

Supplementary Information for

Regulating the terminator: SraL sRNA interaction prevents the premature transcription termination of *rho* mRNA

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Supplementary Materials and Methods
Supplementary References

Other supplementary materials for this manuscript include the following:

Dataset S1

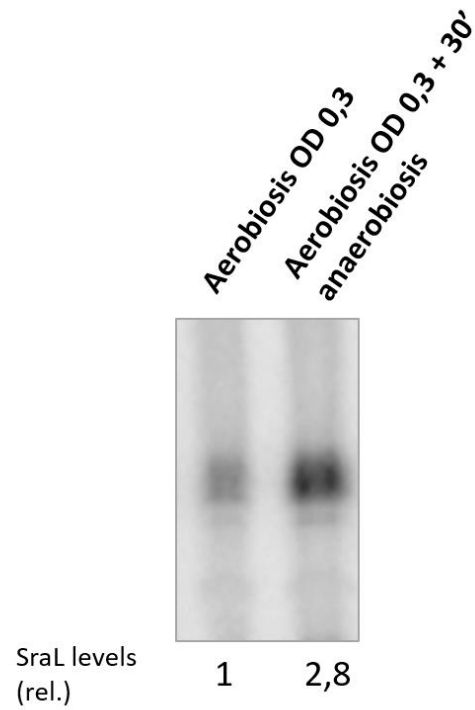
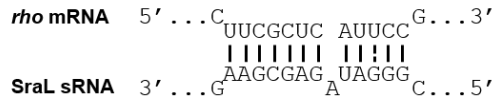


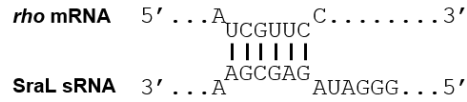
Fig. S1. SraL sRNA expression upon anaerobic shock. Total cellular RNA was extracted from *Salmonella* wild-type strain grown in LB at 37°C and 220 rpm to an OD600 of 0.3 and then placed in a filled 50 ml Falcon tube and incubated at 37°C without agitation during 30 min. Fifteen micrograms of total RNA were separated in a 6% PAA / 8.3 M urea to determine the expression level of SraL.

A) SraLWT / rhoWT

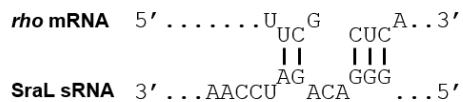
mfe: -19,2 kcal/mol
p-value: 9,999939e-01

**B) SraLWT / rhoMUT**

mfe: -12,4 kcal/mol
p-value: 9,999939e-01

**C) SraLMUT / rhoWT**

mfe: -8,4 kcal/mol
p-value: 9,999939e-01

**D) SraLMUT / rhoMUT**

mfe: -19,2 kcal/mol
p-value: 9,999939e-01

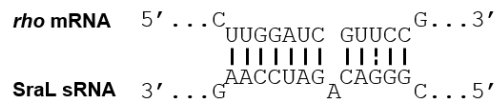


Fig. S2. Predicted interaction regions between SraL and *rho* forms. The interaction regions between SraL sRNA and *rho* mRNA were predicted by IntaRNA software (1) and RNA Hybrid (2). Minimum free energies (mfe) and p-values are indicated in the respective figure.

- (A) Predicted interaction between SraLWT and *rho*WT
- (B) Predicted interaction between SraLWT and *rho*MUT
- (C) Predicted interaction between SraLMUT and *rho*WT
- (D) Predicted interaction between SraLMUT and *rho*MUT

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STM      TTATTCTTAAATTGTCAGGATCTCTGGACGCCCGGCTCGAGTCGTGCTAAGTTAGTATTG      60
STY      TTATTCTTAAATTGTCAGGATCTCTGGACGCCCGGCTCGAGTCGTGCTAAGTTAGTATTG      60
SBO      TTATTCTTAAATTGTCAGGATCTCTGGACGCCCGGCTCGAGTCGTGCTAAGTTAGTATTG      60
SBD      -----CTGGACGCCCGGCTCGAGTCATGCTAAGTTAGTATTG      37
SFL      -----CTGGACGCCCGGCTCGAGTCATGCTAAGTTAGTATTG      37
SDR      -----CTGGACGCCCGGCTCGAGTCATGCTAAGTTAGTATTG      37
ECO      -----CTGGACGCCCGGCTCGAGTCATGCTAAGTTAGTATTG      37
CRT      -----GGATGACTGGACGCCCGGCTCGAGTCGTGCTAAGTTAATGTTG      43
CKO      ---ATTCTAAATTATTCGGATGACTGGACGCCCGGCTCGAGTCGTGCTAAGTTAGTATTG      57
ENT      -----GGATCTCTGGACGCCCGGCTTAGTCTGCTAAGTTACTTATG      43
KPN      -----GGAACTCTGGACGCCCGGAGGAGCTGCTAAGTTAATCCAC      43
          *****          ****          *****          **          *

STM      ACTTCGA--ATTAACATACCTTATTAAGTTTGAATCTGGTTTATCCGTCACCTCCCGT      118
STY      ACTTCGA--ATTAACATACCTTATTAAGTTTGAATCTGGTTTATCCGTCACCTCCCGT      118
SBO      ACTTCGA--ATTAACATACCTTATTAAGTTTGAATCTGGTTTATCCGTCACCTCCCGT      118
SBD      ACTTCGT--ATTAACATACCTTATTAAGTTTGAATCTGTAATTTCCACGCTCCCGT      95
SFL      ACTTCGT--ATTAACATACCTTATTAAGTTTGAATCTGTAATTTCCACGCTCCCGT      95
SDR      ACTTCGT--ATTAACATACCTTATTAAGTTTGAATCTGTAATTTCCACGCTCCCGT      95
ECO      ACTTCGT--ATTAACATACCTTATTAAGTTTGAATCTGTAATTTCCACGCTCCCGT      95
CRT      ACTACGT--AGTAACATACCTTATTAAGTTTGAATCTGTAATTTCCGATACCTCCCGT      101
CKO      ACTTCGT--ATTAACATACCTTATTAAGTTTGAATCTGTTTATCCACGCTCCCGT      115
ENT      ACTTCGATTTAA--ACATACCTTCTC-----GTTCTTGTTTTACCCGATGCTTCCCAT      94
KPN      AGACTTCGTTTAAACATACC---TATTGTTTGAATCTGTTTATCCAAAGCGTCCCGC      100
          *          *****          *          ***          *          **          *          *****

STM      TTTT---CTCGCAGGAGAAGTGGAAGATTCTGG-CTCTTCGCTCATTCGCTCT      171
STY      TTTT---CTCGCACGAGAAGTGGAAGATTCTGG-CTCTTCGCTCATTCGCTCT      171
SBO      TTTT---CTCGCAGGAGAAGTGGAAGATTCTGG-CTCTTCGCTCATTCGCTCT      171
SBD      TTTAT---CTTAAATCGGAAGTGAACAGATTCTGG-CTCTTCACCTCAATCCGCTCT      148
SFL      TTTAT---CTTAAATCGGAAGTGAACAGATTCTGG-CTCTTCACCTCAATCCGCTCT      148
SDR      TTTAT---CTTAAATCGGAAGTGAACAGATTCTGG-CTCTTCACCTCAATCCGCTCT      148
ECO      TTTAT---CTTAAATCGGAAGTGAACAGATTCTGG-CTCTTCACCTCAATCCGCTCT      148
CRT      TTTT---CTCGCGGAGAAGTGGAAGATTCTGG-CTCTTCACCTCAATCCGCTCT      155
CKO      TTTT---CTCGCAGGAGAAGTGAACAGATTCTGG-CTCTTCACCTCAATCCGCTCT      168
ENT      TGTGACACTCACAGTGCCTGAAGTTGC-TAGATTCCGGGCTTTCACCTCAATCCGCTCT      153
KPN      A---G-----ACGATGCGGTGAGGCTAATAATTCGGGCTTATTCACCTCATTCGCTCT      151
          *          *          *          **          **          **          *****

STM      GTCGTTTCAGTTCGCGTACT-TTCCTGTGACCAGACAGCGAACAGACATGAGTTGATAG      230
STY      GTCGTTTCAGTTCGCGTACT-TTCCTGTGACCAGACAGCGAACAGACATGAGTTGATAG      230
SBO      GTCGTTTCAGTTCGCGTACT-TTCCTGTGACCAGACAGCGAACAGACATGAGTTGATAG      230
SBD      GTCGTTTCAGTTCGCGTACT-CTCCTGTGACCAGGACAGCGAAAAGACATGAGTCGATGA      207
SFL      GTCGTTTCAGTTCGCGTACT-CTCCTGTGACCAGGACAGCGAAAAGACATGAGTCGATGA      207
SDR      GTCGTTTCAGTTCGCGTACT-CTCCGTGACCAGGACAGCGAAAAGATATGAGTCGATGA      207
ECO      GTCGTTTCAGTTCGCGTACT-CTCCTGTGACCAGGACAGCGAAAAGACATGAGTCGATGA      207
CRT      GTCGTTTCAGTTCGCGTACT-TTCCTGTGACCAGACAGCGAACAGACATGAATTGATGG      214
CKO      GTCGTTTCAGTTCGCGTACT-TTCCTGTGACCAGACAGCGAACAGACATGAGTTGGTGG      227
ENT      GTCGTTTCAGAGTTCGCTTCTTTCCTGTACCAGACAGCGAACAGACATGAGTTGATGG      213
KPN      GTCGTTTCAGTTCGCGTCT-TTCCTGTGACCAGGCAACGTACAGACAGGATGAAG-      209
          *****          ***          **          **          *          *****          **          *          *          *          *

STM      CCGTAAACAGGCATGGATGACCCCTGCCATACCATTCAACATTAAGTTCGAGATTTACC      290
STY      CCGTAAACAGGCATGGATGACCCCTGCCATACCATTCAACATTAAGTTCGAGATTTACC      290
SBO      CCGTAAACAGGCATGGATGACCCCTGCCATACCATTCAACATTAAGTTCGAGATTTACC      290
SBD      CCGTAAACAGGCATGGATGATCCTGCCATACCATTCAACATTAAGTTCGAGATTTACC      267
SFL      CCGTAAACAGGCATGGATGATCCTGCCATACCATTCAACATTAAGTTCGAGATTTACC      267
SDR      CCGTAAACAGGCATGGATGATCCTGCCATACCATTCAACATTAAGTTCGAGATTTACC      267
ECO      CCGTAAACAGGCATGGATGATCCTGCCATACCATTCAACATTAAGTTCGAGATTTACC      267
CRT      CCGTAAACAGGCATGGATGACCCCTGCCATACCATTCAACATTAAGTTCGAGATTTACC      274
CKO      CCGTAAACAGGCATGGATGATCCTGCCATACCATTCAACATTAAGTTCGAGATTTACC      287
ENT      CCGCAACAGGCATGGATGACCCCTCCATACCATTCAAA-ATTAAGTTCGAGATTTACC      272
KPN      CCGTAAACAGGCATGGATGATCCTGCCATACCATTCAACATTAAGTTCGAGATTTACC      269
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STM CCAAGTTTAAAGAACTCACACCATTATGAATCTTACCGAATTAAGAATACGCCGTTTCT 350
 STY CCAAGTTTAAAGAACTCACACCATTATGAATCTTACCGAATTAAGAATACGCCGTTTCT 350
 SBO CCAAGTTTAAAGAACTCACACCATTATGAATCTTACCGAATTAAGAATACGCCGTTTCT 350
 SBD CCAAGTTTAAAGAACTCACACCATTATGAATCTTACCGAATTAAGAATACGCCGTTTCT 327
 SFL CCAAGTTTAAAGAACTCACACCATTATGAATCTTACCGAATTAAGAATACGCCGTTTCT 327
 SDR CCAAGTTTAAAGAACTCACACCATTATGAATCTTACCGAATTAAGAATACGCCGTTTCT 327
 ECO CCAAGTTTAAAGAACTCACACCATTATGAATCTTACCGAATTAAGAATACGCCGTTTCT 327
 CRT CCAAGTTTAAAGAACTCACACCATTATGAATCTTACCGAATTAAGAATACGCCGTTTCT 334
 CKO CCAAGTTTAAAGAACTCACACCATTATGAATCTTACCGAATTAAGAATACGCCGTTTCT 347
 ENT CCGAGTTTAAAGAACCCACACCATTATGAATCTTACCGAATTAAGAATACGCCGTTTCT 332
 KPN CCGAGTTTAAAGAACCCACACCATTATGAATCTTACCGAATTAAGAATACGCCGTTTCT 329
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STM GAGCTGATCACTCTCGGCGAAAATATGGGGCTGGAAAACCTGGCCCGTATGCGCAAGCAG 410
 STY GAGCTGATCACTCTCGGCGAAAATATGGGGCTGGAAAACCTGGCCCGTATGCGCAAGCAG 410
 SBO GAGCTGATCACTCTCGGCGAAAATATGGGGCTGGAAAACCTGGCCCGTATGCGTAAGCAG 410
 SBD GAGCTGATCACTCTCGGCGAAAATATGGGGCTGGAAAACCTGGCTCGTATGCGTAAGCAG 387
 SFL GAGCTGATCACTCTCGGCGAAAATATGGGGCTGGAAAACCTGGCTCGCATGCGTAAGCAG 387
 SDR GAGCTGATCACTCTCGGCGAAAATATGGGGCTGGAAAACCTGGCTCGTATGCGTAAGCAG 387
 ECO GAGCTGATCACTCTCGGCGAAAATATGGGGCTGGAAAACCTGGCTCGTATGCGTAAGCAG 387
 CRT GAGCTGATCACTCTCGGCGAAAATATGGGGCTGGAAAACCTGGCCCGTATGCGCAAGCAG 394
 CKO GAGCTGATCACTCTCGGCGAAAATATGGGGCTGGAAAACCTGGCTCGTATGCGCAAGCAG 407
 ENT GAGCTGATCACTCTCGGCGAAAATATGGGGCTGGAAAACCTGGCTCGTATGCGCAAGCAG 392
 KPN GAGCTGATCACTCTCGGCGAAAATATGGGGCTGGAAAACCTGGCTCGTATGCGTAAGCAG 389
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STM GACATTATTTTGGCCATCCTGAAGCAGCAGCAAAGAGTGGCGAAGATATCTTTGGCGAC 470
 STY GACATTATTTTGGCCATCCTGAAGCAGCAGCAAAGAGTGGCGAAGATATCTTTGGCGAC 470
 SBO GACATTATTTTGGCCATCCTGAAGCAGCAGCAAAGAGTGGCGAAGATATCTTTGGCGAC 470
 SBD GACATTATTTTGGCCATCCTGAAGCAGCAGCAAAGAGTGGCGAAGATATCTTTGGTGAT 447
 SFL GACATTATTTTGGCCATCCTGAAGCAGCAGCAAAGAGTGGCGAAGATATCTTTGGTGAT 447
 SDR GACATTATTTTGGCCATCCTGAAGCAGCAGCAAAGAGTGGCGAAGATATCTTTGGTGAT 447
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 CRT GACATTATTTTGGCCATCCTGAAGCAGCAGCAAAGAGTGGCGAAGATATCTTTGGTGAC 454
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STM GGTGTGCTGGAGATATTGCAGGATGGATTTGGTTTCTCCGTTCTGCAGACAGCTCCTAC 530
 STY GGTGTGCTGGAGATATTGCAGGATGGATTTGGTTTCTCCGTTCTGCAGACAGCTCCTAC 530
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 SBD GGCCTACTGGAGATATTGCAGGATGGATTTGGTTTCTCCGTTCTGCAGACAGCTCCTAC 507
 SFL GGCCTACTGGAGATATTGCAGGATGGATTTGGTTTCTCCGTTCTGCAGACAGCTCCTAC 507
 SDR GGCCTACTGGAGATATTGCAGGATGGATTTGGTTTCTCCGTTCTGCAGACAGCTCCTAC 507
 ECO GGCCTACTGGAGATATTGCAGGATGGATTTGGTTTCTCCGTTCTGCAGACAGCTCCTAC 507
 CRT GGTGTGCTGGAGATATTGCAGGATGGATTTGGTTTCTCCGTTCTGCAGACAGCTCCTAC 514
 CKO GGTGTGCTGGAGATATTGCAGGATGGATTTGGTTTCTCCGTTCTGCAGACAGCTCCTAC 527
 ENT GGTGTACTGGAGATATTGCAAGACGGATTTGGTTTCTCCGTTCTGGAGACAGCTCCTAC 512
 KPN GGCCTACTGGAGATATTGCAGGATGGATTTGGATTCCTCCGTTCTGCAGACAGCTCCTAC 509
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STM CTCGCCGGTCTGATGATATCTACGTTTCCCCAGCCAAATCCGCCGTTTCAACCTCCGC 590
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 SBO CTCGCCGGTCTGATGATATCTACGTTTCCCCAGCCAAATCCGCCGTTTCAACCTCCGC 590
 SBD CTCGCCGGTCTGATGATATCTACGTTTCCCCAGCCAAATCCGCCGTTTCAACCTCCGC 567
 SFL CTCGCCGGTCTGATGATATCTACGTTTCCCCAGCCAAATCCGCCGTTTCAACCTCCGC 567
 SDR CTCGCCGGTCTGATGATATCTACGTTTCCCCAGCCAAATCCGCCGTTTCAACCTCCGC 567
 ECO CTCGCCGGTCTGATGATATCTACGTTTCCCCAGCCAAATCCGCCGTTTCAACCTCCGC 567
 CRT CTTGCCGGTCTGATGATATCTACGTTTCTCCAGCCAAATCCGCCGTTTCAACCTCCGC 574
 CKO CTCGCCGGTCTGATGATATCTACGTTTCTCCAGCCAAATCCGCCGTTTCAACCTCCGC 587
 ENT CTCGCCGGTCTGATGATATCTACGTTTCCCCAGCCAAATCCGCCGTTTCAACCTCCGT 572
 KPN CTCGCCGGTCTGATGATATCTACGTTTCCCCAGCCAAATCCGCCGTTTCAACCTCCGC 569
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STY ACTGGTGAACCATTTCGGTAAGATTCGCCCGCCGAAAGAAGGTGAACGCTATTTTGCG 650
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SBD ACTGGTGAACCATCTCTGGTAAGATTCGCCCGCCGAAAGAAGGTGAACGCTATTTTGCG 627
SFL ACTGGTGAACCATCTCTGGTAAGATTCGCCCGCCGAAAGAAGGTGAACGCTATTTTGCG 627
SDR ACTGGTGAACCATCTCTGGTAAGATTCGCCCGCCGAAAGAAGGTGAACGCTATTTTGCG 627
ECO ACTGGTGAACCATCTCTGGTAAGATTCGCCCGCCGAAAGAAGGTGAACGCTATTTTGCG 627
CRT ACTGGTGAACCATCTCTGGTAAGATTCGCCCGCCGAAAGAAGGTGAACGCTATTTTGCG 634
CKO ACTGGTGAACCATTTCGGTAAGATTCGCCCGCCGAAAGAAGGTGAACGCTATTTTGCG 647
ENT ACTGGTGAACCATTTCGGTAAGATTCGGTCTCTCTAAAGAGGTGAACGCTATTTTGCG 632
KPN ACTGGTGAACCATTTCGGTAAGATTCGGTCTCTCTAAAGAGGTGAACGCTATTTTGCA 629
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STM CTGTTGAAAGTTAACGAAGTTAACTACGACAAACCGGAAAACGCCCGTAACAAAATCCTC 710
STY CTGTTGAAAGTTAACGAAGTTAACTACGACAAACCGGAAAACGCCCGTAACAAAATCCTC 710
SBO CTGTTGAAAGTTAACGAAGTTAACTACGACAAACCGGAAAACGCCCGTAACAAAATCCTG 710
SBD CTGCTGAAAGTTAACGAAGTTAACTTCGACAAACCTGAAAACGCCCGCAACAAAATCCTC 687
SFL CTGCTGAAAGTTAACGAAGTTAACTTCGACAAACCTGAAAACGCCCGCAACAAAATCCTC 687
SDR CTGCTGAAAGTTAACGAAGTTAACTTCGACAAACCTGAAAACGCCCGCAACAAAATCCTC 687
ECO CTGCTGAAAGTTAACGAAGTTAACTTCGACAAACCTGAAAACGCCCGCAACAAAATCCTC 687
CRT CTGCTGAAAGTTAACGAAGTTAACTACGACAAACCGGAAAACGCCCGTAACAAAATCCTC 694
CKO CTGTTGAAAGTTAACGAAGTTAACTACGACAAACCTGAAAACGCCCGTAACAAAATCCTC 707
ENT CTGTTGAAAGTTAACGAAGTTAACTACGATAAACCTGAAAACCTCGCCAAATAGATCCTG 692
KPN CTGTTGAAAGTTAACGAAGTTAACTACGACAAACCGGAAAACGCCCGTAACAAAGATCCTG 689
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STY TTTGAGAACTTAACCCCGCTGCACGCAAACTCTCGTCTGCGTATGGAGCGTGGTAACGGT 770
SBO TTTGAGAACTTAACCCCGCTGCACGCAAACTCTCGTCTGCGTATGGAGCGTGGTAACGGT 770
SBD TTTGAGAACTTAACCCCGCTGCACGCAAACTCTCGTCTGCGTATGGAACTGGTGGTAACGGT 747
SFL TTTGAGAACTTAACCCCGCTGCACGCAAACTCTCGTCTGCGTATGGAACTGGTGGTAACGGT 747
SDR TTTGAGAACTTAACCCCGCTGCACGCAAACTCTCGTCTGCGTATGGAACTGGTGGTAACGGT 747
ECO TTTGAGAACTTAACCCCGCTGCACGCAAACTCTCGTCTGCGTATGGAACTGGTGGTAACGGT 747
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CKO TTTGAGAACTTAACCCCGCTGCACGCAAACTCACTCTGCGTATGGAACTGGTGGTAACGGT 767
ENT TTTGAGAACTTAACCCCGCTGCACGCAAACTCTCGCTGCGCATGGAGCGTGGTAAACGGT 752
KPN TTTGAGAACTTAACCCCGCTGCACGCAAACTCTCGCTGCGTATGGAACTGGTGGTAACGGT 749
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STM TCTACGAAGACTTAACGGCGCGCGTTCTGGATCTGGCTTCGCCGATCGGTGCGGCCAG 830
STY TCTACGAAGACTTAACGGCGCGCGTTCTGGATCTGGCTTCGCCGATCGGTGCGGCCAG 830
SBO TCTACGAAGACTTAACGGCGCGCGTTCTGGATCTGGCTTCGCCGATCGGTGCGGCCAG 830
SBD TCTACGAAGACTTAACCGCTCGCGTACTGGATCTGGCATCACTATCGGTGCGTGGTTCAG 807
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SDR TCTACGAAGACTTAACGCTCGCGTACTGGATCTGGCATCACTATCGGTGCGTGGTTCAG 807
ECO TCTACTGAAGACTTAACGCTCGCGTACTGGATCTGGCATCACTATCGGTGCGTGGTTCAG 807
CRT TCTACGAAGACTTAACGGCGCGCGTCTGGATCTGGCTTCGCCAATCGGTGCGGCCAG 814
CKO TCTACTGAAGACTTAACGGCTCGCGTACTGGATTTGGCTTCGCCGATGGCCGTGGTTCAG 827
ENT TCTACGAAGACTTACGGCTCGCGTCTGGATCTGGCTTCCTCAATGGTTCGTGGTTCAG 812
KPN TCTACGAAGACTTAACCGCTCGCGTACTGGATCTGGCTTCGCCGATGGCCGCGGCCAG 809
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STM CGCGGTCTGATTGTCGCGCCGCCGAAAGCGGGTAAAACCATGCTGCTGCAGAACATCGCG 890
STY CGCGGTCTGATTGTCGCGCCGCCGAAAGCGGGTAAAACCATGCTGCTGCAGAACATCGCG 890
SBO CGTGGTCTGATTGTTGCGCCGCCGAAAGCGGGTAAAACCATGCTGCTGCAGAAATATCGCG 890
SBD CGTGGTCTGATTGTTGCCACCGCCGAAAGCGGGTAAAACCATGCTGCTGCAGAACATGCT 867
SFL CGTGGTCTGATTGTTGCCACCGCCGAAAGCGGGTAAAACCATGCTGCTGCAGAACATGCT 867
SDR CGTGGTCTGATTGTTGCCACCGCCGAAAGCGGGTAAAACCATGCTGCTGCAGAACATGCT 867
ECO CGTGGTCTGATTGTTGCCACCGCCGAAAGCGGGTAAAACCATGCTGCTGCAGAACATGCT 867
CRT CGTGGTCTGATTGTTGCCACCGCCGAAAGCGGGTAAAACCATGCTGCTGCAGAAATATCGCG 874
CKO CGTGGTCTGATTGTTGCCACCGCCGAAAGCGGGTAAAACCATGCTGTTGCAGAACATCGCG 887
ENT CGTGGCTGATGTTGCCACCGCCGAAAGCGGTAAAGACCATGCTGCTGCAGAACATCGCG 872
KPN CGTGGTTTGGATTGTTGCGCCGCCGAAAGCGGGTAAAACCATGCTGCTGCAGAACATCGCG 869
** **

STM	CAGAGCATCGCGTATAACCA CCCGACTGCGTGCTGATGGT GCTGCTGATGACGAACGT	950
STY	CAGAGCATCGCGTATAACCA CCAGACTGCGTGCTGATGGT GCTGCTGATGACGAACGT	950
SBO	CAGAGCAT TGCGTACAACCA TCCAGACTGCGTGCTGATGGTCTTGCTGATGACGAACGT	950
SBD	CAGAGCAT TGCTTACAACCA CCCGGATTGTGTGCTGATGGTTCGCTGATCGACGAACGT	927
SFL	CAGAGCAT TGCTTACAACCA CCCGGATTGTGTGCTGATGGTTCGCTGATCGACGAACGT	927
SDR	CAGAGCAT TGCTTACAACCA CCCGGATTGTGTGCTGATGGTTCGCTGATCGACGAACGT	927
ECO	CAGAGCAT TGCTTACAACCA CCCGGATTGTGTGCTGATGGTTCGCTGATCGACGAACGT	927
CRT	CAGAGCATCGCTTACAACCA TCCGGACTGCGTGCTGATGGT GCTGCTGATGACGAACGT	934
CKO	CAGAGCAT TGCTTACAACCA CCCTGACTGCGTACTGATGGTGTGCTGATCGACGAACGT	947
ENT	CAGAGCAT TGCTTACAACCA TCCGTGACTGCGTACTGATGGTTCGCTGATCGATGAGCGT	932
KPN	CAGAGCATCGCTTACAACCA CCCGGACTGCGTGCTGATGGT GCTGCTGATCGACGAACGT	929
	***** ** ** ***** ** ** ** * ***** ***** ** ** ** *	
STM	CCGGAAGAAGT GACCCGAGATGCAGCGTCTGGT GAAAGGCGAAGTGGTTGCTTCTACCTTT	1010
STY	CCGGAAGAAGT AACCCGAGATGCAGCGTCTGGT GAAAGGCGAAGTGGTTGCTTCTACCTTT	1010
SBO	CCGGAAGAAGT GACCCGAGATGCAGCGTCTGGT GAAAGGCGAAGTGGTTGCTTCTACCTTT	1010
SBD	CCGGAAGAAGT AACCCGAGATGCAGCGTCTGGT A AAAGG TGAAGTGGTTGCTTCTACCTTT	987
SFL	CCGGAAGAAGT AACCCGAGATGCAGCGTCTGGT A AAAGG TGAAGTGGTTGCTTCTACCTTT	987
SDR	CCGGAAGAAGT AACCCGAGATGCAGCGTCTGGT A AAAGG TGAAGTGGTTGCTTCTACCTTT	987
ECO	CCGGAAGAAGT AACCCGAGATGCAGCGTCTGGT A AAAGG TGAAGTGGTTGCTTCTACCTTT	987
CRT	CCGGAAGAAGT GACCCGAGATGCAGCGTCTGGT GAAAGGCGAAGTGGTTCCTTCCACCTTC	994
CKO	CC A GAAAGT AACCCGAAATGCAGCGTCTGGT A AAAGG TGAAGTGGTTGCTTCCACCTTC	1007
ENT	CC A GAAAGT AACCCGAGATGCAGCGTCTGGT GAAAGGCGAAGTGGTTGCTTCCACCTTC	992
KPN	CCGGAAGAAGT GACCCGAGATGCAGCGTCTGGT GAAAGGCGAAGTGGTTGCTTCCACCTTT	989
	** ***** ** ** ***** ***** ***** * ** ** *****	
STM	GACGAACCGGCATCCGCCACGTT CAGGTTGCC GAAATGGTATCGAGAAGGCCAAGCGT	1070
STY	GACGAACCGGCATCCGCCACGTT CAGGTTGCC GAAATGGTATCGAGAAGGCCA AAC CGT	1070
SBO	GACGAACCGGCATCCGCCACGTT CAGGTTGCC GAAATGGTATCGAGAAGGCCA AAC CGC	1070
SBD	GACGAACCGGCATCTCGCCACGTT CAGGTTGCC GAAATGGTATCGAGAAGGCCA AAC CGC	1047
SFL	GACGAACCGGCATCTCGCCACGTT CAGGTTGCC GAAATGGTATCGAGAAGGCCA AAC CGC	1047
SDR	GACGAACCGGCATCTCGCCACGTT CAGGTTGCC GAAATGGTATCGAGAAGGCCA AAC CGC	1047
ECO	GACGAACCGGCATCTCGCCACGTT CAGGTTGCC GAAATGGTATCGAGAAGGCCA AAC CGC	1047
CRT	GACGAACCGGCATCCGTCACGTT CAGGTTAGCT GAAATGGTATCGAGAAGGCCA AAC CGT	1054
CKO	GACGAACCGGCATCCGTCACGTT CAGGTTAGCT GAAATGGTATCGAGAAGGCCA AAC CGT	1067
ENT	GATGAGCCAGCCTCTCGCCACGTT CAGGTTGCT GAAATGGTATCGAGAAGGCCA AAC CGT	1052
KPN	GACGAGCCGGCATCCGCCACGTT CAGGTTGCC GAGATGGTATCGAGAAGGCCA AAC CGT	1049
	** ** ** ** ** ** ** ** ** ** ** ** ***** ***** ** ** ***** ** ***** ** ** ** *	
STM	CTGGTTGAA CACAAGAAAGACGTTATCAT CCTGCTCGACTCCATCACCCTGCTGGCGGC	1130
STY	CTGGTTGAA CACAAGAAAGACGTTATCAT CCTGCTCGACTCCATCACCCTGCTGGCGGT	1130
SBO	CTGGTTGAA CACA AAAAGACGTTATCATCCTGCTCGACTCTAT TAC ACGCTGCTGGCGGT	1130
SBD	CTGGTTGAA CACAAGAAAGACGTTATCAT TCTGCTCGACTCCATCACTCGTCTGGCGGC	1107
SFL	CTGGTTGAA CACAAGAAAGACGTTATCAT TCTGCTCGACTCCATCACTCGTCTGGCGGC	1107
SDR	CTGGTTGAA CACAAGAAAGACGTTATCAT TCTGCTCGACTCCATCACTCGTCTGGCGGC	1107
ECO	CTGGTTGAA CACAAGAAAGACGTTATCAT TCTGCTCGACTCCATCACTCGTCTGGCGGC	1107
CRT	CTGGTTGAA CATAAAGAAAGACGTTATCAT TCTGCTCGACTCCATCACCCTGCTGGCGGT	1114
CKO	CTGGTTGAA CACA AAAAGACGTTATCATCCTGCTCGACTCCATCACTCGTCTGGCTCGC	1127
ENT	CTGGT CGAG CACAAGAAAGACGTTAT TAT TCTGCTCGACTCCATCACTCGTCTGGCGGT	1112
KPN	CTGGTTGAA CACAAGAAAGACGTTATCAT CCTGCTCGACTCCAT TACT CGTCTGGCGGA	1109
	***** ** ** ** ***** ***** ***** ** ** ***** ***** ** *	
STM	GCCTACAACACCGTGGT GCCGGCTTCCGGTAAAGT ATTGACCGGTGGTGTGGACGCTAAC	1190
STY	GCCTACAACACCGTGGT GCCGGCTTCCGGTAAAGT ACTGACCGGTGGTGTGGATGCTAAC	1190
SBO	GCCTACAACACCGTGGT GCCAGCTTCTGGTAAAGT GCTGACTGGTGGTGTGGATGCCAAC	1190
SBD	GCTTACAACACTGTTGTTCCGGCGTCAGGTA AAAGT GTTGACCGGTGGTGTGGACGCCAAT	1167
SFL	GCTTACAACACTGTTGTTCCGGCGTCAGGTA AAAGT GTTGACCGGTGGTGTGGACGCCAAT	1167
SDR	GCTTACAACACCGTGGT TCCGGCGTCAGGTA AAAGTGTTGACTGGTGGTGTGGACGCCAAT	1167
ECO	GCTTACAACACCGTGGT TCCGGCGTCAGGTA AAAGTGTTGACCGGTGGTGTGGATGCCAAC	1167
CRT	GCGTATAACACCGTGGT TCCGGCTTCCGGTAAAGT GTTGACCGGTGGTGTGGATGCCAAC	1174
CKO	GCCTACAACACCGTGGT GCCGGCTTCTGGTAAAGT CCTGACCGGTGGTGTGGACGCCAAC	1187
ENT	GCTTACAACACCGTAGTT CCTGCTTCCGGTAAAGT ACTGACCGGTGGTGTGGATGCCAAC	1172
KPN	GCCTACAACACCGTGGT ACC GGCTTCTGGTAAAGTCTGACCGGTGGTGTGGACGCCAAC	1169
	** ** ***** ** ** ** ***** ***** ***** ***** ***** ** ** *	

STM GCCCTGCATCGTCCGAAACGCTTTCTTCGGCGCGGCGTAACGTGGAAGAGGGCGGTAGC 1250
STY GCCCTGCATCGTCCGAAACGCTTTCTTCGGCGCGGCGTAACGTGGAAGAGGGCGGTAGC 1250
SBO GCCCTGCATCGTCCGAAACGCTTTCTTCGGTGC CGGCGTAACGTGGAAGAGGGCGGCAGC 1250
SBD GCCCTGCATCGTCCGAAACGCTTTCTTCGGTGC CGGCGTAACGTGGAAGAGGGCGGCAGC 1227
SFL GCCCTGCATCGTCCGAAACGCTTTCTTCGGTGC CGGCGTAACGTGGAAGAGGGCGGCAGC 1227
SDR GCCCTGCATCGTCCGAAACGCTTTCTTCGGTGC CGGCGTAACGTGGAAGAGGGCGGCAGC 1227
ECO GCCCTGCATCGTCCGAAACGCTTTCTTCGGTGC CGGCGTAACGTGGAAGAGGGCGGCAGC 1227
CRT GCCCTGCACCGTCCGAAACGCTTTCTTCGGCGCGGCGTAACGTGGAAGAGGGCGGTAGC 1234
CKO GCCCTGCACCGTCCGAAACGCTTTCTTCGGCGCGGCGTAACGTGGAAGAGGGCGGCAGC 1247
ENT GCATACACCGTCCGAAACGCTTTCTTCGGTGC CGGCGTAACGTGGAAGAGGGAGGAACG 1232
KPN GCCCTGCACCGTCCGAAACGCTTTCTTCGGTGC CGGCGTAACGTGGAAGAGGGCGGCAGC 1229
** * * * * * ** * * * * * ** * * * * * ** * * * * * ** * * * * * ** * * * * *

STM CTGACTATCATCGCGACGGCGCTAATCGATACCGGTTCCAAGATGGACGAAGTTATCTAC 1310
STY CTGACTATCATCGCGACGGCGCTAATCGATACCGGTTCCAAGATGGACGAAGTTATCTAC 1310
SBO CTGACTATCATCGCGACGGCGTTGATCGACACCGGCTCCAAGATGGATGAAGTTATCTAC 1310
SBD CTGACCATATCGCGACGGCGCTTATCGATACCGGTTCTAAAATGGACGAAGTTATTTAC 1287
SFL CTGACCATATCGCGACGGCGCTTATCGATACCGGTTCTAAAATGGACGAAGTTATTTAC 1287
SDR CTGACCATATCGCGACGGCGCTTATCGATACCGGTTCTAAAATGGACGAAGTTATTTAC 1287
ECO CTGACCATATCGCGACGGCGCTTATCGATACCGGTTCTAAAATGGACGAAGTTATCTAC 1287
CRT CTGACCATCATCGCGACCGCGCTTATCGATACCGGCTCCAAGATGGACGAGTTATCTAC 1294
CKO CTGACCATATCGCAACGGCGCTGATCGACACCGGTTCTAAGATGGATGAAGTATCTAC 1307
ENT CTGACGATATCGCAACCGCTCTGGTTGATACCGGCTCTAAAATGGATGAAGTTATCTAC 1292
KPN CTGACCATCATTCGCGACGGCGCTGATTCGACACCGGCTCCAATGGACGAAGTATCTAC 1289
***** ** *

STM GAAGAGTTTAAAGGCACCGTAACATGGAGCTGCATCTCTCGCGTAAGATCGCTGAAAAA 1370
STY GAAGAGTTTAAAGGCACCGTAACATGGAGCTGCATCTCTCGCGTAAGATCGCTGAAAAA 1370
SBO GAAGAGTTTAAAGGTACAGGCAACATGGAACCTGCACCTCTCTCGTAAGATCGCTGAAAAA 1370
SBD GAAGAGTTTAAAGGTACAGGCAACATGGAACCTGCACCTCTCTCGTAAGATCGCTGAAAAA 1347
SFL GAAGAGTTTAAAGGTACAGGCAACATGGAACCTGCACCTCTCTCGTAAGATCGCTGAAAAA 1347
SDR GAAGAGTTTAAAGGTACAGGCAACATGGAACCTGCACCTCTCTCGTAAGATCGCTGAAAAA 1347
ECO GAAGAGTTTAAAGGTACAGGCAACATGGAACCTGCACCTCTCTCGTAAGATCGCTGAAAAA 1347
CRT GAAGAGTTCAAGGCGACCGTAACATGGAACCTGCACCTTCCCGTAAGATCGCGGAAAAA 1354
CKO GAAGAGTTTAAAGGCACCGTAACATGGAGCTGCACCTCTCCCGTAAGATCGCTGAAAAA 1367
ENT GAAGAGTTTAAAGGCACCGTAACATGGAGCTGCACCTGGCACGTAATAATCGCCGAGAAAG 1352
KPN GAAGAGTTTAAAGGCACCGTAACATGGAGCTGCACCTCTCTCGTAAGATGCGGAAAAA 1349
***** ** *

STM CGTGTCTTCCCGGTATCGACTACAACCGTTCCGGTACCGGTAAGAAGAGCTGCTCACC 1430
STY CGTGTCTTCCCGGTATCGACTACAACCGTTCCGGTACCGGTAAGAAGAGCTGCTCACC 1430
SBO CGCGTCTTCCCGGTATCGACTACAACCGTTCCGGTACCGGTAAGAAGAGCTGCTTACC 1430
SBD CGCGTCTTCCCGGTATCGACTACAACCGTTCCGGTACCGGTAAGAAGAGCTGCTCACC 1407
SFL CGCGTCTTCCCGGTATCGACTACAACCGTTCCGGTACCGGTAAGAAGAGCTGCTCACC 1407
SDR CGCGTCTTCCCGGTATCGACTACAACCGTTCCGGTACCGGTAAGAAGAGCTGCTCACC 1407
ECO CGCGTCTTCCCGGTATCGACTACAACCGTTCCGGTACCGGTAAGAAGAGCTGCTCACC 1407
CRT CGCGTCTTCCCGGCATTGACTACAACCGTTCCGGTACCGGTAAGAAGAGCTGCTTACC 1414
CKO CGCGTCTTCCCGGTATCGACTACAACCGTTCCGGTACCGGTAAGAAGAGCTGCTCACC 1427
ENT CGCGTCTTCCCGGATTGATTACAACCGTTCCAGGACCGGTAAGAAGAGCTGCTCACC 1412
KPN CGCGTCTTCCCGGTATCGATTACAACCGTTCCGGCACCGGTAAGAAGAAGCTGCTCACC 1409
** * * * * * ** * * * * * ** * * * * * ** * * * * * ** * * * * * ** * * * * *

STM ACTCAGGAAGAGCTGCAGAAAATGTGGATCCTGCGTAAAATCATCCATCCGATGGGTGAA 1490
STY ACTCAGGAAGAGCTGCAGAAAATGTGGATCCTGCGTAAAATCATCCATCCGATGGGTGAG 1490
SBO ACTCAGGAAGAGCTGCAGAAAATGTGGATCCTACGTAAAATCATTCATCCATGGGTGAA 1490
SBD ACTCAGGAAGAAGCTGCAGAAAATGTGGATCCTGCGCAAAAATCATTCACCCGATGGGCGAA 1467
SFL ACTCAGGAAGAAGCTGCAGAAAATGTGGATCCTGCGCAAAAATCATTCACCCGATGGGCGAA 1467
SDR ACTCAGGAAGAAGCTGCAGAAAATGTGGATCCTGCGCAAAAATCATTCACCCGATGGGCGAA 1467
ECO ACTCAGGAAGAAGCTGCAGAAAATGTGGATCCTGCGCAAAAATCATTCACCCGATGGGCGAA 1467
CRT ACTCAGGAAGAAGCTGCAGAAAATGTGGATCCTGCGTAAAGATCATTCACCAATGGGCGAA 1474
CKO ACTCAGGAAGAAGCTACAGAAAATGTGGATCCTGCGCAAAAATCATCCATCCGATGGGTGAA 1487
ENT ACTCAGGAAGAGCTGCAGAAAATGTGGATCCTGCGTAAAGATCATTCACCCGATGGGCGAA 1472
KPN ACTCAGGAAGAGCTGCAGAAAATGTGGATCCTGCGCAAAAATCATTCATCCGATGGGTGAG 1469
***** ** *

STM	ATTGACGC GATGGAAATTCCTCATTAAC AAACTGGCGATGACCAA AACT AATGACGACTTT	1550
STY	ATTGACGC GATGGAAATTCCTCATTAAC AAACTGGCGATGACCAA AACT AATGACGACTTT	1550
SBO	ATCGACGC AAATGGAAATTCCTCAT CAATAA ATGGCGATGACCAA ACC AATGATGACTTC	1550
SBD	ATCGATGC AAATGGAAATTCCTCATTA ATAA CTGGCCATGACCAA AGCC AATGACGATTTTC	1527
SFL	ATCGATGC AAATGGAAATTCCTCATTA ATAA CTGGCCATGACCAA AGCC AATGACGATTTTC	1527
SDR	ATCGATGC AAATGGAAATTCCTCATTA ATAA CTGGCAATGACCAA AGCC AATGACGATTTTC	1527
ECO	ATCGATGC AAATGGAAATTCCTCATTA ATAA CTGGCAATGACCAA AGCC AATGACGATTTTC	1527
CRT	ATCGACGC GATGGAAATTTCTCATTA ACAAG CTGGCAATGACCAA AGCC AA CGACGACTTC	1534
CKO	ATCGACGC GATGGAAATTCCTCATTA ACAAG CTGGCAATGACCAA AGCC AA CGACGACTTC	1547
ENT	ATCGACGC AAATGGAAATTCCTCAT CAATAA AGCTGGCAATGAC AAAGCC AA CGATGATTTTC	1532
KPN	ATCGATGC AAATGGAAATTCCTCATTA ACAAG CTGGCGATGACCAA AGCC AATGATGACTTC	1529
	** *	
STM	TTCGAGATGATGAA CGCTCATAA	1574
STY	TTCGAGATGATGAA CGCTCATAA	1574
SBO	TTT GAGATGATGAA CGCTCATAA	1574
SBD	TTCGAAATGATGAA CGCTCATAA	1551
SFL	TTCGAAATGATGAA CGCTCATAA	1551
SDR	TTCGAAATGATGAA CGCTCATAA	1551
ECO	TTCGAAATGATGAA CGCTCATAA	1551
CRT	TTCGACATGATGAA CGTTCGTAA	1558
CKO	TTCGACATGATGAA CGTTCGTAA	1571
ENT	TTCGACATGATGAA CGCTCGTAA	1556
KPN	TTCGATATGATGAA CGCTCGTAA	1553
	** *	

Fig. S3. Alignment of *rho* genes including the upstream promoter region in several enterobacteria. All nucleotides are colored regarding their degree of conservation (red: high conservation; blue: partial conservation; black: little or no conservation). The asterisks (*) below the sequences are indicating the nucleotides conserved between all the species analyzed. *rho* coding sequence is given in bold letters. The *rho* interaction region with SraL is also indicated in yellow. STM: *Salmonella enterica* serovar Typhimurium; STY: *Salmonella enterica* serovar Typhi; SBO: *Salmonella bongori*; SBD: *Shigella boydii*; SFL: *Shigella flexneri*; SDR: *Shigella dysenteriae*; ECO: *Escherichia coli*; CRT: *Citrobacter rodentium*; CKO: *Citrobacter koseri*; ENT: *Enterobacter*; KPN: *Klebsiella pneumoniae*

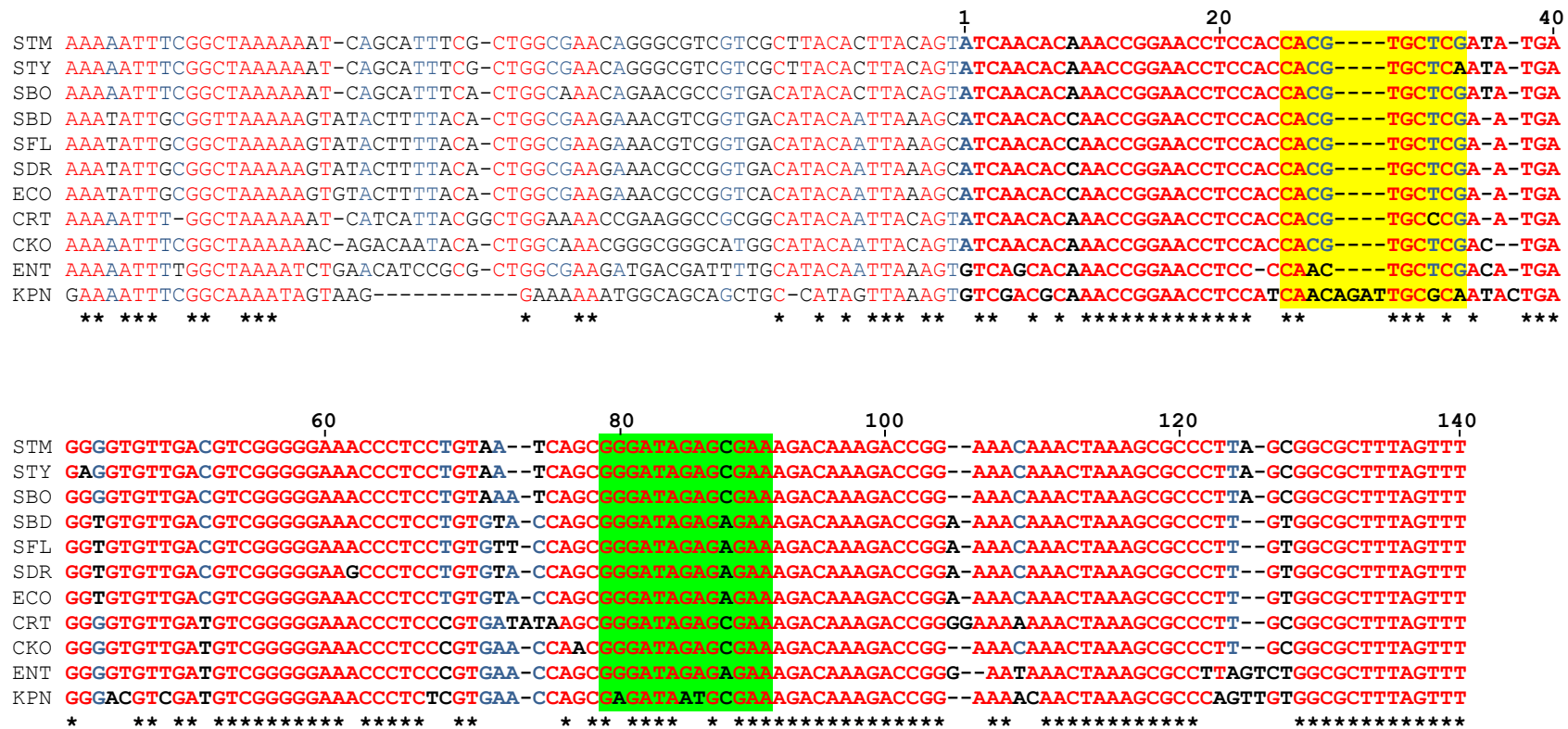
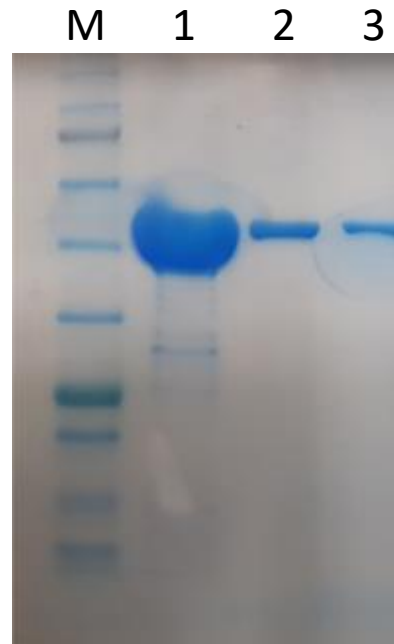


Fig. S4. Alignment of *sraL* genes including the upstream promoter region in several enterobacteria. All nucleotides are colored regarding their degree of conservation (red: high conservation; blue: partial conservation; black: little or no conservation). The asterisks (*) below the sequences are indicating the nucleotides conserved between all the species analyzed. The SraL interaction regions with *tig* mRNA and *rho* mRNA are also indicated in yellow and green, respectively. STM: *Salmonella enterica* serovar Typhimurium; STY: *Salmonella enterica* serovar Typhi; SBO: *Salmonella bongori*; SBD: *Shigella boydii*; SFL: *Shigella flexneri*; SDR: *Shigella dysenteriae*; ECO: *Escherichia coli*; CRT: *Citrobacter rodentium*; CKO: *Citrobacter koseri*; ENT: *Enterobacter*; KPN: *Klebsiella pneumoniae*



- 1 – Rho protein after purification (17,2 μM)
- 2 – Rho protein in Buffer 2 without glycerol (2,8 μM)
- 3 – Rho protein in Buffer 2 (1,4 μM)

Fig. S5. SDS-PAGE analysis of purified Rho protein.

Protein samples were visualized by BlueSafe (Nzytech). Molecular weight marker (NZYBlue Protein Marker - Nzytech) is shown on the left side of the image. Purified recombinant *Salmonella* Rho (~42,7 kDa) after purification and after buffer exchange (without and with 50% glycerol) were separated on a 10 % polyacrylamide gel.

Table S1. List of Oligonucleotides used in this work

Oligo	Sequence 5' to 3'
pIS-021	ATCAACACAAACCGGAAC
pIS-022	GTTTTTTTTTTAATACGACTCACTATAGGGAGGTAAGGGCGCTTTAGTTG
pIS-023	CTACGGCGTTTCACTTCTGAGTTC
pIS-024	ACGGCTACCTTGTTACGACTT
pIS-027	TGGTAAGGCAACGCCAGCTC
pIS-030	GATGAGCTGGACGGTTGTATC
pIS-041	AGGGCGCTGTATGGTCAGGCGTCTGTCAATTCGACGAATGATTAAGGTATCAGTGTAGGCTGGAGCTGCTTC
pIS-042	TCCTGACAATTTAAGAATAACGTAAGGACAACAGACGCCACGAACTGCCGGTCCATATGAATATCCTCCTTAG
pIS-043	AAACGGCGAAGTGGCGGCAAC
pIS-044	GTGGAAAGATTCTGGCTCTTGGATCGTTCCGCTTGTCGTTTCA
pIS-045	TGAAACGACAAGACGGAACGATCCAAGAGCCAGGAATCTTCCAC
pIS-046	TCGCCGAGAGTGATCAGCTCAG
pIS-047	CCCTCCTGTAATCAGCGGGACAGATCCAAAGACAAAGACCGGAAA
pIS-048	TTCCGGTCTTTGTCTTTGGATCTGTCCCCTGATTACAGGAGGG
pIS-049	GTTTTTTTTTTAATACGACTCACTATAGTTCACCAGACGCTGCATCTC
pIS-050	CGTTCTGGATCTGGCTTCGC
pIS-051	CGATGCTCTGCGCGATGTTT
pIS-052	ATCACTCTCGGCGAAAGTATGG
pIS-053	TCCATCCTGCAATATCTCCAGC
pIS-054	CTCGCACGAGAAGTGGAAAG
pIS-055	CGCTGTCTGGTCACAGGAAAG
pIS-056	TAAGCGTCGCTGCCG
pIS-057	AAAGAAAGCGTAATAGCTCACTGGTC
pIS-058	GTTTTTTCATATGAATCTTACCGAATTAAAG

pIS-059	GTTTTTTAAGCTTGCGCATATCCCGATGACCAGTTT
pIS-060	GTCTAACCTATAGGATACTTACAGCCGACTTCGAATTAACATACCTTATTAAG
pIS-061	TTACCACGCTCCATACGCAGACGAGAGTTTGCG
pIS-062	TTATCAAAAAGAGTATTGACTTAAAGTCTAACCTATAGGATACTTACAGCC
pIS-063	GTTTTTTTTAATACGACTCACTATAGGATCAACACAAACCGGAACCTC
pIS-064	AAAATAAGCGCCGCTAAG
pIS-065	GGAATGAGCGAA
pIS-066	GTTTTTTTTAATACGACTCACTATAGGACATCAGATTCCTGGTGTAACG
pIS-067	AAAAAATCCCGGCCCTACGGGTC
pIS-068	CAACGGATAACGTAGCAATTACTGATGGCGTCATTATAATGTGTAGGCTGGAGCTGCTTC
pIS-069	CGGTTTATCAGCGCGAGGTTTACGTTACGCGCCTTCTTTACGGTCCATATGAATATCCTCCTTAG
pIS-070	GTCGCACAGCTTGAAACC
pIS-071	CTGCGCGTACAATCAAAGAC
pIS-072	GTTTTTCTAGATGACGCCTGTTTCATGCCTGCTG
pIS-073	GTTTTTTAAGCTTATGCAAGCAGGCCAGCTAAC
EM1724	GACTGAGAATTCCAGACCCTGATGGTGTCTG
EM2883	CCGCTCGAGCGTACACCATCAGGGTACGTTTT
EM2884	CAGTTGAATTCATCAACACAAACCGGAACCTC
EM2885	CCCAAGCTTCACTCGGCCATCGGGCTG
EM2886	CCGCTCGAGATCAACACAAACCGGAACCTC
EM2888	CCTCGAGCCCGGGGAGCTCG
EM2889	AATTCGAGCTCCCCGGGCTCGAGGACGT

Table S2. List of strains used in this work

Strain	Relevant Markers / Genotype	Source/Reference
<i>S. Typhimurium</i> , SL1344	Str ^R <i>hisG rpsL xyl</i>	(3)
<i>E. coli</i> DH5	<i>recA1 endA1 gyrA96 thi-hsdR17 supE44 relA1 ΔlacZYA-arg</i> FU169 f80dLacZDM15	New England Biolabs
BL21(DE3)	<i>fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS</i> <i>λ DE3 = λ sBamHI ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5</i>	New England Biolabs
CMA-651	SL1344 <i>sraL</i> (Δ <i>sraL</i> ::Cm ^R)	(4)
CMA-654	SL1344 [<i>sraL</i> - <i>STM4267</i>]IG::Cm ^R	(4)
CMA-659	SL1344 [<i>trxA-rho</i>]IG::Cm ^R	This study
CMA-660	SL1344 [<i>trxA-rho</i>]IG::Cm ^R <i>rho</i> _{C98G/C100A/A103G}	This study
CMA-661	SL1344 [<i>sraL</i> - <i>STM4267</i>]IG::Cm ^R <i>sraL</i> _{U83C/G87U/G89C}	This study
CMA-662	SL1344 <i>sraL</i> _{U83C/G87U/G89C}	This study
CMA-663	SL1344 [<i>trxA-rho</i>]IG::Cm ^R <i>rho</i> _{C98G/C100A/A103G} <i>sraL</i> _{U83C/G87U/G89C}	This study
CMA-664	SL1344 <i>proQ</i> (Δ <i>proQ</i> ::Cm ^R)	This study
CMA-665	SL1344 <i>sraL</i> (Δ <i>sraL</i>)	This study
CMA-666	SL1344 <i>sraL proQ</i> (Δ <i>sraL</i> Δ <i>proQ</i> ::Cm ^R)	This study
JVS-0255	SL1344 Δ <i>hfq</i> ::Cm ^R	(5)
MA11598	LT2 <i>pgaA1</i> (Δ)- <i>lacZY aph</i> Δ <i>csrA</i> ::cat <i>sup2</i>	(6)

Table S3 - List of plasmids used in this work

Plasmid	Comments	Origin/Marker	Source / Reference
pKD3	Template for mutants construction; carries chloramphenicol-resistance cassette	oriR γ /Amp ^R	(7)
pKD46	Temperature-sensitive lambda-red recombinase expression plasmid	oriR101/Amp ^R	(7)
pCP20	Temperature-sensitive FLP recombinase expression plasmid	Amp ^R /Cm ^R	(7)
pET-28a	Inducible expression vector, N-terminal His Tag	Kan ^R	Novagen
pWSK29	Constitutive expression plasmid	pSC101/Amp ^R	(8)
pZE12Luc	P _{LlacO} promoter; constitutive expression plasmid	ColE1/Amp ^R	(9)
pISVA-001	pZE12luc derivative; P _{LlacO} promoter; constitutive plasmid expressing SraL	ColE1/Amp ^R	(4)
pISVA-004	pET-28a encoding His-Rho protein	Kan ^R	This study
pISVA-005	pWSK29 derivative; constitutive plasmid expressing ProQ	pSC101/Amp ^R	This study
pBAD-MS2	pBAD24 + MS2 aptamer; arabinose inducible plasmid	pBR322/Amp ^R	(10)
pBR- <i>plac</i>	P _{LlacO} promoter expression vector	pBR322/Amp ^R	(11)
pBR- <i>plac2</i>	P _{LlacO} promoter expression vector	pBR322/Amp ^R	This study
pBR- <i>plac2-sraL</i>	P _{LlacO} promoter expression vector; plasmid expressing SraL; IPTG inducible promoter	pBR322/Amp ^R	This study
pBR- <i>plac2 MS2</i>	P _{LlacO} promoter expression vector	pBR322/Amp ^R	This study
pBR- <i>plac2 MS2-sraL</i>	P _{LlacO} promoter expression vector; plasmid expressing MS2-SraL; IPTG inducible promoter	pBR322/Amp ^R	This study
pSVA-6	pWSK29 derivative; constitutive plasmid expressing Hfq	pSC101/Amp ^R	(12)

Supplementary Materials and Methods

Bacterial strains and plasmids

The chromosomal point mutants in *rho* (CMA-660) and *sraL* (CMA-661) were obtained by a multiple-step PCR process. The strain CMA-659 was constructed by inserting a Cm^R cassette 89 nucleotides upstream of *rho* transcription start site, in the intergenic region between *rho* and its upstream gene *trxA*. The Cm^R was amplified from plasmid pKD3 using the primers pair pIS-041/pIS-042. The resulting PCR product was integrated into SL1344 wild-type chromosome using the λ -red recombinase method (7). Subsequently, DNA fragments containing the Cm^R cassette and the nucleotides substitutions in *rho* were prepared using two primers pairs pIS-043/pIS-045 and pIS-044/pIS-046 and using CMA-659 genomic DNA as template. In the case of *sraL*, the DNA fragments were prepared using the primers pairs pIS-027/pIS-048 and pIS-047/pIS-030 and the previously constructed CMA-654 (4) genomic DNA as template. The two resulting DNA fragments of each gene were mixed and used as PCR templates to obtain a DNA fragment containing Cm^R cassette and the nucleotides substitutions using the primers pair pIS-043/pIS-046 for *rho* and pIS-027/pIS-030 for *sraL*. The resulting DNA fragments were purified and integrated into SL1344 by the λ -red recombinase method (7). The mutants were subsequently transferred to a fresh genetic background (SL1344 strain) by P22 HT105/1 int-201 transduction (13). The presence of the expected substitutions was confirmed by DNA sequencing (STAB Vida, Portugal). The strains CMA-662 and CMA-665 were obtained by eliminating chloramphenicol resistance gene from strains CMA-661 and CMA-651, respectively, using plasmid pCP20 (7). The double mutants (CMA-663 and CMA-666) were constructed using P22 HT105/1 int-201 transduction method (13).

proQ null mutant (CMA-664) was also constructed using the λ -red recombinase method (7) using the primer pair pIS-068/pIS-069. Chloramphenicol-resistance cassette of plasmid pKD3 replaces nucleotides -56 to +439 of *proQ* gene. Gene deletion was confirmed by colony PCR with primer pair pIS-070/pIS-071.

The pBR-*plac2-sraL* plasmid was constructed as follow. The multi-cloning site of the pBR-*plac* (11) was modified to introduce new endoribonuclease cleavage sites (XhoI, SmaI, SacI) by annealing oligonucleotides EM2888 and EM2889 and cloned into pBR-*plac* AatII/EcoRI-digested. The resulting plasmid was called pBR-*plac2*. The *sraL* gene was PCR-amplified from *Salmonella* Typhimurium SL1344 with primers EM2886 and EM2885. PCR product was then digested with XhoI and HindIII and inserted in pBR-*plac2* cut with the same enzymes to generate the pBR-*plac2-sraL* plasmid. Note: *sraL* was not cloned directly at the +1 of the pBR-*plac2* plasmid due to the presence of an AatII cleavage site in its sequence.

The plasmid pBR-*plac2 MS2-sraL* was constructed as follow. Plasmid pBR-*plac2 MS2* was generated using a PCR reaction from the plasmid pBAD-*MS2* (14) with the following oligonucleotides, EM2883 and EM1724. The PCR product was digested with appropriate restriction enzymes and ligated into an equivalently digested pBR-*plac2*. Plasmid pBR-*plac2 MS2-sraL* was generated using a PCR reaction with EM2884 and EM2885 with *Salmonella* Typhimurium SL1344 genomic DNA as matrix. The PCR product and plasmid pBR-*plac2 MS2* were then digested with EcoRI and HindIII and ligated. In this construct, *MS2* aptamer was fused to the 5'-end of *SraL*.

To overexpress *Salmonella* Rho protein, SL1344 *rho* coding region was amplified with primer pair pIS-058/pIS-059. The purified PCR product was digested with NdeI and

HindIII and ligated to the pET-28a plasmid previously digested with the same enzymes, giving rise to pISVA-004. The plasmid was first transformed into *E. coli* DH5 α and was subsequently cloned into BL21(DE3) strain.

To obtain pISVA-005 plasmid expressing ProQ, the entire *proQ* sequence was amplified from SL1344 chromosome using the primer pair pIS-072/pIS-073. The resultant PCR fragment was digested with XbaI and HindIII and ligated into pWSK29 plasmid previously digested with the same enzymes.

All these constructs were verified by sequencing.

MS2-affinity purification coupled with RNA sequencing

Salmonella Typhimurium SL1344 strains were grown in LB medium at 37°C (100 mL). Cells were harvested in (A) exponential phase after anaerobic shock and in (B) late stationary phase. MAPS was performed as previously described in (14, 15). Briefly, the expression of MS2-*sraL* (or *sraL* control) was induced by addition of 1 mM IPTG for 10 minutes. Then, harvested cells were chilled for 20 minutes on ice. Cells were then centrifuged, resuspended in 1 mL of buffer A (20 mM Tris-HCl at pH 8.0, 150 mM KCl, 1 mM MgCl₂, 1 mM DTT). Cells were then transferred to screw cap tubes containing 200 μ L of glass beads (0.1 mm bead diameter; BioSpec). Cells were disrupted using Precellys®24 Homogenizer (three cycles: 6500 rpm for 20 seconds, followed by 1 minute on ice; Bertin). The lysate was centrifuged at 17,000g for 30 minutes at 4°C and the supernatant was applied to the column for affinity purification. MS2-affinity purification was performed as previously described (15). Both lysates were applied on the same column (200 pmol of the MS2-MBP protein immobilized on an amylose resin). Afterward, RNA samples (output) were treated with TURBO™DNase (Ambion). cDNA libraries were

prepared with ScriptSeq™ v3 RNA-Seq Library Preparation Kit (Illumina) and sequenced (MiSeq sequencing system; Illumina). We used Galaxy Project (16) to analyze data. The whole list of genes enriched is available in Supplementary Dataset S1. The GEO accession number is GSE108234.

In vitro transcription termination assays

DNA templates for the *in vitro* transcription assays were prepared by PCR using genomic DNA from SL1344 wild-type strains for the wild-type transcripts, and the chromosomal DNA from the CMA-660 for the *rho*MUT transcript, and the following primer pairs: pIS-060/pIS-061 and pIS-062/pIS-061 for *rho*WT; pIS-063/pIS-064 for *sraL*; and pIS-066/pIS-067 for *dsrA*. *In vitro* transcription using T7 RNA polymerase and “riboprobe *in vitro* transcription system” (Promega) was performed to obtain *sraL* and *dsrA* transcripts. The transcripts were then purified by electrophoresis on an 8.3 M urea / 8% polyacrylamide gel, identified by ethidium bromide staining, and cut out from the gel. The RNA was eluted (3 M ammonium acetate at pH 5.2, 1 mM EDTA, 2.5% [v/v] phenol at pH 4.3) overnight at room temperature. The RNA was ethanol precipitated and resuspended in RNase free water. Transcription termination experiments were performed as described previously (17). Briefly, DNA template (5 nM; *rho*WT or *rho*MUT), *E. coli* RNA polymerase (0.0135 U/μl; New English Biolabs), Rho (0 nM or 70 nM, hexamer concentration) and RNasin (0,4 U/μl; Promega) were mixed in transcription buffer (40 mM Tris-HCl pH 8, 5 mM MgCl₂, 1.5 mM DTT, 0.05 mg/ml BSA and 100 mM KCl). After 10 min at 37°C, the indicated sRNAs (*SraL*, *DsrA* or Anti) were added at a final concentration of 0.433 μM. After addition of transcription initiation mixture (0.2 mM rATP, 0.2 mM rCTP, 0.2 mM rGTP, 0.02 mM rUTP, 0.2 μCi/μl of ³²P-αUTP, 25 μg/ml rifampicin in transcription buffer) the samples

were incubated for 20 min at 37°C. Each reaction was stopped with 4 µl of EDTA (0.5 M pH 7.5), 2 µl of Yeast tRNA (0.25 mg/ml; Invitrogen) and 10 µl of sodium acetate (3 M pH 5.2), and subjected to phenol-chloroform extraction and ethanol precipitation as described (17). Reaction pellets were dissolved in resuspension buffer (10 mM MOPS, 1 mM EDTA, pH 7.5), mixed with RNA loading buffer (95% [v/v] formamide, 0.1% [w/v] xylene cyanol, 0.1% [w/v] bromophenol blue, 10 mM EDTA) and analysed by denaturing 8.3 M urea / 6% polyacrylamide gel electrophoresis.

β-Galactosidase activity assays

β-Galactosidase activity was assayed as previously described by Miller (18), with some modifications. Bacterial cells in middle exponential phase (OD₆₀₀ of 1), stationary phase (OD₆₀₀ of 2 plus 6 h) and upon anaerobic shock were harvested and resuspended in Buffer 1 (60 mM Na₂HPO₄·7H₂O, 40 mM NaH₂PO₄·H₂O, 10 mM KCl, 1 mM MgSO₄, and 50 mM β-mercaptoethanol, pH 7). The optical density at 600 nm was determined and a specific volume of cells was used to assess LacZ expression using *o*-nitrophenyl- β-D-galactopyranoside (ONPG; Sigma) as substrate. The reaction was stopped with 1 M of Na₂CO₃ when the suspension reached a light-yellow color. OD₄₂₀ and OD₅₀₀ were measured and β-Galactosidase activity expressed in Miller Units. Paired t-test statistical analysis was carried out using GraphPad Prism 6 software.

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Additional data table S1 (separate file)

Dataset S1 - Summary table of all enriched RNAs using MAPS in *sraL* background. Genes are named according to *Salmonella enterica* serovar Typhimurium SL1344 nomenclature (NCBI Genome Database NC_016810.1). Reads were normalized by coverage. gene1_gene2 represents the intergenic region between gene1 and gene2 (including 5' and 3'-UTR). The first entry corresponds to SraL; the previous described SraL target (trigger factor) and Rho are highlighted.