of target-sf*gfp* fusions in the *rne701* mutant strain. A) Basal activity (0 ng/ml aTc) or B-F) regulated activity (20-100 ng/ml aTc) of *ptsG*, *manX*, *purR*, *asdI*, *asdI-II* and *yigL* fusions plotted as a function of IPTG concentrations (0, 0.1, 0.25, 0.5, 1 and 1.5 mM IPTG). Regulated activity plotted as a function of basal activity for G) *ptsG*, H) *manX*, I) *purR,* J) *asdI,* K) *asdI-II* and L) *yigL* fused to *sfgfp* reporter gene in the *rne701* mutant strain. The plots with slopes =1 indicate lack of SgrS regulation, <1 indicate repression and >1 indicate activation by SgrS.