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Supplementary Material

Accessibility and conservation - general features of bacterial small RNA-mRNA interactions?

Andreas S. Richter and Rolf Backofen

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Supplementary Tables

Table S1: Dataset of interactions in *E. coli* used in this study. Target interaction site positions are given as distance to the annotated translation start site (TLS) and to the transcription start site (TSS) used in this study. Genes that are located within an operon are indicated. Interactions are given in bracket notation, where the '&' symbol concatenates the sRNA with its target, matching brackets represent base pairs between the two sequences and dots represent unpaired positions. 'Compens. mutations' indicates that the sRNA-target interaction was validated by the introduction of compensatory mutations in sRNA and target.

sRNA	Target	sRNA site	Target site [TLS]	[TSS]	Interaction	Validation	Ref.
ArcZ	*rpoS*	66 – 91	-120 – -99	/ 449 – 470	`(((((((..(((.((((.(((.((((&)))))))))))))).)))))))`	compens. mutations	20
ChiX	*chbC*	38 – 58	-69 – -49	/ [operon]	`(((((((..((((((((((((&))))))))))))..)))))))`	sRNA mutations	22
ChiX	*chiP*	45 – 56	-19 – -8	/ 52 – 63	`((((((((((((&))))))))))))`	compens. mutations	31
ChiX	*dpiB*	46 – 57	-37 – -26	/ 27 – 38	`((((((((((((&))))))))))))`	compens. mutations	19
CyaR	*luxS*	35 – 49	-12 – 3	/ 134 – 148	`(....(.((((((((&)))))))).)....)`	compens. mutations	8
CyaR	*nadE*	35 – 49	-11 – 3	/ 20 – 33	`(...(((((.(((((&))))))))))...)`	compens. mutations	8
CyaR	*ompX*	38 – 48	-9 – 2	/ 231 – 241	`(.(((((((((&))))))))).)`	compens. mutations	8
CyaR	*yqaE*	31 – 50	-4 – 16	/ 54 – 73	`(.(.(((((((((......(&)......))))))))).).)`	compens. mutations	8
DsrA	*hns*	31 – 43	7 – 19	/ 43 – 55	`(((((((((((((&)))))))))))))`	compens. mutations	16
DsrA	*rpoS*	8 – 32	-119 – -95	/ 450 – 474	`(.(((((((..((((((((((((((&))))))))))))))..))))))).)`	compens. mutations	17
FnrS	*folE*	1 – 12	-27 – -15	/ 121 – 133	`(((((((.((((&))))..)))))))`	sRNA mutations	10
FnrS	*folX*	1 – 6	-7 – -2	/ 18 – 23	`((((((&))))))`	sRNA mutations	10
FnrS	*gpmA*	38 – 57	-13 – 4	/ 75 – 91	`((((((...(((((.(((((&)))))))))).))))))`	compens. mutations	10
FnrS	*maeA*	31 – 65	-21 – 10	/ 13 – 43	`(((.(.(((((.(((.((((((..(((((((((((&` `))))))))))))))))))))).))))).))))`	compens. mutations	10
FnrS	*sodB*	1 – 8	13 – 20	/ 66 – 73	`((((((((&))))))))`	compens. mutations	10
GcvB	*sstT*	64 – 99	-34 – 2	/ 50 – 85	`(((.(...(((.(.((((((((((......((.(((&` `))).))......)))))))))).).)))...).)))`	compens. mutations	30
GlmZ	*glmS*	150 – 169	-40 – -22	/ 118 – 136	`((((((((.....(((((((&)))))))....))))))))`	compens. mutations	38
MicA	*ompA*	8 – 24	-21 – -6	/ 153 – 168	`((((.((((((((((((&))))))))))))))))`	compens. mutations	37
MicA	*phoP*	6 – 31	-15 – 8	/ 30 – 52	`((((((((((.((((..((.((((((&)))))))).))))))))))))))`	compens. mutations	7
MicC	*ompC*	1 – 30	-41 – -15	/ 47 – 73	`((((((((((((((((........((((((&))))))).....))))))))))))))))`	compens. mutations	6

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sRNA	Target	sRNA site	Target site [TLS]	[TSS]	Interaction	Validation	Ref.
MicF	*ompF*	1 – 33	-16 – 10	/ 95 – 120	`(((.((((((((......((((((((((.((((&)))).)))))))))))))))))))))`	*in vitro* probing	34
OmrA	*cirA*	2 – 24	-35 – -10	/ 126 – 151	`((((((((((((...((((((((&))))))))....))).)))))).)))`	compens. mutations	13
OmrA	*csgD*	2 – 20	-79 – -61	/ 69 – 87	`((((((((((((((.((((&)))).))))))))))))))`	compens. mutations	14
OmrA	*ompR*	1 – 19	-29 – -11	/ 134 – 152	`(((((((((.(..(.((((&)))).)..).)))))))))`	compens. mutations	13
OmrA	*ompT*	1 – 33	-12 – 20	/ 20 – 51	`(((((((((.(((.(((((((((...(((..((&))..))).)))))).))).))).)))))))))`	compens. mutations	13
OmrB	*cirA*	2 – 24	-35 – -10	/ 126 – 151	`((((((((((((..(((((((((&)))))))))...))).)))))).)))`	compens. mutations	13
OmrB	*csgD*	2 – 20	-79 – -61	/ 69 – 87	`(((((((((((((((((((&)))))))))))))))))))`	compens. mutations	14
OmrB	*ompR*	1 – 19	-29 – -11	/ 134 – 152	`(((((((((.(..(..(((&)))..)..).)))))))))`	compens. mutations	13
OmrB	*ompT*	1 – 32	-12 – 20	/ 20 – 51	`(((((((((.(((.(.(((((((....(((((&)))))...)))))).).).))).)))))))))`	compens. mutations	13
OxyS	*fhlA*	22 – 30	34 – 42	/ 78 – 86	`(((((((((&)))))))))`	compens. mutations	1
		98 – 104	-15 – -9	/ 30 – 36	`(((((((&)))))))`		
RprA	*rpoS*	33 – 62	-117 – -94	/ 452 – 475	`(((((..((((((.........((((((((&))))))))...))))))..)))))`	compens. mutations	18
RyhB	*cysE*	34 – 46	-4 – 9	/ 116 – 128	`(((((((((((((&)))))))))))))`	compens. mutations	33
RyhB	*fur*	38 – 76	-96 – -47	/ 84 – 133	`((((((((((..(.(((((..((.(((.....(((((((&))).))))))).))..))......))).).......))))))....))))`	compens. mutations	39
RyhB	*iscS*	40 – 68	-26 – 3	/ [operon]	`((((((((..............(((((((&)))))))..............))))))))`	*in vitro* probing	9
RyhB	*shiA*	44 – 55	-59 – -48	/ 19 – 30	`((((((((((((&))))))))))))`	compens. mutations	28
RyhB	*sodB*	38 – 46	-4 – 5	/ 50 – 58	`(((((((((&)))))))))`	*in vitro* probing	12
SgrS	*manX*	159 – 172	24 – 37	/ 137 – 150	`((..((((((((((&))))))))))..))`	compens. mutations	32
SgrS	*ptsG*	168 – 187	-28 – -9	/ 213 – 232	`((((..((((((((.(((((&))))).))))))))..))))`	compens. mutations, *in vitro* probing	15,32
Spot42	*galK*	20 – 61	-19 – 21	/ [operon]	`((((.(.((((((((((.((((.....((((..(((((((((&))))))))).))))..))))...)))))))))).).))))`	*in vitro* probing	21
Spot42	*gltA*	4 – 13	-131 – -122	/ 310 – 319	`((((((((((&))))))))))`	sRNA mutations	3
Spot42	*nanC*	1 – 17	-33 – -16	/ 395 – 412	`(((((((((((((((((&)))).)))))))))))))`	compens. mutations	3
Spot42	*srlA*	20 – 34	-15 – -1	/ 90 – 104	`((((((.((((((((&)))))))).))))))`	compens. mutations	3
Spot42	*sthA*	48 – 55	15 – 22	/ 47 – 54	`((((((((&))))))))`	compens. mutations	3

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sRNA	Target	sRNA site	Target site			Interaction	Validation	Ref.
			[TLS]		[TSS]			
Spot42	*xylF*	1 – 33	2 – 40	/	64 – 102	`((.((((.((((((((.((((((((...(((((&` `)))))....)))))))).)).....)))))).)))).))`	sRNA mutations	[3]

Table S2: Dataset of interactions in *Salmonella* used in this study. Target interaction site positions are given as distance to the annotated translation start site (TLS) and to the transcription start site (TSS) used in this study. Genes that are located within an operon are indicated. Interactions are given in bracket notation, where the '&' symbol concatenates the sRNA with its target, matching brackets represent base pairs between the two sequences and dots represent unpaired positions. 'Compens. mutations' indicates that the sRNA-target interaction was validated by the introduction of compensatory mutations in sRNA and target.

sRNA	Target	sRNA site	Target site [TLS] / [TSS]	Interaction	Validation	Ref.
ArcZ	*sdaC*	62 – 71	-13 – -3 / 67 – 77	((((((((((&))))).)))))	compens. mutations	25
ArcZ	STM3216	63 – 87	-25 – -5 / 67 – 87	(((((((((.((((.....((((((&)))))).)))).)))))))))	compens. mutations	25
ArcZ	*tpx*	66 – 83	10 – 26 / 44 – 60	((((((.......(((((&)))))......))))))	compens. mutations	25
ChiX	*chbC*	35 – 55	-66 – -46 / [operon]	(((((((..((((((((((((&))))))))))))..)))))))	mRNA mutations	11
ChiX	*chiP*	42 – 53	-19 – -8 / 52 – 63	((((((((((((&))))))))))))	compens. mutations	11
CyaR	*ompX*	35 – 66	-30 – 3 / 210 – 242	((..(((((((((..((((....((((..(((&))).)))).....)).))...))))))))).))	compens. mutations compens. mutations	24 24
GcvB	*argT*	75 – 91	-57 – -42 / 90 – 105	(((((((.(((((((((&))))))))))))))))	*in vitro* probing	35
GcvB	*cycA*	72 – 85	-34 – -19 / 51 – 66	((((((((((((.(&).)).)))))))).))	*in vitro* probing	36
		138 – 161	-24 – -8 / 61 – 77	((((((((.......(((((((((&)))))))))))))))))		
GcvB	*dppA*	65 – 82	-30 – -14 / 136 – 152	((((((((.(((((((((&)))))))))))))))))	*in vitro* probing	35
GcvB	*gltI*	65 – 76	-38 – -27 / 70 – 81	((((((((((((&))))))))))))	*in vitro* probing	35
GcvB	*livJ*	63 – 87	-51 – -28 / 54 – 77	((((((.((((.(((((((.(((((&))))).))))))))))).))))))	*in vitro* probing	35
GcvB	*livK*	65 – 77	-29 – -17 / 370 – 382	(((((((((((((&)))))))))))))	*in vitro* probing	35
GcvB	*oppA*	65 – 89	-8 – 16 / 505 – 528	(((((((((((...(((((((((((&)))))))))))..)))))))))))	*in vitro* probing	35
GcvB	STM4351	69 – 79	-54 – -43 / 12 – 23	(((((((((((&)).)))))))))	*in vitro* probing	35
InvR	*ompD*	33 – 42	56 – 65 / 122 – 131	((((((((((&))))))))))	*in vitro* probing	27
MicA	*lamB*	8 – 36	-9 – 18 / [operon]	(((((...((.((((((((..((((((((&)))))))).)))))))).))..)))))	compens. mutations	4
MicC	*ompD*	1 – 12	67 – 78 / 133 – 144	((((((((((((&))))))))))))	compens. mutations	26
RybB	*chiP*	1 – 7	12 – 18 / 82 – 88	(((((((&)))))))	compens. mutations	2
RybB	*fadL*	1 – 8	49 – 56 / 150 – 157	((((((((&))))))))	compens. mutations	23
RybB	*ompA*	1 – 13	21 – 32 / 194 – 205	((((((((...((&))..))))))))	compens. mutations	23
RybB	*ompC*	1 – 10	-50 – -41 / 38 – 47	((((((((((&))))))))))	*in vitro* probing	2, 23
RybB	*ompD*	1 – 9	18 – 26 / 84 – 92	(((((((((&)))))))))	compens. mutations	2, 23
		1 – 10	10 – 20 / 76 – 86	((((((((((&))).)))))))		

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sRNA	Target	sRNA site	Target site		Interaction	Validation	Ref.
			[TLS]	[TSS]			
RybB	*ompF*	1 – 9	-46 – -38 /	67 – 75	`(((((((((&)))))))))`	compens. mutations	[23]
RybB	*ompN*	1 – 16	5 – 20 /	80 – 95	`((((.(((((((((((&))))))))))).))))`	compens. mutations	[5]
RybB	*ompS*	1 – 14	7 – 20 /	73 – 86	`((((..((((((((&))))))))..))))`	sRNA deletion mutant	[23]
RybB	*ompW*	1 – 13	3 – 20 /	32 – 49	`(((((((.(((((&)))))......)))))))`	compens. mutations	[23]
RybB	*tsx*	1 – 16	-26 – -7 /	86 – 105	`(((((((...((((((&))))))).......)))))))`	compens. mutations	[23]

Table S3: Dataset of non-functional interactions used in this study. Interaction site positions in the mRNA are given as distance to the annotated translation start site. Interactions are given in bracket notation, where the '&' symbol concatenates the sRNA with its target, matching brackets represent base pairs between the two sequences and dots represent unpaired positions. The last column gives for each non-functional interaction the true target of corresponding verified interaction.

Organism	sRNA	mRNA	sRNA site	mRNA site	Predicted non-functional interaction	True target
E. coli	ArcZ	*ypdB*	26 – 51	-133 – -112	(((((((....(((.(((((((((((&)))).)))))))))))))))))	*rpoS*
E. coli	ChiX	*ltaE*	4 – 24	-371 – -351	(((((((((((((..((((((&))))).)))))).))))))))	*chbC*
E. coli	ChiX	*ybaK*	12 – 23	-144 – -133	((((((((((((&))))))))))))	*chiP*
E. coli	ChiX	*ybaK*	2 – 13	-134 – -123	((((((((((((&))))))))))))	*dpiB*
E. coli	CyaR	*hofO*	69 – 83	-182 – -168	(....(.((((((((&)))))))).)....)	*luxS*
E. coli	CyaR	*livF*	66 – 80	-127 – -114	(((...(((.(((((&))))).)))..)))	*nadE*
E. coli	CyaR	*pabC*	60 – 79	-7 – 13	((((......((.((.((((&)))).)).))......))))	*yqaE*
E. coli	CyaR	*rlpA*	49 – 59	-256 – -246	(.(((((((((&))))))))).)	*ompX*
E. coli	DsrA	*barA*	73 – 85	34 – 46	(((((((((((((&)))))))))))))	*hns*
E. coli	DsrA	*ykgE*	56 – 83	-58 – -27	((((...(((((((((((((.((..(((&)))))..)))))))).....)))))...))))	*rpoS*
E. coli	FnrS	*lspA*	110 – 115	-132 – -127	((((((&))))))	*folX*
E. coli	FnrS	*rmuC*	86 – 105	-12 – 5	((.(((((((((...(((((&))))).)))))))))))	*gpmA*
E. coli	FnrS	*rsmH*	15 – 22	132 – 139	((((((((&))))))))	*sodB*
E. coli	FnrS	*ubiE*	27 – 38	-60 – -48	(((((((.((((&))))..)))))))	*folE*
E. coli	FnrS	*yaeI*	77 – 116	-115 – -73	(.....(.(((((..((.((((((((((.((.((((((((&))))))))...........)).)))))))))).)).)))))))	*maeA*
E. coli	GcvB	*yfeY*	6 – 41	-44 – -9	((.(..(((((((....((((....(((.((((.((&)).)))).)))))))..)))).)))..)......))	*sstT*
E. coli	GlmZ	*gabT*	65 – 84	-174 – -156	((((...(((((((..((((&)))))))))))....))))	*glmS*
E. coli	MicA	*aceF*	45 – 61	-380 – -365	(((((((((((.(((((&))))))))))))))))	*ompA*
E. coli	MicA	*tauC*	36 – 68	-146 – -113	(((((.(((......((((.(((.((..(((((&)))))..))...))).)))).....))).)))))	*phoP*
E. coli	MicC	*yiiD*	41 – 70	-50 – -24	(((((..((((((((.((((...((..(((&)))..)).)))).))).))))))))))	*ompC*
E. coli	MicF	*ileS*	56 – 90	-353 – -316	((((..(((.(((((..((((((((..((((...(&)...)))).)))).....))))....))))))))))))	*ompF*
E. coli	OmrA	*gspM*	43 – 75	-41 – -10	(.((((((((..(((((((..((((.(((((.(&))))))).)))))))))).))))))...)).)	*ompT*
E. coli	OmrA	*insI*	27 – 45	-18 – 1	(((((...((.((((((((&)).))))))))...)))))	*ompR*
E. coli	OmrA	*marA*	25 – 47	-70 – -45	((((((((..(((((((((.(((&).)))))))).)))...)).))))))	*cirA*

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Organism	sRNA	mRNA	sRNA site	mRNA site	Predicted non-functional interaction	True target
E. coli	OmrA	*ygcO*	64 – 82	-41 – -23	`((((.((((((((((((((&)))))))).))))))))))`	*csgD*
E. coli	OmrB	*hofC*	36 – 54	-59 – -41	`(((((.(.((..((((.((&))..))))..)).))))))`	*ompR*
E. coli	OmrB	*mcbA*	31 – 63	-56 – -23	`(((((.(.((...(((.........((((((((&))))))))............))).)).).)))))`	*csgD*
E. coli	OmrB	*pyrB*	35 – 57	-146 – -121	`((((((((((((((((((((..(&).)).))))..))))).)))))))))`	*cirA*
E. coli	OmrB	*yfdQ*	35 – 66	-85 – -54	`(((((...((((((((.(((.(.((((((.((&)).))))))).)))...)))).)))).)))))`	*ompT*
E. coli	OxyS	*apaH*	7 – 13	-30 – -24	`(((((((&)))))))`	*fhlA*
E. coli	OxyS	*caiT*	73 – 81	76 – 84	`(((((((((&)))))))))`	*fhlA*
E. coli	RprA	*paaC*	67 – 96	-37 – -14	`(((((..((..((.(((((......(((((&)))))...)))))))..)))))))`	*rpoS*
E. coli	RyhB	*sdhC*	48 – 60	-161 – -149	`(((((((((((((&)))))))))))))`	*cysE*
E. coli	RyhB	*yagV*	77 – 85	-397 – -389	`(((((((((&)))))))))`	*sodB*
E. coli	RyhB	*yeaW*	3 – 37	-131 – -75	`(.(((((..((((.(((((((((((.(((.(((.(&` `))))..))).)).........))))).)).))....))))).....)))))......)`	*fur*
E. coli	RyhB	*yicS*	1 – 29	-180 – -152	`(((....(........((((((.(.((((&)))).))))))).........)....)))`	*iscS*
E. coli	RyhB	*yigE*	77 – 88	-188 – -177	`((((((((((((&))))))))))))`	*shiA*
E. coli	SgrS	*hlyE*	66 – 79	43 – 56	`((..((((((((((&))))))))))..))`	*manX*
E. coli	SgrS	*putP*	64 – 83	-121 – -102	`((.((((((..(((((((((&)))))))))..)))))).))`	*ptsG*
E. coli	Spot42	*aat*	43 – 59	-28 – -11	`(((((((((((((((((&))))))).))))))))))`	*nanC*
E. coli	Spot42	*arnT*	80 – 89	-37 – -28	`((((((((((&))))))))))`	*gltA*
E. coli	Spot42	*cynX*	82 – 89	18 – 25	`((((((((&))))))))`	*sthA*
E. coli	Spot42	*rhlE*	65 – 106	-40 – -1	`((.(((((((((((((((..((.((....((((((..(((((&` `)))))..).))))).))))..))))))))).)))))).))`	*galK*
E. coli	Spot42	*xylH*	61 – 75	-123 – -109	`((((((.((((((((&)))))))).))))))`	*srlA*
E. coli	Spot42	*yggU*	64 – 96	15 – 53	`(((((((...(((((((((.((((.((.(((((&` `))))).)).))))))...)).....)))))..)))))))`	*xylF*
Salmonella	ArcZ	STM3651	92 – 116	-125 – -105	`((..((((((....(((((((((((&)))))))))))))))))..))`	STM3216
Salmonella	ArcZ	*pduJ*	73 – 82	-178 – -168	`((((((((((&))))).)))))`	*sdaC*
Salmonella	ArcZ	*purA*	5 – 22	108 – 124	`((((((.......(((((&)))))......))))))`	*tpx*
Salmonella	ChiX	*atpB*	4 – 24	-177 – -157	`((((((((((((..(((((((&))))))).)))))).))))))`	*chbC*
Salmonella	ChiX	*ybaK*	2 – 13	-135 – -124	`((((((((((((&))))))))))))`	*chiP*
Salmonella	CyaR	STM1787	1 – 32	-110 – -78	`((((....(((..((((...((((((.(((((&))))).)))))).).))).....)))...))))`	*ompX*

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Organism	sRNA	mRNA	sRNA site	mRNA site	Predicted non-functional interaction	True target
Salmonella	GcvB	STM1049	57 – 80	-165 – -149	`(((((((((((.......((((((&)))))))))))))))))`	*cycA*
Salmonella	GcvB	STM2598	49 – 60	-169 – -158	`((((((((((((&))))))))))))`	*gltI*
Salmonella	GcvB	STM2768	20 – 37	-142 – -126	`((((((.(((((((((((&)))))))))))))))))`	*dppA*
Salmonella	GcvB	STM4002	49 – 65	-159 – -144	`(((((((.(((((((((&))))))))))))))))`	*argT*
Salmonella	GcvB	STM4032.2N	36 – 60	-20 – 4	`(((((((((.(((.(((.(((((((&))))))).))).))))))))))))`	*livJ*
Salmonella	GcvB	STM4032.2N	37 – 61	-21 – 3	`((((((((.(((.(((.((((((((&)))))))).))).)))))))))))`	*oppA*
Salmonella	GcvB	*flgJ*	32 – 44	-139 – -127	`(((((((((((((&)))))))))))))`	*livK*
Salmonella	GcvB	*rfaJ*	49 – 62	-422 – -407	`((((((((((((.(&).)).)))))))).))`	*cycA*
Salmonella	GcvB	*sspH2*	40 – 50	-112 – -101	`(((((((((((&)).)))))))))`	STM4351
Salmonella	InvR	*dps*	50 – 59	63 – 72	`((((((((((&))))))))))`	*ompD*
Salmonella	MicA	STM0952	41 – 69	-52 – -26	`((((.(((((((((((((((.....((((&))))))).))))))))))))...))))`	*lamB*
Salmonella	MicC	*yaeH*	92 – 103	19 – 30	`((((((((((((&))))))))))))`	*ompD*
Salmonella	RybB	STM1632	32 – 40	-40 – -32	`(((((((((&)))))))))`	*ompF*
Salmonella	RybB	STM1636	30 – 45	-192 – -173	`(((((((...((((((&)))))).......)))))))`	*tsx*
Salmonella	RybB	STM3356	62 – 77	3 – 18	`((((.(((((((((((&))))))))))).))))`	*ompN*
Salmonella	RybB	*pmrF*	66 – 79	60 – 73	`((((..((((((((&))))))))..))))`	*ompS*
Salmonella	RybB	*pps*	19 – 28	69 – 79	`((((((((((&))).)))))))`	*ompD*
Salmonella	RybB	*pyrG*	44 – 56	24 – 35	`((((((((...((&))..))))))))`	*ompA*
Salmonella	RybB	*rstB*	65 – 74	-78 – -69	`((((((((((&))))))))))`	*ompC*
Salmonella	RybB	*secF*	20 – 32	131 – 148	`(((((((.(((((&)))))......)))))))`	*ompW*
Salmonella	RybB	*stfC*	36 – 42	45 – 51	`(((((((&)))))))`	*chiP*
Salmonella	RybB	*wcaG*	32 – 40	44 – 52	`(((((((((&)))))))))`	*ompD*
Salmonella	RybB	*yabI*	45 – 52	92 – 99	`((((((((&))))))))`	*fadL*

Table S4: Organisms used in this study and the respective NCBI RefSeq database genome accession numbers.[29]

Organism	RefSeq genome accession number
Citrobacter koseri	NC_009792
Citrobacter rodentium	NC_013716
Cronobacter sakazakii	NC_009778
Enterobacter sp. 638	NC_009436
Escherichia coli K-12	NC_000913
Escherichia fergusonii	NC_011740
Klebsiella pneumoniae	NC_009648
Pectobacterium carotovorum	NC_012917
Photorhabdus luminescens	NC_005126
Proteus mirabilis	NC_010554
Salmonella Typhimurium	NC_003197
Salmonella Typhi	NC_003198
Serratia proteamaculans	NC_009832
Shigella boydii	NC_007613
Shigella dysenteriae	NC_007606
Shigella flexneri	NC_004337
Shigella sonnei	NC_007384
Sodalis glossinidius	NC_007712
Yersinia enterocolitica	NC_008800
Yersinia pestis	NC_003143
Yersinia pseudotuberculosis	NC_006155

Supplementary Figures

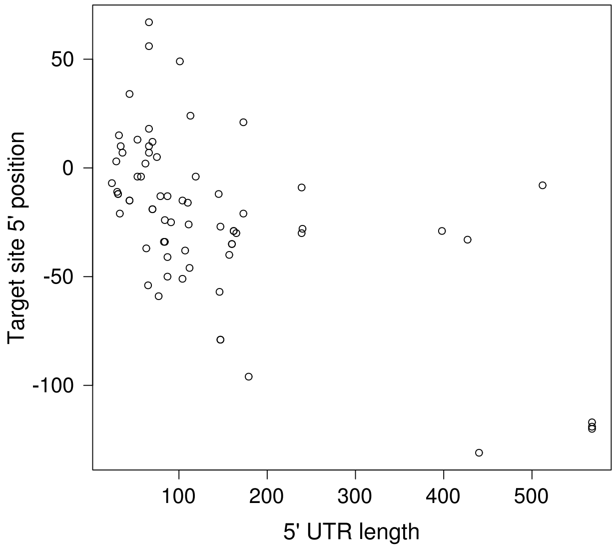


Figure S1: Positions of the 5’ end of all target sites from *E. coli* and *Salmonella* vs. the lengths of the 5’ UTR of the target genes. Target site positions are given as distance to the annotated translation start site. All genes that are located within an operon are excluded. Target site location and 5’ UTR length show modest negative correlation (Spearman’s correlation coefficient $\rho = -0.54$).

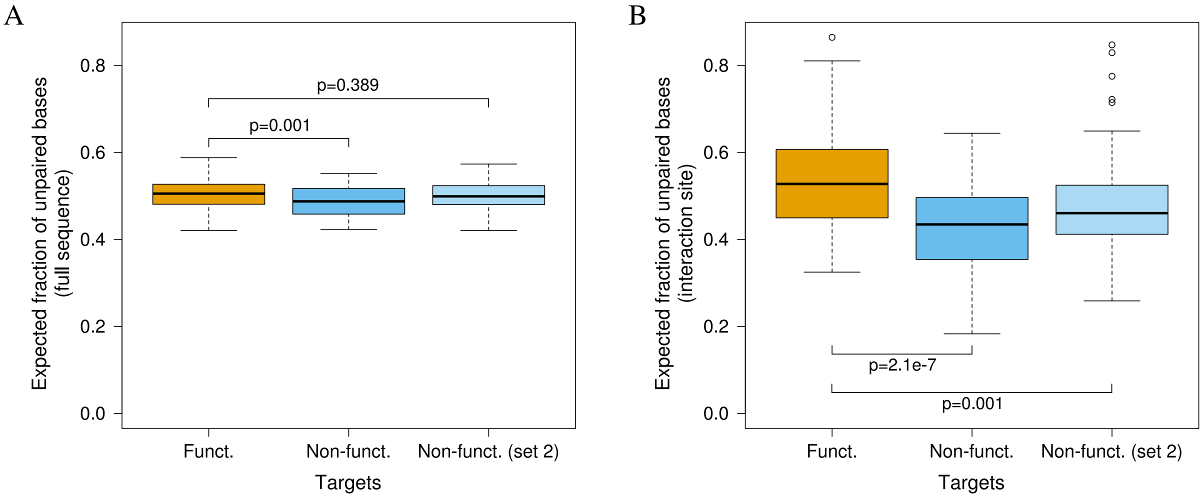


Figure S2: Comparison of target gene accessibility between a functional (orange) and two non-functional data sets (blue and light blue). The plots show the accessibility of **(A)** the full 5’ UTR and 150 nt CDS sequence, and **(B)** the interaction site only, respectively. In non-functional data set 2 (light blue), each non-functional target was not selected by the interaction free energy, but by the overall accessibility, which had to be as similar as possible to the overall accessibility of the corresponding true target. For both negative data sets, the difference in mean accessibility between functional and non-functional targets is larger and much more significant for the interaction sites only than for the whole sequence. All *p*-values were calculated by Wilcoxon rank sum test.

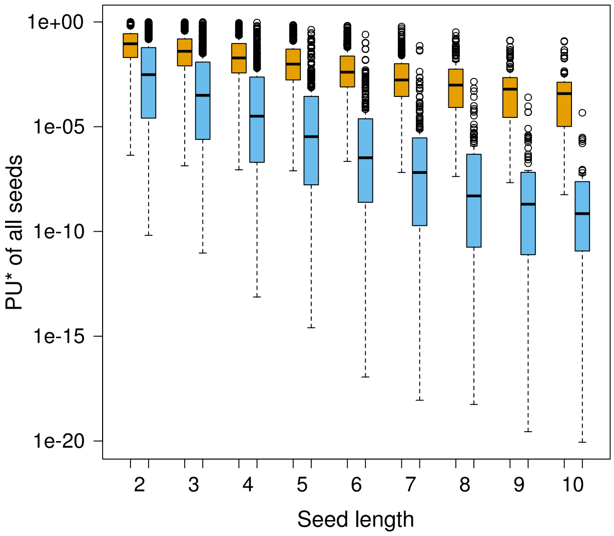
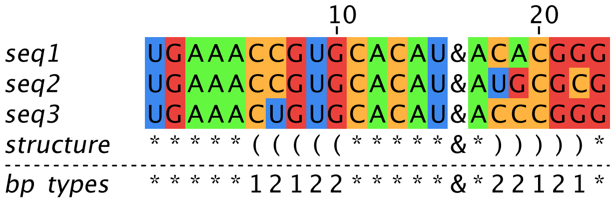


Figure S3: Comparison of the interaction site accessibility between functional (orange) and non-functional (blue) interactions. The plot shows the joint probability of being unpaired (PU^*) of all interaction seeds, i.e., all perfectly matching sub-interactions, of length two to ten. Interaction seeds in the experimentally verified interactions are significantly more accessible than in the non-functional interactions ($p < 4.8 \times 10^{-19}$ calculated by Wilcoxon rank sum test).



```
                 10        20
seq1       UGAAACCGUGCACAU&ACACGGG
seq2       UGAAACCGUGCACAU&AUGCGCG
seq3       UGAAACUGUGCACAU&ACCCGGG
structure  *****(((((*****&*)))))*
-------------------------------------
bp types   *****12122*****&*22121*
```

Figure S4: Schematic illustration of different interaction base pairings between two interacting RNAs. The sequence alignment shows two multiple RNA alignments concatenated by the linker symbol '&'. The structure contains round brackets indicating intermolecular base pairs between the two RNAs and stars indicating positions that do not participate in the interaction. The number of different interaction base pairings is given for each interaction position. For example, alignment columns 9 and 19 support the base pair `U-A` in *seq1* and `G-U` in *seq2*, whereas *seq3* contains a mismatch. Consequently, alignment columns 9 and 19 contain two different base pair combinations. The average number of different interaction base pair combinations is 1.6 in this example.

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