Supplemental Tables and Figures.

Functional analyses of the RsmY and RsmZ small non-coding regulatory RNAs in *Pseudomonas aeruginosa*

Janssen et al.

Table S1. Strain and plasmids used in this study

Strains	Relevant characteristics	Source
E. coli		
DH5a	*supE44* D*lacU169* (f80 *lacZ*D*M15*) *hsdR17 recA1 endA1 gyrA96 thi-1relA1*	Hanahan
SM10	*thi thr leu tonA lacY supE recA*::RP4-2Tc::Mu Km	Simon et al
P. aeruginosa		
PA103		
PA103 Δ*rsmYZ*		Intile et al
PA103 Δ*rsmA*		Marden et al
PA103 Δ*rsmF*		Marden et al
PA103 Δ*rsmAF*		Marden et al
PA103 Δ*rsmAYZ*		Marden et al
PA103 Δ*rsmFYZ*		This study
PA103 Δ*rsmAFYZ*		This study
Plasmids		
pEXG2	cloning vector for allelic exchange	Rietsch et al
pEXG2 Δ*rsmF*	cloning vector for allelic exchange ΔrsmF	Marden et al
pJN105	Arabinose inducible expression vector	Newman and Fuqua
pminiCTX	Cloning vectore for chromosomal integration at CTX site	Hoang et al
pminiCTX-$P_{tssA1'-'lacZ}$	mini-CTX with $P_{tssA1'-'lacZ}$ fusion	Marden et al
pRsmA (p2UY83)	RsmA expression vector, pJN105 backbone	Marden et al
pRsmF (p2UY37)	RsmF expression vector, pJN105 backbone	Marden et al
pRsmY	RsmY expression vector, pJN105 backbone	Intile et al
pRsmY GGA2	pRsmY bearing GGA2 substitution	This study
pRsmY GGA5	pRsmY bearing GGA5 substitution	This study
pRsmY GGA7	pRsmY bearing GGA7 substitution	This study
pRsmY GGA25	pRsmY bearing GGA25 substitutions	This study
pRsmY GGA57	pRsmY bearing GGA57 substitutions	This study
pRsmY GGA27	pRsmY bearing GGA27 substitutions	This study
pRsmY GGA257	pRsmY bearing GGA257 substitutions	This study
pRsmZ (p3UY22)	pRsmZ expression vector, pJN105 backbone	Intile et al
pRsmZ GGA1	pRsmZ bearing GGA1 substitution	This study
pRsmZ GGA2	pRsmZ bearing GGA2 substitution	This study
pRsmZ GGA5	pRsmZ bearing GGA5 substitution	This study
pRsmZ GGA6	pRsmZ bearing GGA6 substitution	This study
pRsmZ GGA25	pRsmZ bearing GGA25 substitutions	This study
pRsmZ GGA56	pRsmZ bearing GGA56 substitutions	This study
pRsmZ GGA26	pRsmZ bearing GGA26 substitutions	This study
pRsmZ GGA256	pRsmZ bearing GGA256 substitutions	This study
pRsmZ GGA1256	pRsmZ bearing GGA1256 substitutions	This study

Table S2. Plasmid and probe construction details.

Plasmid	Primers pairs used to generate PCR product(s)		Template
pRsmY GGA2	118488206-119108926	118351239-119108925	pRsmY
pRsmY GGA5	118488206-119108928	118351239-119108927	pRsmY
pRsmY GGA7	118488206-118488188	118351239-118488187	pRsmY
pRsmY GGA25	118488206-119108926	118351239-119108925	pRsmY GGA5
pRsmY GGA57	118488206-119554393	118351239-119554392	pRsmY GGA5
pRsmY GGA27	118488206-119108926	118351239-119108925	pRsmY GGA7
pRsmY GGA257	118488206-119108926	118351239-119108925	pRsmY GGA57
pRsmZ GGA1	118488206-126681303	118351239-126681302	pRsmZ
pRsmZ GGA2	118488206-118351233	118351239-118351232	pRsmZ
pRsmZ GGA5	118488206-118351235	118351239-118351234	pRsmZ
pRsmZ GGA6	118488206-118351237	118351239-118351236	pRsmZ
pRsmZ GGA25	118488206-118759540	118351239-118618111	pRsmZ GGA2
pRsmZ GGA56	118488206-118759539	118351239-118656985	pRsmZ GGA6
pRsmZ GGA26	118488206-118351237	118351239-118351236	pRsmZ GGA2
pRsmZ GGA256	118488206-119108996	118351239-119108995	pRsmZ GGA56
pRsmZ GGA1256	118488206-126681305	118351239-126681304	pRsmZ GGA256

Probe	Primers pairs used to generate PCR product(s)	Template
RsmY	59928377-59928380	pRsmY
RsmY GGA2	59928377-59928380	pRsmY GGA2
RsmY GGA5	59928377-59928380	pRsmY GGA5
RsmY GGA7	59928377-59928380	pRsmY GGA7
RsmY GGA25	59928377-59928380	pRsmY GGA25
RsmY GGA57	59928377-59928380	pRsmY GGA57
RsmY GGA27	59928377-59928380	pRsmY GGA27
RsmY GGA257	59928377-59928380	pRsmY GGA257
RsmY GGA134	130634797-130634798	130851998
RsmY GGA 123457	130634797-130634798	130629833
RsmZ	47607884-47607885	pRsmZ
RsmZ GGA1	130520981-47607884	pRsmZ GGA1
RsmZ GGA2	47607884-47607885	pRsmZ GGA2
RsmZ GGA5	47607884-47607885	pRsmZ GGA5
RsmZ GGA6	47607884-47607885	pRsmZ GGA6
RsmZ GGA15	130520981-47607884	pRsmZ GGA15
RsmZ GGA25	47607884-47607885	pRsmZ GGA25
RsmZ GGA56	47607884-47607885	pRsmZ GGA56
RsmZ GGA26	47607884-47607885	pRsmZ GGA26
RsmZ GGA256	47607884-47607885	pRsmZ GGA256
RsmZ GGA1256	130520981-47607884	pRsmZGGA1256
RsmZ GGA147	130520981-47607884	130269402
RsmZ GGA124567	130520981-47607884	130269401

Table S3. Primers and gBlocks used in this study.

Primers

ID	Name	Sequence
117830775	pBAD Gibson Mlu	5'- CCAAAGCCATGACAAAAACGCGTAAC
117830776	pBAD Gibson	5'- ATGGAGAAACAGTAGAGAGTTGCGATAAA
118351232	RsmZ GAA1 back	5'- GTACAGGGAACACGCAACCCCGAACCTTC GGGGAAGGGACGTCGCCAGGG
118351233	RsmZ GAA1 front	5'- CCCTGGCGACGTCCCTTCCCCGAAGGTTC GGGGTTGCGTGTTCCCTGTAC
118351234	RsmZ GGA2 back	5'- GATCGGGGAAGGGACGTCGCCAGCCTGGC GATTCATCAGGATGATGACGAGG
118351235	RsmZ GAA2 front	5'- CCTCGTCATCATCCTGATGAATCGCCAGGC TGGCGACGTCCCTTCCCCGATC
118351236	RsmZ GAA3 back	5'- GTCGCCAGGGAGGCGATTCATCACCTTGA TGACGAGGGACTGAAGAGTGGG
118351237	RsmZ GAA3 front	5'- CCCACTCTTCAGTCCCTCGTCATCAAGGT GATGAATCGCCTCCCTGGCGAC
118351239	pJN PvuI Gibson	5'- GCAACTGTTGGGAAGGGCGATCGG
118488187	RsmY GGA3 back	5'- ATGGTGGCGTAGCACGGATGTCACCTTAG AGGTCTGCAAAACCCCGCCCA
118488188	RsmY GGA3 front	5'- TGGGCGGGGTTTTGCAGACCTCTAAGGTG ACATCCGTGCTACGCCACCAT
118488206	pJN Nru Gibson	5'- CCCACTGGTGATACCATTCGCGAGCC
118618111	RsmZ GGA1 + GGA2	5'- GTACAGGGAACACGCAACCCCGAACCTTC GGGGAAGGGACGTCGCCAGCC
118759539	RsmZ GGA2 to 3 front	5'- CCCACTCTTCAGTCCCTCGTCATCAAGGT GATGAATCGCCAGGCTGGCGAC
118759540	RsmZGGA(1) to 2 front	5'- GGCTGGCGACGTCCCTTCCCCGAAGGTTC GGGGTTGCGTGTTCCCTGTAC
118656985	RsmZ GGA2 to 3(back)	5'- GTCGCCAGCCTGGCGATTCATCACCTTGAT GACGAGGGACTGAAGAGTGGG
119108925	RsmY GGA1 back PA103	5'- TTTCTCCATGTCAGGACATTGCGCACCTA GCGCCAAAGACAATACGGAAACCAA
119108926	RsmY GGA1 front PA103	5'- TTGGTTTCCGTATTGTCTTTGGCGCTAGG TGCGCAATGTCCTGACATGGAGAAA
119108927	RsmY GGA2 back PA103	5'- TACGGAAACCAAGGGAATCCACCATCCTT GGTGGCGTAGCACGGATGTCAGG
119108928	RsmY GGA2 front PA103	5'- CCTGACATCCGTGCTACGCCACCAAGGA TGGTGGATTCCCTTGGTTTCCGTA
119108995	RsmZ final GGAs back	5'- CCTTCGGGGAAGGGACGTCGCCAG
119108996	RsmZ final GGAs front	5'- CTGGCGACGTCCCTTCCCCGAAGG
119554392	PA103 RsmY GGA3 into GGA2 bac	5'- TTGGTGGCGTAGCACGGATGTCACCTTAG AGGTCTGCAAAACCCCGCCCA
119554393	PA103 RsmY GGA3 into GGA2 fron	5'- TGGGCGGGGTTTTGCAGACCTCTAAGGTG ACATCCGTGCTACGCCACCAA
121115383	rsmA Gibson 5'	5'- GCCATGGTACCCGGGGATCCAAGGAGATA TACCATGCTGATTCTGACTCGTCGGGTC

Table S3. Primers and gBlocks used in this study (cont.).

Primers

ID	Name	Sequence
122501196	rsmZ_forward	5'- GTACAGGGAACACGCAACCC
124280319	rsmZ_reverse	5'- CACTCTTCAGTCCCTCGTCATCA
122503154	rsmY_forward	5'- GCGCCAAAGACAATACGGAAAC
122503155	rsmY_reverse	5'- CGGGGTTTTGCAGACCTCTA
123193558	RimM_Forward	5- ATCACGCCGAGCAACTG
123193559	RimM_Reverse	5'- CGGTTACGAGATCTGCATCC
126681302	RsmZ GGA0 back	5'- CTCTCTACTGTTTCTCCATCGTACAGCCT ACACGCAACCCCGAAGGATCGGG
126681303	RsmZ GGA0 front	5'- CCCGATCCTTCGGGGTTGCGTGTAGGCTG TACGATGGAGAAACAGTAGAGAG
126681304	RsmZ quad back	5'- CTCTCTACTGTTTCTCCATCGTACAGCCT ACACGCAACCCCGAACCTTCGGG
126681305	RsmZ quad front	5'- CCCGAAGGTTCGGGGTTGCGTGTAGGCTG TACGATGGAGAAACAGTAGAGAG
127879425	RsmZ GGA0_Forward	5'- GTACAGCCTACACGCAACCC
130520981	RsmZ_gblock_mutant _T7_Forward	5'- TAATACGACTCACTATAGCGTACAGC
130634797	RsmYgBlockmutantF	5'- TAATACGACTCACTATAGGTCACCT
130634798	RsmYgBlockmutantR	5'- AAAACCCCGCCTTTTGGGCGGGG

SHAPE-MaP and Illumina Sequencing primers

ID	Name	Sequence
NA	Rnd1Fwd	5'- CCCTACACGACGCTCTTCCGATCTNNNNN GGCCTTCGGG CCAA
NA	Rnd1Rev	5'- GACTGGAGTTCAGACGTGTGCTCTTCCGATCT NNNNNGA ACCGGACCGAAGCCCG
NA	Rnd2Fwd	5'- AATGATACGGCGACCACCGAGATCTACACT CTTTCCCTA CACGACGCTCTTCCG
NA	Rnd2Rev	5'-CAAGCAGAAGACGGCATACGAGATXXXXXXXX GACTGG AGTTCAGAC where X represents a unique barcode

gBlocks

ID	Name	Sequence
130269401	RsmZ_GGA124567	5'- TAATACGACTCACTATAGCGTACAGCCTA CACGCAACCCCGAACCTTCGGGGAAGCCT CGTCGCCAGCCTGGCGATTCATCACCTTGA TGACGAGCCTCTGAAGAGTGGGCGGGGTA ATACCCCGCCCCTTTTTT
130269402	RsmZ_GGA147	5'- TAATACGACTCACTATAGCGTACAGCCTACA CGCAACCCCGAAGGATCGGGGAAGCCTCG TCGCCAGGGAGGCGATTCATCAGGATGATG ACGAGCCTCTGA AGAGTGGGCGGGGTAAT ACCCCGCCCCTTTTTT

Table S3. Primers and gBlocks used in this study (cont.).

gBlocks

ID	Name	Sequence
130629833	RsmY_GGA123457	5'- TATTTCCTCAAGTGACGGGATGCCGCTAT TGAGTATGCATAATACGACTCACTATAGG TCACCTCATTGCGCACCTAGCGCCAAAGA CAATACCCTAACCAAGCCTATCCACCATCC TTGGTGGCGTAGCACGGATGTCACCTTAG AGGTCTGCAAAACCCCGCCCAAAAGGCGG GGTTTTCTTCTCAACTTTCAACTCTTTCGCC AACGGAACTGGAGTCGTTAAGC
130851998	RsmY_GGA134	5'- TATTTCCTCAAGTGACGGGATGCCGCTAT TGAGTATGCATAATACGACTCACTATAGG TCACCTCATTGCGCAGGAAGCGCCAAAG ACAATACCCTAACCAAGCCTATCCACCAT GGATGGTGGCGTAGCACGGATGTCAGGA TAGAGGTCTGCAAAACCCCGCCCAAAAGG CGGGGTTTTCTTCTCAACTTTCAACTCTTT CGCCAACGGAACTGGAGTCGTTAAGC
NA	RsmY_SHAPE_gBlock	5'- TAATACGACTCACTATAGGGGGCCTTCGG GCCAAGTCAGGACATTGCGCAGGAAGCG CCAAAGACAATACGGAAACCAAGGGAATC CACCATGGATGGTGGCGTAGCACGGATGT CAGGATAGAGGTCTGCAAAACCCCGCCCA AAAGGCGGGGTTTTTCGATCCGGTTCGCC GGATCCAAATCGGGCTTCGGTCCGGTTC
NA	RsmZ_SHAPE_gBlock	5'- TAATACGACTCACTATAGGGGGCCTTCGG GCCAACGTACAGGGAACACGCAACCCCG AAGGATCGGGGAAGGGACGTCGCCAGGG AGGCGATTCATCAGGATGATGACGAGGG ACTGAAGAGTGGGCGGGGTAATACCCCG CCCCTTTTTTCGATCCGGTTCGCCGGATC CAAATCGGGCTTCGGTCCGGTTC

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	RsmA/F consensus	CAnGGAyG	
RsmY	GGA site 1	CAgGGAuC	4/8
	GGA site 2	GAaGGAuC	5/8
	GGA site 3	UAcGGAaA	4/8
	GGA site 4	CAgGGAaU	4/8
	GGA site 5	CAuGGAuG	7/8
	GGA site 6	CAcGGAuG	7/8
	GGA site 7	UCaGGAuA	4/8

	RsmA/F consensus	CAnGGAyG	
RsmZ	GGA site 1	UCaGGAcA	4/8
	GGA site 2	CGcGGAaG	5/8
	GGA site 3	UCgGGAaG	4/8
	GGA site 4	AAgGGAaC	4/8
	GGA site 5	CAgGGAgG	6/8
	GGA site 6	UCaGGAuG	5/8
	GGA site 7	CGaGGAcU	5/8

Figure S1. Comparison of each GGA sequence in RsmY and RsmZ to the full 8 nt consensus binding sites for RsmA and RsmF. The GGA sequence is highlighted in red typeface and the nt positions that match consensus at the remaining positions (non-GGA) are highlighted in blue typeface.

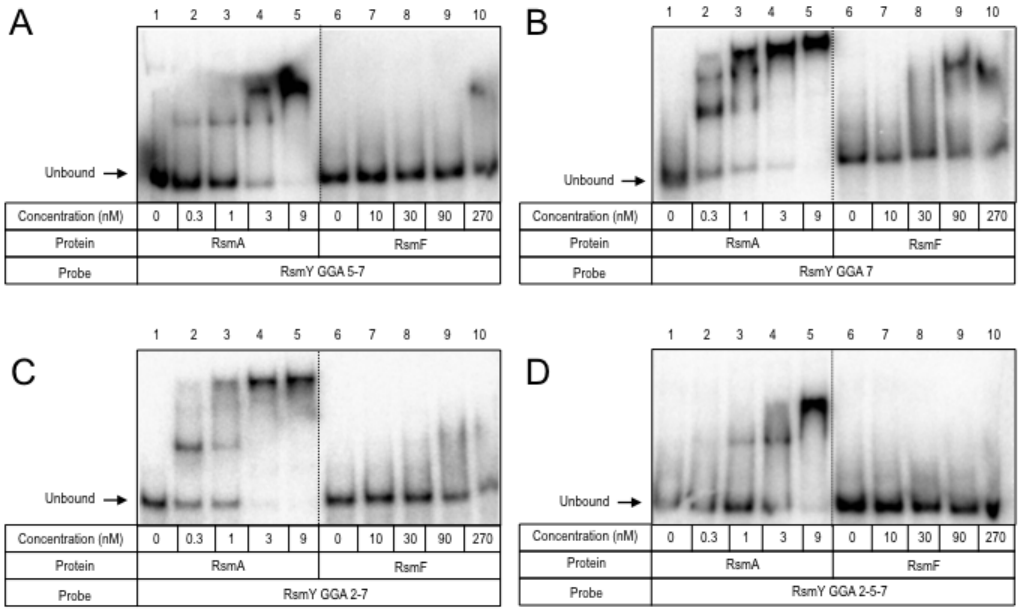

Figure S2. RsmA and RsmF binding to RsmY GGA single and double mutants. RsmY mutant RNA was radiolabeled and used in electrophoretic mobility shift assays with RsmA (lanes 1-5) and RsmF (lanes 6-10) Unbound RNA is indicated by an arrow.

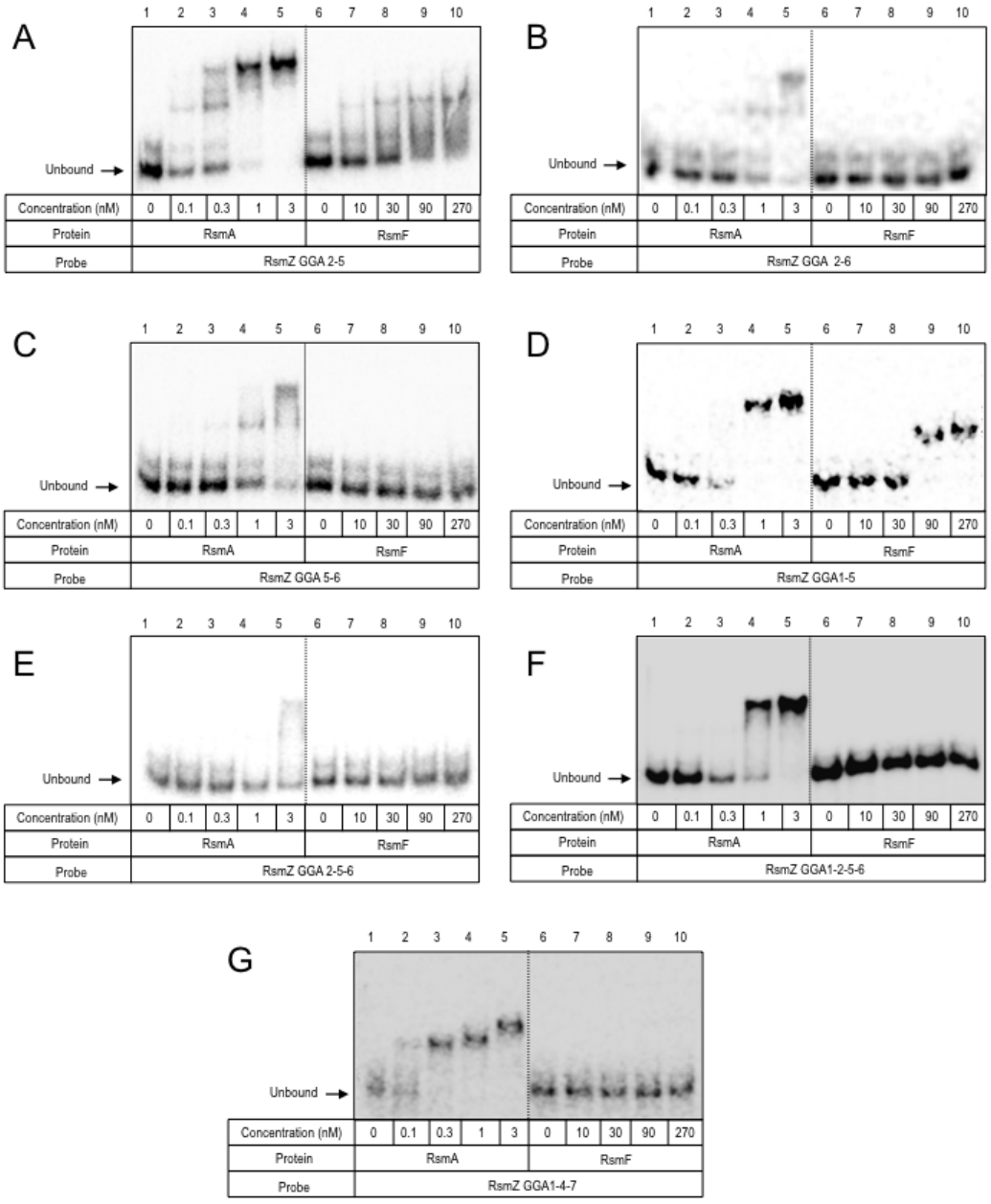

Figure S3. RsmA and RsmF binding to RsmZ GGA single, double, and triple mutants. RsmZ mutant RNA was radiolabeled and used in electrophoretic mobility shift assays with RsmA (lanes 1-5) and RsmF (lanes 6-10). Unbound RNA is indicated by an arrow.

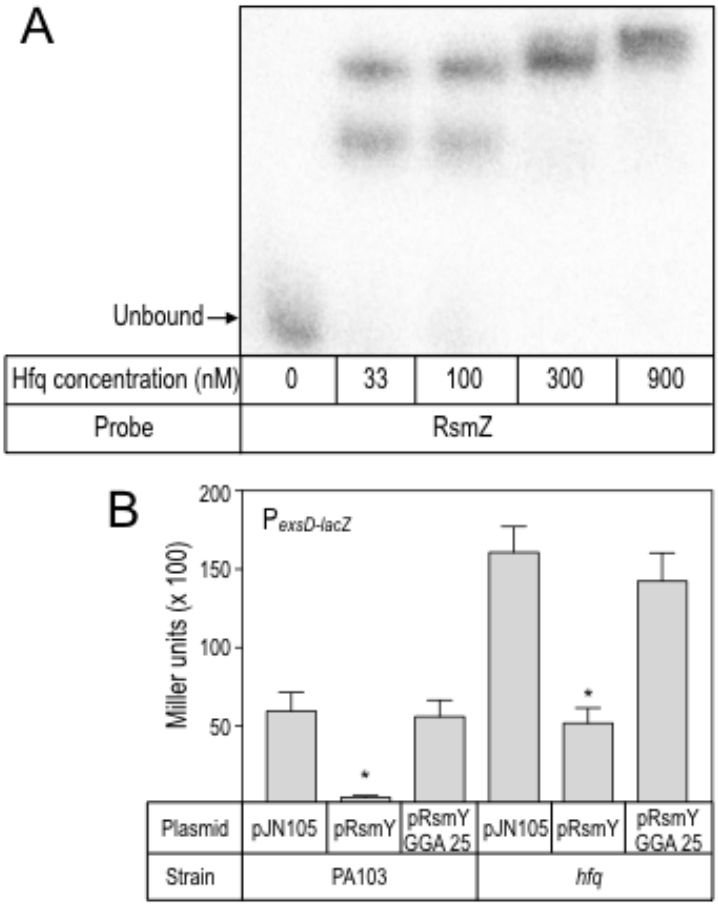

Figure S4. A) Hfq binding to RsmZ. RsmZ RNA was radiolabeled and used in electrophoretic mobility shift assays. B) PA103 or the Δ*hfq* mutant carrying the $P_{exsD\text{-}lacZ}$ transcriptional reporter were transformed with either vector control, wild type RsmY, or the RsmY GGA2 expression plasmids. The resulting strains were cultured in the presence of 0.1% arabinose to induce expression of the respective RNAs and assayed for ß-galactosidase activity. Reported values represent the average of two experiments with the standard error indicated. P-value <0.05 when compared to expression of the wt vector is indicated (*).