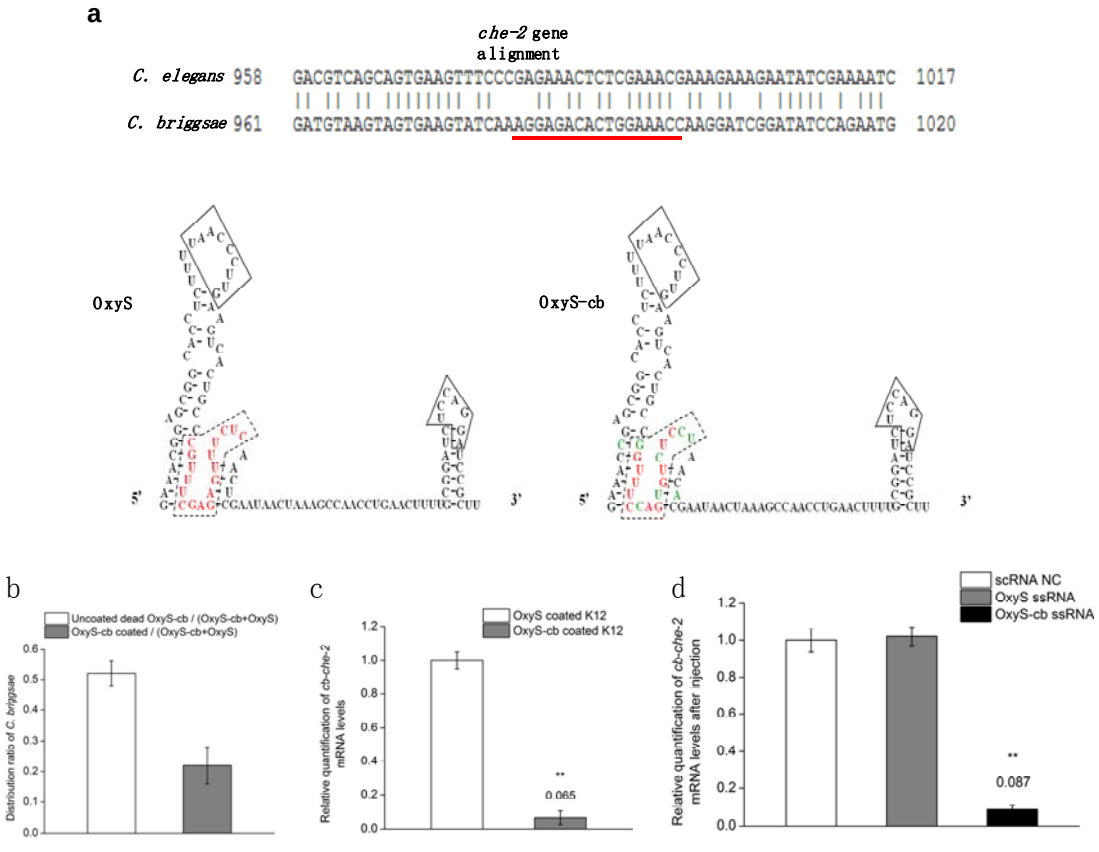
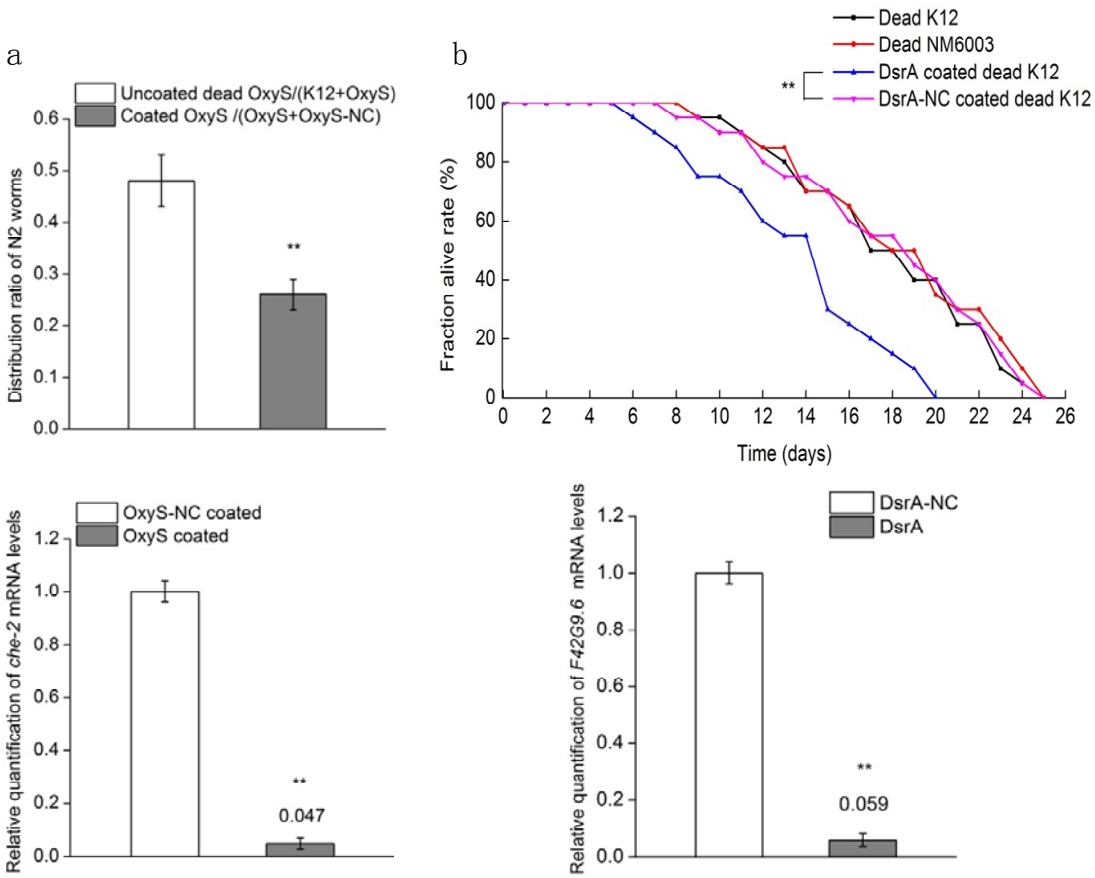
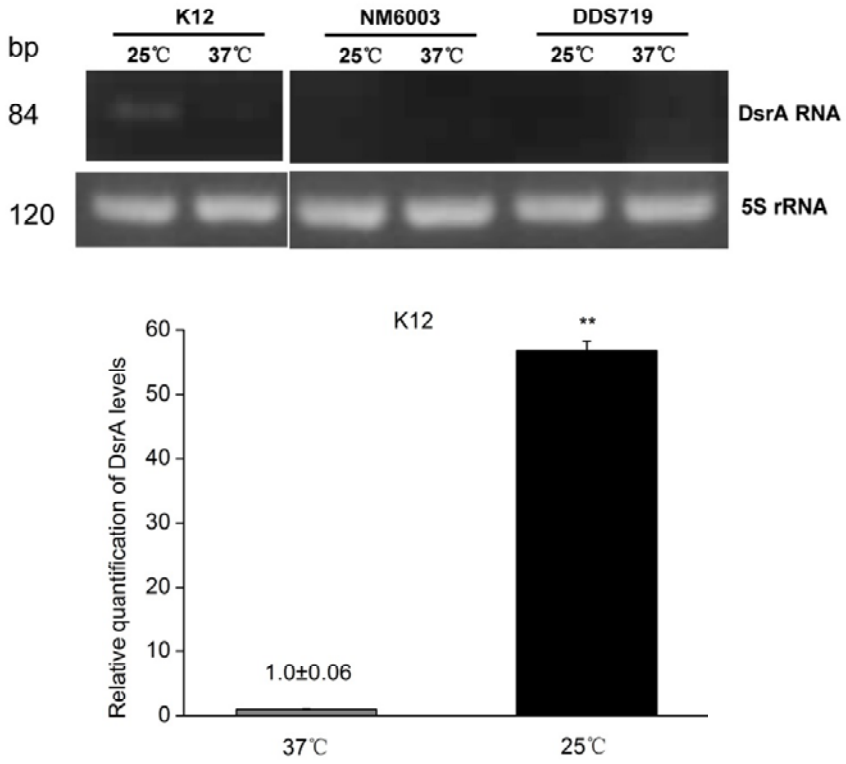
Supplementary Figures



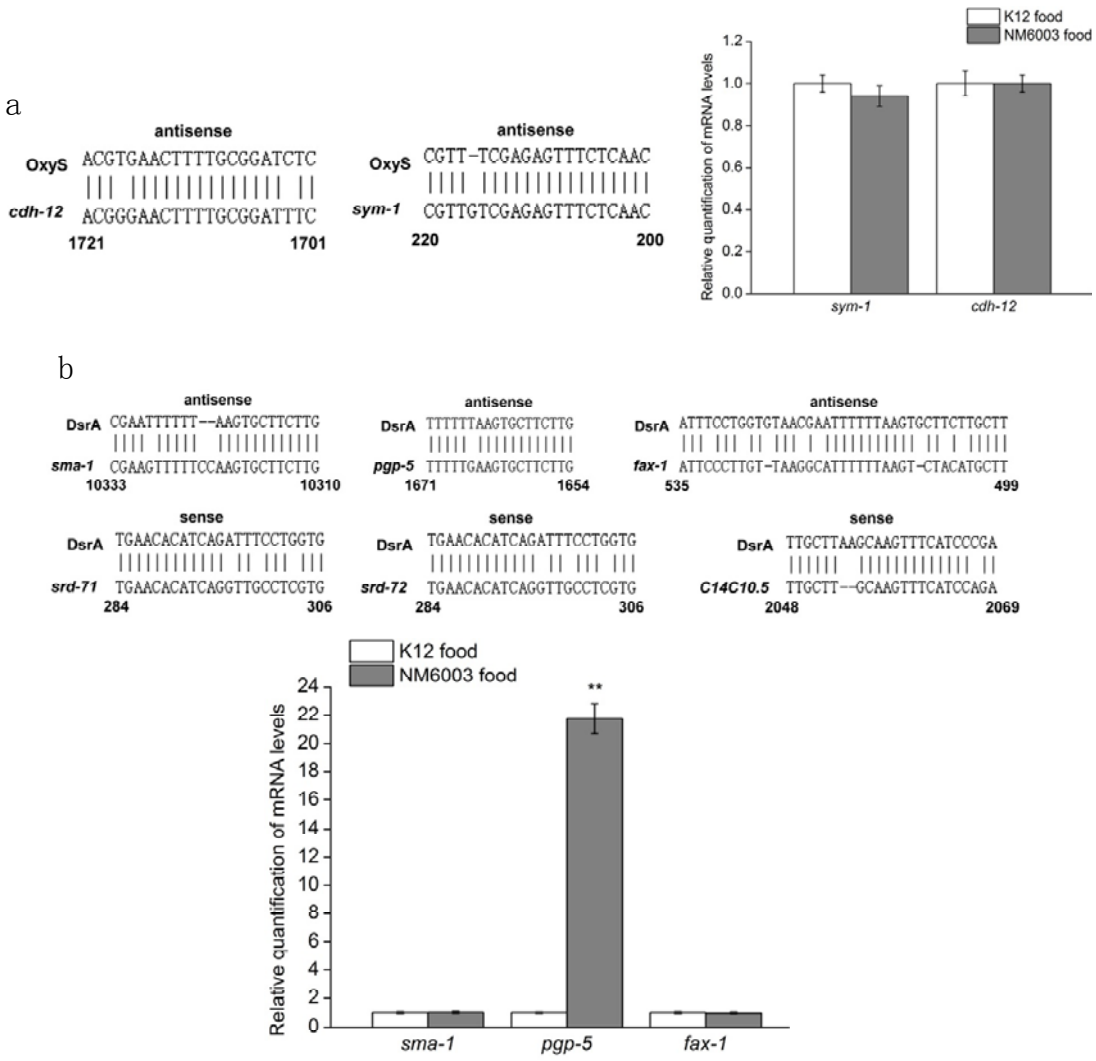
Supplementary Figure S1 | OxyS-cb sequences and effect of OxyS-cb & OxyS-cb small RNA. (**a**) The OxyS targeting sequence in the *C. elegans che-2* is not conserved in the *C. briggsae che-2* (underlined with red). To construct OxyS-cb plasmid that expresses OxyS-cb, the 17 nt OxyS sequence complementary to *C. elegans che-2* was changed to complement with *C. briggsae che-2*. The overall structure of OxyS was kept in the artificial OxyS-cb. The changed nucleotides were shown in green. (**b**) *C. briggsae* did not show discrimination to UV killed bacteria no matter they once expressed OxyS or OxyS-cb, while showed discrimination between OxyS-cb RNA (with RNase inhibitor) coated and negative control OxyS RNA (with RNase inhibitor) coated dead bacteria. (**c**) Real-time PCR revealed a decrease in *cb-che-2* level in *C. briggsae* fed with OxyS-cb coated dead bacteria. Boiled or UV killed bacteria gave similar experimental results, and data from UV killed bacteria were shown. (**d**) Injection of 17 nt small RNAs (OxyS-cb ssRNA) targeting to *C. briggsae che-2* (*cb-che-2*) could down-regulate *cb-che-2*; 17 nt OxyS small RNAs targeting to *C. elegans che-2* (OxyS ssRNA) and 17 nt small RNAs with a scramble sequence (scRNA NC) were used as negative controls. N=40 worms for each group of behavioral or longevity assays. The Student's t-test was used to calculate *P* values. ** *P* value <0.01. All data are from three repeat experiments. Error bars represent s.e.m.



Supplementary Figure S2 | Physiological effects on *C. elegans* of dead *E. coli* coated with OxyS or DsrA. (**a**) *C. elegans* did not show discrimination to UV killed bacteria no matter they once expressed OxyS or not, while showed discrimination between OxyS RNA (with RNase inhibitor) coated and negative control RNA (OxyS-NC, with RNase inhibitor) coated dead bacteria (food comparison assays for 24 hours). Real-time PCR revealed a decrease in *che-2* level in worms fed with OxyS coated dead bacteria for 24 hours. (**b**) No significant difference in longevity between worms lived on dead bacteria no matter they once expressed DsrA or not, while showed significant difference in longevity on DsrA RNA (fresh RNA added every day with RNase inhibitor) and negative control RNA (DsrA-NC, with RNase inhibitor) coated dead bacteria. Real-time PCR revealed a decrease in *F42G9.6* level in worms fed with DsrA coated dead bacteria for 48 hours. Boiled or UV killed bacteria gave similar experimental results, and data from UV killed bacteria were shown. N=40 worms for each group of behavioral assays, and 20 worms for longevity assay. Real-time PCR data were collected from three repeats. The log-rank test was used to calculate *P* values for longevity assays. The Student's t-test was used to calculate *P* values for other data. ** *P* value <0.01. All data are from three repeat experiments. Error bars represent s.e.m.



Supplementary Figure S3 | DsrA expression levels at 37 °C and 25 °C of *E. coli* strains. Semi-quantitative RT-PCR (top) and real-time PCR (bottom) results were shown. Two DsrA deletion strains, NM6003 and DDS719, expressed no DsrA at both temperatures. K12 bacteria expressed DsrA RNA at much higher level at 25 °C. The Student's t-test was used to calculate *P* values. ** *P* value <0.01. All data are from three repeat experiments. Error bars represent s.e.m.

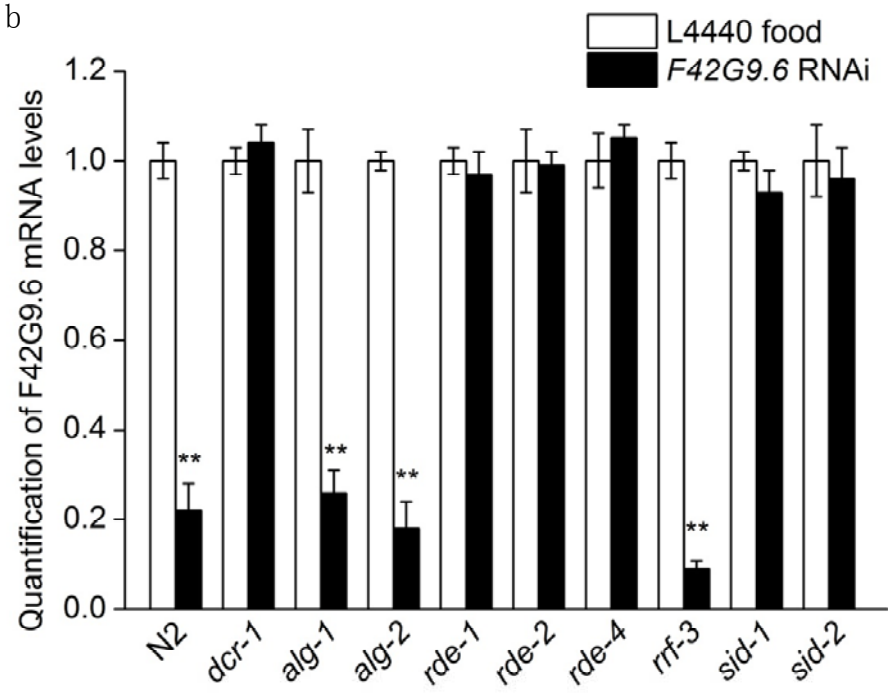


Supplementary Figure S4 | Other potential *C. elegans* target genes of OxyS and DsrA. (**a**) We examined the expression of *sym-1* and *cdh-12* with real-time PCR. These two genes have comparable stretches of identity to OxyS. No significant difference in their expression levels between worms fed on *E. coli* with OxyS expressing and those on *E. coli* without OxyS expressing. (**b**) Six *C. elegans* genes, *pgp-5*, *sma-1*, *fax-1*, *srd-71*, *srd-72*, and *C14C10.5* contain DsrA related (sense or antisense) sequence in the coding region of their mRNA. Real-time PCR to compare their expression levels in *C. elegans* fed with K12 or NM6003 revealed that *pgp-5* but not *sma-1* or *fax-1* might also be a target of DsrA. We did not examine *srd-71*, *srd-72* and *C14C10.5* for mRNA levels. Our data above suggested that *F42G9.6* is the major DsrA target related to longevity. The Student's t-test was used to calculate *P* values. ** *P* value <0.01. All data are from three repeat experiments. Error bars represent s.e.m.

a

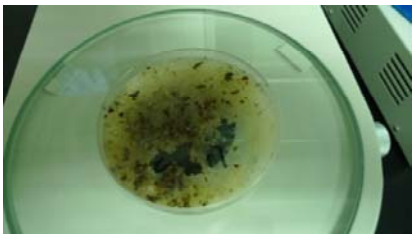
Genotype	che-2 feeding RNAi effective trials/total trials
N2	4/20
dcr-1	0/20
alg-1	3/20
alg-2	5/20
rde-1	0/20
rde-2	1/20
rde-4	0/20
rrf-3	13/20
sid-1	0/20
sid-2	0/20

b



Supplementary Figure S5 | Effect of dsRNA feeding RNAi in mutants of the RNAi pathway. Feeding was continued for 48 hours before the behavioral or real-time PCR assays (instead of examining the progeny, worms subjected to feeding RNAi were examined directly). (**a**) *che-2* dsRNA feeding RNAi in several mutants; 20 trials of *che-2* feeding RNAi were performed for each mutant, and the numbers of trials resulted in worms showed significant defect in "food searching ability assay" were shown. (**b**) *F42G9.6* dsRNA feeding RNAi in several mutants. The Student's t-test was used to calculate *P* values. ** *P* value <0.01. All data are from three repeat experiments. Error bars represent s.e.m.

Setup for the experiment



No-worm Plate after the experiment

Supplementary Figure S6 | Mice spread *C. elegans* and *E. coli* in a laboratory setup. A chamber of 1.0 m long and 0.4 m wide was used. Two 10 cm NMG plates without cover were placed at the opposite corners. One plate (worm plate) had worms with mixed ages well fed on OP50 (no dauers, medium density worms). The other plate (no-worm plate) had no worm or *E. coli* on it. One small fruit (e.g., apricot) was put on each plate as bait for mice. One mouse (C57BL/6) was allowed to stay for 12 hours in the chamber. The "no-worm" plate was examined for the presence of worms and *E. coli* four days after the experiment. In all three experiments, mice could transfer *C. elegans* and *E. coli* from the "worm plate" into the "no-worm plate". Some bacteria (including *E. coli*) on "no-worm" plates might also be from mouse droppings.

Supplementary Table S1

	ncRNA_ID	Potential Target numbers	Target Gene Names
1	Ecoli_CO299-3-AE016759	1	*K01A12.3*
2	Ecoli_CO465-2-AE005411	1	*pqn-32*
3	Ecoli_CO465-3-AE016762	1	*pqn-32*
4	Ecoli_CO465-4-U00096	2	*F54G8.1, pqn-32*
5	Ecoli_CO716-8-U00096	1	*B0303.11*
6	Ecoli_CO716-9-AE016766	1	*B0303.11*
7	Ecoli_CO716-10-AJ617685	1	*B0303.11*
8	Ecoli_CsrB-5-AE005507	2	*K02F6.1, sru-25*
9	Ecoli_CsrB-6-AE016765	2	*sru-25, K02F6.1*
10	Ecoli_CsrB-7-AF031251	2	*K02F6.1, sru-25*
11	Ecoli_CsrC-5-AE016770	2	*C16D9.1, ZC239.6*
12	Ecoli_CsrC-6-AP002567	2	*C16D9.1，ZC239.6*
13	Ecoli_CsrC-7-D16509	2	*C16D9.1, ZC239.6*
14	Ecoli_DicF-5-AP002557	1	*F35H10.2*
15	Ecoli_DicF-6-U00096	1	*F35H10.2*
16	Ecoli_DsrA-10-U00096	7	*F42G9.6, pgp-5, sma-1, fax-1, srd-71, srd-72, C14C10.5*
17	Ecoli_FinP-9-X55896	1	*shc-1*
18	Ecoli_GcvB-2-U00096	3	*T28F2.7, prx-13, C26B9.1*
19	Ecoli_IS061-7-U00096	2	*cyp-35A5, F26C11.3*
20	Ecoli_IS061-8-AE016760	1	*F26C11.3*
21	Ecoli_IS102-2-AJ586887	1	*ZC250.2*
22	Ecoli_IS102-4-U24429	3	*ZC250.2, oac-58, oac-57*
23	Ecoli_IS102-5-U00096	3	*ZC250.2, oac-58, oac-57*
24	Ecoli_IS128-7-U00096	1	*str-38*
25	Ecoli_MicC-9-AP002556	4	*skr-4, Y48A6B.6, F49C12.15, C49G7.7*
26	Ecoli_MicC-10-AE016760	1	*skr-4*
27	Ecoli_MicC-2-U00096	4	*skr-4, Y48A6B.6, F49C12.15, C49G7.7*
28	Ecoli_MicF-7-AP002560	2	*tag-65, C10G8.8*
29	Ecoli_MicF-8-D90850	2	*tag-65, C10G8.8*
30	Ecoli_MicF-9-U00096	2	*tag-65, C10G8.8*
31	Ecoli_OxyS-7-J04553	1	*che-2*
32	Ecoli_QUAD-8-AE016767	3	*C37H5.5, C18E3.3, E04D5.4*
33	Ecoli_QUAD-9-U00096	3	*C37H5.5, C18E3.3, E04D5.4*
34	Ecoli_QUAD-10-AP002564	2	*C37H5.5, C18E3.3*
35	Ecoli_QUAD-3-AP002563	2	*C06B8.7, ncl-1*
36	Ecoli_QUAD-4-AP002563	2	*C06B8.7, ncl-1*

37	Ecoli_QUAD-7-AP002560	2	*T08B6.1, F45C12.1*
38	Ecoli_RtT-3-U00096	2	*Y70C5C.1, F53C11.5*
39	Ecoli_RybB-5-U00096	2	*ZK418.9, act-5*
40	Ecoli_RyeE-2-U00096	2	*K12H4.4, sre-23*
41	Ecoli_RyfA-7-AE005482	1	*srx-87*
42	Ecoli_RyhB-8-U00096	1	*drsh-1*
43	Ecoli_SraB-8-AP002555	1	*Y51B9A.5*
44	Ecoli_SraC-5-AE005406	1	*F40G9.12*
45	Ecoli_SraC-6-AE016761	2	*E01G6.3, F40G9.12*
46	Ecoli_SraC-7-U00096	2	*E01G6.3, F40G9.12*
47	Ecoli_SraG-3-U00096	1	*F26E4.3*
48	Ecoli_SraJ-8-U00096	1	*B0334.13*
49	Ecoli_SroB-3-U00096	1	*Y66D12A.9*
50	Ecoli_tke1-9-AE016764	1	*epi-1*
51	Ecoli_tke1-10-U00096	1	*epi-1*
52	Ecoli_6S-4-X01238	0	
53	Ecoli_CO299-4-U73842	0	
54	Ecoli_CO343-6-AE016760	0	
55	Ecoli_CO343-7-X52647	0	
56	Ecoli_DicF-3-AP002554	0	
57	Ecoli_DicF-4-AP002556	0	
58	Ecoli_FinP-6-X55894	0	
59	Ecoli_FinP-7-U01159	0	
60	Ecoli_FinP-8-M20941	0	
61	Ecoli_IS102-3-AE016766	0	
62	Ecoli_IstR-1-8-U00096	0	
63	Ecoli_IstR-2-5-BA000007	0	
64	Ecoli_IstR-2-6-AE005174	0	
65	Ecoli_IstR-2-7-U00096	0	
66	Ecoli_QUAD-10-AE016763	0	
67	Ecoli_QUAD-2-U00096	0	
68	Ecoli_QUAD-4-AP002560	0	
69	Ecoli_QUAD-5-AE016766	0	
70	Ecoli_QUAD-5-U00096	0	
71	Ecoli_QUAD-6-AE016763	0	
72	Ecoli_QUAD-6-U00096	0	
73	Ecoli_QUAD-7-AP002564	0	
74	Ecoli_QUAD-8-AE005433	0	
75	Ecoli_QUAD-9-U00096	0	
76	Ecoli_RprA-9-U00096	0	
77	Ecoli_RtT-10-K01197	0	
78	Ecoli_RtT-2-U00096	0	
79	Ecoli_RtT-4-AP002556	0	

80	Ecoli_RtT-4-U00096	0	
81	Ecoli_RtT-5-AE016760	0	
82	Ecoli_RtT-5-S64921	0	
83	Ecoli_RtT-6-AE005340	0	
84	Ecoli_RtT-7-AP002556	0	
85	Ecoli_RtT-8-AP002556	0	
86	Ecoli_RtT-9-U00096	0	
87	Ecoli_RydB-2-AE016761	0	
88	Ecoli_RydB-3-AP002558	0	
89	Ecoli_RydB-4-U00096	0	
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95	Ecoli_RyfA-9-U00096	0	
96	Ecoli_Spot42-5-X01895	0	
97	Ecoli_SraB-9-AE016759	0	
98	Ecoli_SraD-4-AP002562	0	
99	Ecoli_SraD-5-U00096	0	
100	Ecoli_SraE_RygA_RygB-2-U00096	0	
101	Ecoli_SraE_RygA_RygB-3-U00096	0	
102	Ecoli_SraH-2-AP002564	0	
103	Ecoli_SraH-3-U00096	0	
104	Ecoli_SroC-10-U00096	0	
105	Ecoli_SroC-8-AP002552	0	
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108	Ecoli_SroH-4-U00096	0	
109	Ecoli_SroH-5-M88701	0	
110	Ecoli_t44-2-AE016755	0	

Supplementary Table S1

Sequence analysis for 110 unique *E. coli* sRNA sequences against *C. elegans* mRNA transcripts. *C. elegans* genes (mRNAs) with 17 nt or longer related sequence (complementary or identical) to each individual sRNA were shown. 1 or 2 nt of mismatches were allowed to interrupt the 17 nt or longer related sequence. The likelihood to find a 17 nt sequence by chance is $1/4^{17}$ (which is about 10^{-10}, and the genome size of *C. elegans* is 10^{8}).

Supplementary Table S2

GSM297742 Embryo

GSM297743 L1

GSM297744 L2

GSM297745 L3

GSM297746 L4

GSM297747 Adult (A)

GSM297748 Dauer

GSM297749 Glp-4 mutant

GSM297750 Mixed stage

Library Name: GSM297743 2158251

	Length (nt)	Count	count per 10^7
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.....TGGTGTAACGAATTTTTTAA.....	20	1	5
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.....GTGCTTCTTGCTTAAGCAAG.....	20	1	5
.....CTTCTTGCTTAAGCAAGTT.....	19	4	19
.....CTTCTTGCTTAAGCAAGTTTC.....	21	1	5
.....TTAAGCAAGTTTCATCCC.....	18	2	9
.....ACCCCCTCAGGGTCGGGATT.	20	2	9
.....ACCCCCTCAGGGTCGGGAT..	19	5	23
.....CCCCCTCAGGGTCGGGAT..	18	1	5
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.....CTAAAGGACCACATTGCTT.....	19	1	5
.....CTAAAGGACCACATTGCTTAAA.....	22	5	23
.....CTAAAGGACCACATTGCTTAAAA.....	23	1	5
.....CTAAAGGACCACATTGCTTA.....	20	4	19
.....TAAAGGACCACATTGCTTA.....	19	2	9
.....TAAAGGACCACATTGCTT.....	18	2	9
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...........AAGGACCACATTGCTTAAA.....	19	5	23
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...........AGGACCACATTGCTTAAA.....	18	4	19
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....................GCTTAAAAAATTCACGAAGAAC.....	22	1	5
........................AAAAAATTCACGAAGAAC.....	18	2	9
........................AAAATTCACGAAGAACGAATTC.....	22	1	5
........................AAATTCACGAAGAACGAAT.....	19	1	5
........................AATTCACGAAGAACGAAT.....	18	1	5
................................TCACGAAGAACGAATTCGTT.....	20	1	5
....................................ACGAATTCGTTCAAAGTAG.....	19	1	5
....................................CGAATTCGTTCAAAGTAG.....	18	18	83
....................................CGAATTCGTTCAAAGTAGGGCTGGG.....	25	5	23
....................................GAATTCGTTCAAAGTAGGGCTGGG.....	24	2	9
....................................TTCGTTCAAAGTAGGGCTGGG.....	21	1	5
....................................AAAGTAGGGCTGGGGGAGTCCC.....	22	2	9
....................................AAAGTAGGGCTGGGGGAGT.....	19	3	14

......................TTGAAGTCACTGCCCGTTTCGAG.....	23	2	9
..........AAAGCCAACGTGAACTTTTGCGGAT.....	25	1	5
GAAACGGAGCGGCACCTCTTTTAACCCTTGAAGTCACTGCCCGTTTCGAGAGTTTCTCAACTCGAATAACTAAAGCCAACGTGAACTTTTGCGGATCTCCAGGATCCGCT	S	Oxys	
CTTTGCCTCGCCGTGGAGAAAATTGGGAACTTCAGTGACGGGCAAAGCTCTCAAAGAGTTGAGCTTATTGATTTCGGTTGCACTTGAAAACGCCTAGAGGTCCTAGGCGA	AS		

Library Name: GSM297744 2093815

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.....ATCAGATTTCCTGGTGTAACGA.....	22	1	5
........AGATTTCCTGGTGTAACGA.....	19	2	10
.........GATTTCCTGGTGTAACGA.....	18	1	5
..........ATTTCCTGGTGTAACGAATTTTTTA.....	25	1	5
...........TTTCCTGGTGTAACGAAT.....	18	1	5
......................TTTTTTAAGTGCTTCTTGC.....	19	1	5
..........TTAAGTGCTTCTTGCTTAAGCAA.....	23	1	5
....................TTTCATCCCGACCCCCTCAGGGT.....	23	2	10
..........ACCCCCTCAGGGTCGGGAT..	19	2	10
AACACATCAGATTTCCTGGTGTAACGAATTTTTTAAGTGCTTCTTGCTTAAGCAAGTTTCATCCCGACCCCCTCAGGGTCGGGATTT	**S**	**dsrA**	
TTGTGTAGTCTAAAGGACCACATTGCTTAAAAAATTCACGAAGAACGAATTCGTTCAAAGTAGGGCTGGGGGAGTCCCAGCCCTAAA	**AS**		

.....TCTAAAGGACCACATTGCTT.....	20	1	5
.....TCTAAAGGACCACATTGCTTA.....	21	3	14
.....CTAAAGGACCACATTGCTT.....	19	1	5
.....CTAAAGGACCACATTGCTTAA.....	21	1	5
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.....GCTTAAAAAATTCACGAAGAA.....	21	2	10
.....CGAATTCGTTCAAAGTAG.....	18	5	24
.....CGAATTCGTTCAAAGTAGGGCTGGG.....	25	2	10
.....GAATTCGTTCAAAGTAGGGCTGGG.....	24	1	5
.....CAAAGTAGGGCTGGGGGAGT.....	20	1	5
.....AAAGTAGGGCTGGGGGAGTCCCA.....	23	1	5
.....AAAGTAGGGCTGGGGGAGT.....	19	1	5

.....ACTTTTGCGGATCTCCAGGATC....	22	2	10
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Library Name: GSM297745 3978045

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.....ATCAGATTTCCTGGTGTAACGA.....	22	2	5
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.....TTAAGTGCTTCTTGCTTAAGCA.....	22	1	3
.....TTAAGCAAGTTTCATCCC.....	18	2	5
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.....ACCCCCTCAGGGTCGGGATT.	20	3	8
.....ACCCCCTCAGGGTCGGGAT..	19	4	10
.....ACCCCCTCAGGGTCGGGA...	18	2	5
.....CCCTCAGGGTCGGGATTT	18	1	3

AACACATCAGATTTCCTGGTGTAACGAATTTTTTAAGTGCTTCTTGCTTAAGCAAGTTTCATCCCGACCCCCTCAGGGTCGGGATTT	**S**	**dsrA**	
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.......GTCTAAAGGACCACATTGCTTAAA.....	24	1	3
.......GTCTAAAGGACCACATTGCTTA.....	22	1	3
........TCTAAAGGACCACATTGCTTA.....	21	3	8
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..........TAAAGGACCACATTGCTTAA.....	20	1	3
...........AAAGGACCACATTGCTTAAA.....	20	1	3
...........AAAGGACCACATTGCTTA.....	18	6	15
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................CATTGCTTAAAAAATTCACGAAGAA.....	25	2	5
....................GCTTAAAAAATTCACGAAGAA.....	21	1	3
....................GCTTAAAAAATTCACGAAGAAC.....	22	1	3
........................AAAAATTCACGAAGAACGAAT.....	21	1	3
........................AAATTCACGAAGAACGAAT.....	19	1	3
..........CGAATTCGTTCAAAGTAG.....	18	12	30
..........CGAATTCGTTCAAAGTAGGGCTGGG.....	25	1	3
..........TCGTTCAAAGTAGGGCTGGGGGAGT.....	25	1	3
..........AAAGTAGGGCTGGGGGAGTCCCA.....	23	1	3
..........AAGTAGGGCTGGGGGAGT.....	18	1	3
..........GTAGGGCTGGGGGAGTCCCAG.....	21	1	3
..........ATAACTAAAGCCAACGTGAAC.....	21	2	5
..........ACTAAAGCCAACGTGAACTTTTGCG.....	25	1	3
GAAACGGAGCGGCACCTCTTTTAACCCTTGAAGTCACTGCCCGTTTCGAGAGTTTCTCAACTCGAATAACTAAAGCCAACGTGAACTTTTGCGGATCTCCAGGATCCGCT	**S**	**Oxys**	
CTTTGCCTCGCCGTGGAGAAAATTGGGAACTTCAGTGACGGGCAAAGCTCTCAAAGAGTTGAGCTTATTGATTTCGGTTGCACTTGAAAACGCCTAGAGGTCCTAGGCGA	**AS**		

Library Name: GSM297746 3942404

.....ATCAGATTTCCTGGTGTAACGA.....	22	1	3
.........GATTTCCTGGTGTAACGAAT.....	20	1	3
...........TTTCCTGGTGTAACGAATTTTTTAA.....	25	1	3
..........GACCCCCTCAGGGTCGGGATT.	21	1	3
AACACATCAGATTTCCTGGTGTAACGAATTTTTTAAGTGCTTCTTGCTTAAGCAAGTTTCATCCCGACCCCCTCAGGGTCGGGATTT	**S**	**dsrA**	
TTGTGTAGTCTAAAGGACCACATTGCTTAAAAAATTCACGAAGAACGAATTCGTTCAAAGTAGGGCTGGGGGAGTCCCAGCCCTAAA	**AS**		

Library Name: GSM297747	4302109		
..........TTTCCTGGTGTAACGAATTTTTTAA.....	25	1	2
..........TTTCCTGGTGTAACGAATT.....	19	2	5
........................TTTTTTAAGTGCTTCTTGCTTA.....	22	2	5
AACACATCAGATTTCCTGGTGTAACGAATTTTTTAAGTGCTTCTTGCTTAAGCAAGTTTCATCCCGACCCCCTCAGGGTCGGGATTT	**S**	**dsrA**	
TTGTGTAGTCTAAAGGACCACATTGCTTAAAAAATTCACGAAGAACGAATTCGTTCAAAGTAGGGCTGGGGGAGTCCCAGCCCTAAA	**AS**		
.....CGAATTCGTTCAAAGTAGGGCTGGG.....	25	1	2

Supplementary Table S2

Analysis of published deep sequencing data (NCBI GEO accession: GSE11734) showed that small RNAs corresponding to DsrA were present in *C. elegans* at L1, L2, and L3 stages. Small RNAs corresponding to OxyS may also be present in *C. elegans* with much fewer counts at L1, L2, and L3 stages. Low abundance of small RNAs originated from OxyS may be explained by the fact that *E. coli* is generally not under oxidative stress to express OxyS under laboratory setup for raising *C. elegans*. There were no small RNAs corresponding to DsrA in embryos and dauers, and this could be due to the fact that there is no or little feeding at these stages of *C. elegans*. For some unknown reason, there was almost no small RNAs originated from DsrA detected in L4, adult, and worms with mixed stages. With the fact that those small RNAs were detected from L1 to L3 stages, it was possible that *C. elegans* expressed relatively large amount of other small noncoding RNAs (e.g., small ncRNAs from germ line) from L4 stage, and thus “diluted” the amount of DsrA originated small RNAs. Four lines of evidence to support these small RNAs were not contamination from *E. coli*. First, there is no report about the existence of a DsrA antisense transcript in *E. coli*, and also our negative RT-PCR result supported its absence. Second, more small RNAs with DsrA antisense than sense sequence were present. Third, almost no small RNAs originated from DsrA detected in L4, adult, and worms with mixed stages; bacterial contamination would still be present for these samples. Fourth, unlike non-specific degenerative small RNAs with similar distribution with various sizes, DsrA antisense small RNAs had enrichment in sizes of 16-20 nt. Three quarters of antisense small RNAs were with these sizes, and the peak sizes were 17- and 18 nt (about one-fourth for each size in all DsrA antisense small RNAs). The sequences involved in targeting *F42G9.6* or *che-2* is underlined in DsrA or OxyS gene sequences.

Supplementary Table S3 Primers used in this study.

Primer names	**Sequence**	**Amplicon**	**Purpose**
Pche-2-F	GCCCGCGGGATAGT GTGAACTAGGCCC	2.1 kb	Pche-2::gfp construction
Pche-2-R	GCGGATCCCATTCTGCAAAGTTGAA AGCC	2.1 kb	Pche-2::gfp construction
PF42G9.6-F	GTCTAGATTCTTATAAGCGTGATCAC C	2.1 kb	PF42G9.6::gfp construction
PF42G9.6-R	GACCGGTGCGACTAGCGAGGGCAT CGCA	2.1 kb	PF42G9.6::gfp construction
F42G9.6 cDNA-F	GCAGGATCCATGCCCTCGCTAGTCG CTTTTAAC	2.5 kb	Pdpy-30::F42G9.6::gfp construction
F42G9.6 cDNA-R	GACGGTACCTTAACTGGTTGACTAG ACACGATTTCTTC	2.5 kb	Pdpy-30::F42G9.6::gfp construction
F42G9.6 5'RNAi-F	TTCCGCTTGCCTGTTCTACT	517 bp	F42G9.6 5'RNAi construction
F42G9.6 5'RNAi-R	CCTGCAACAACATCACTTGG	517 bp	F42G9.6 5'RNAi construction
che-2-RT-F	GG ATGTTTACTGCCAACTGG	224 bp	che-2 RT-PCR
che-2-RT-R	GATTTCGTTGATCAGGGAAAC	224 bp	che-2 RT-PCR
che-2 Real-F	AGGTTCGAGACGTCAGCAGT	N.A.	che-2 Real-time PCR
che-2 Real-R	GGATGATGTGACCACAATCAGATA	N.A.	che-2 Real-time PCR
cb-che-2 Real-F	AACGGTATGCTCCGTTCTGT	N.A.	C. briggsae che-2 Real-time PCR
cb-che-2 Real-R	ATTTAAGAGGAGCCACTTGC	N.A.	C. briggsae che-2 Real-time PCR
F42G9.6 RT-F	GACTGTAGATGTGGATCAAG	220 bp	F42G9.6 RT-PCR
F42G9.6 RT-R	TGTTAGAAGAGAACCGACTC	220 bp	F42G9.6 RT-PCR
F42G9.6 Real-F	AATGCTCAGAAGCGCTCGAT	N.A.	F42G9.6 Real-time PCR
F42G9.6 Real-R	CTACCAATTGATAGGAGGGA	N.A.	F42G9.6 Real-time PCR
E .coli 16S-F	AGAGTTTGATCCTGGCTCAG	1.5 kb	*E .coli* 16S rDNA PCR
E .coli 16S-R	CTACGGCTACCTTGTTACGA	1.5 kb	*E .coli* 16S rDNA PCR
C. elegans 18S-F	TACTGTCAGTTTCGACTGACTC	0.8 kb	*C. elegans* 18S rDNA PCR
C. elegans 18S-R	ATACGAACCCGAAGATTCGCC	0.8 kb	*C. elegans* 18S rDNA PCR
DsrA-F	AAATCCCGACCCTGAGGGG	84 bp	DsrA RT-PCR & Real-time PCR
DsrA-R	AACACATCAGATTTCCTGGT	84 bp	DsrA RT-PCR & Real-time PCR

E. coli 5S-F	ATGCCTGGCAGTTCCCTACT	120 bp	E. coli 5S RT-PCR PCR
E. coli 5S-R	TGCCTGGCGGCCGTAGCG	120 bp	E. coli 5S RT-PCR PCR
OxyS-F	GGCACCTCTTTTAACCCTTG	100 bp	OxyS RT-PCR & Real-time PCR
OxyS-R	CGGATCCTGGAGATCCGCAA	100 bp	OxyS RT-PCR & Real-time PCR
sym-1 Real-F	ATCTCCATTCCTGAGACACT	N.A.	sym-1 Real-time PCR
sym-1 Real-R	GTACTCGGCTTCATCTTCCT	N.A.	sym-1 Real-time PCR
pgp-5 Real-F	ATCAAGCTGTGGAGGAGGAT	N.A.	pgp-5 Real-time PCR
pgp-5 Real-R	GCTCGAACAGTTCTGACGTT	N.A.	pgp-5 Real-time PCR
cdh-12 Real-F	CACAGTTCTATCATGAGCCA	N.A.	cdh-12 Real-time PCR
cdh-12 Real-R	ACATCAATCGATAACTCTCT	N.A.	cdh-12 Real-time PCR
sma-1 Real-F	CGAGCATGTGGATAATCTGA	N.A.	sma-1 Real-time PCR
sma-1 Real-R	AAATGATGATAGTACAAGGA	N.A.	sma-1 Real-time PCR
fax-1 Real-F	ACTCAATGGCCGCGTCTCCT	N.A.	fax-1 Real-time PCR
fax-1 Real-R	GGCGAAACGTCTAAACGTCG	N.A.	fax-1 Real-time PCR
daf-2 Real-F	TGCATCGAAGTCTACAGCCA	N.A.	daf-2 Real-time PCR
daf-2 Real-R	GGCCTCCAATTACACGAAGA	N.A.	daf-2 Real-time PCR
daf-15 Real-F	GTGCATGTATGCATCAGATG	N.A.	daf-15 Real-time PCR
daf-15 Real-R	AATTGAGATACATAAGATGG	N.A.	daf-15 Real-time PCR
E. coli 5S Real-F	CATGCCGAACTCAGAAGTGA	N.A.	E. coli 5S Real-time PCR
E. coli 5S Real-R	CTGGCAGTTCCCTACTCTCG	N.A.	E. coli 5S Real-time PCR
actin-F	TCGTCCTCGACTCTGGAGAT	102 bp	actin RT-PCR & Real-time PCR
actin-R	AGATCACGTCCAGCCAAGTC	102 bp	actin RT-PCR & Real-time PCR
OXYS-CB-F1	TGAGAATTCCTCACAGAAACCGAG	N.A.	molecular cloning of OxyS-cb
OXYS-CB-R1	GTTAGGAGACACTGGAAACCGGCAGTG	N.A.	molecular cloning of OxyS-cb
OXYS-CB-F2	CACTGCCGGTTTCCAGTGTCTCCTAAC	N.A.	molecular cloning of OxyS-cb
OXYS-CB-R2	TGAGCCAAGCTTATCGCCGGG	N.A.	molecular cloning of OxyS-cb
T7-PKK-OXYS-F	CCCAAGCTTACAATTTCACACAGGAAACA	N.A.	For in vitro synthesis of OxyS
T7-F	CCGTCTAGATAATACGACTCACTATAGGGCCCAAGCTT	N.A.	For in vitro synthesis of OxyS
T7-PKK-OXYS-R	CCGGAATTCAGTTATTCGAGTTGAGAA	N.A.	For in vitro synthesis of OxyS
T7-DSRA-F	CCCAAGCTTGCGGATAAGGTGATGAACACA	N.A.	For in vitro synthesis of DsrA
T7-DSRA-R	GCGTCTCTGAAGTGAATCGTT	N.A.	For in vitro synthesis of DsrA

Supplementary Methods

Locomotion assays. L4 and adult worms were examined at 20 °C on NGM plates seeded with the corresponding *E. coli* strain. Individual animals were allowed to cut tracks for 10 min before paths were measured. Tracks were measured from photographs. Locomotion was quantitated by measuring the amplitude of the path and the distance between successive peaks in the path[34].

Lifespan assay. Animals were kept on 60mm NGM plates at 25 °C. Young adult animals were transferred to plates with the corresponding bacteria, and allowed to lay eggs for about 6 hours and then removed. The time point was then defined as the onset of the lifespan assays. Worms were transferred to new plates with adequate food away from the progeny every other day until no eggs were laid. Animals were checked every day, and scored as dead when they no longer moved or responded to gentle prodding with a platinum wire.

Feeding RNAi with *E. coli* expressing dsRNA. N2 and *daf-2*(*e1370*) synchronized worms were grown on 60mm NGM plates seeded with the bacteria expressing dsRNA against 5′-end of *F42G9.6*. *che-2* feeding RNAi were performed with the *C. elegans* ORF-RNAi Feeding Library v1.1, clone X-1C17. Bacteria carrying the empty vector (L4440) were used as negative control[35]. The lifespan assay was performed at 20°C for better effect of feeding RNAi. Behavioral assays were performed to evaluate the effects of *che-2* feeding RNAi. Real-time PCRs were also performed for some feeding RNAi against *F42G9.6* or *che-2* in multiple *C. elegans* mutants.

Oxidative stress to *E. coli*. K12 *E. coli* was treated with 60 μM (final concentration) of hydrogen peroxide for 15 minutes, and the expression level of OxyS was then evaluated with real-time PCR.

***C. elegans* oxidative stress assay.** Oxidative stress assay was conducted at 20°C. The young adult N2 and *daf-2*(*e1370*) worms, which were incubated with either L4440 or RNAi against 5′-end of *F42G9.6,* were immersed in S-media containing 160 mM of paraquat (1,1-dimethyl-4,4- bipyridinium dichloride, Sigma-Aldrich)[36]. Worms were scored every hour until all worms were scored as dead.

Molecular biology. Synchronized worms from two 60-mm plates were collected, washed three times with M9 buffer, spun down, and lysed in 1 ml TRIzol (Invitrogen) to isolate total RNA. For isolation of total bacterial RNA, the bacteria samples were precipitated and lysed in 1 ml TRIzol. First strand cDNA was synthesized with SuperScript II reverse transcriptase (Invitrogen) and oligo$(dT)_{18}$ (for worms) or random hexamers (for bacteria). Real-time PCR were performed using 7500 Real-Time PCR System and SYBR Green Master Mix (Applied Biosystems) with the gene specific primer pairs. Actin (*act-2*) or 5S rRNA was used as internal control for worm and bacteria respectively. For the real-time PCR of worms injected for 17 nt small RNAs targeting to *C. briggsae che-2* (and the negative controls), RNA was isolated from 30 worms survived from the injection for each group (injection, RNA isolation, and real-time PCR were done with three repeats). For the data about *daf-2* and *daf-15* expression levels on *B. mycoides* verses on *E. coli* presented in Figure 6f, we used L3 worms raised on *E. coli*, and then split them onto *E. coli* and *B. mycoides* to grow up to young adult for comparison. For semi-quantitative RT-PCR, PCR was generally performed for 25 cycles to avoid saturation. Primers used and amplicon information are included in Supplementary Table S3.

Plasmid constructions. To construct oxyS-cb plasmid, OxyS sequence complementary to the *C. elegans che-2* was altered to complement with the *che-2* sequence of *C. briggsae* using fusion PCR. First, two PCR fragments were generated with OxyS specific two primer pairs, in which mutagenic nucleotides, HindIII, or EcoRI site are included. Then, these two fragments were fused by PCR amplification.

The resultant PCR products were cleaved with HindIII and EcoRI, and inserted into HindIII- and EcoRI-cleaved ppk223-3 vector. To generate *F42G9.6* 5' RNAi construct, 5' end of *F42G9.6* mRNA was amplified from oligo$(dT)_{18}$ primed first strand cDNA. The resultant PCR fragments were then inserted into the L4440 vector.

For the construction of *Pche-2::gfp* and *PF42G9.6::gfp*, a 2.1 kb *che-2* and *F42G9.6* promoter was amplified respectively from N2 genomic DNA by PCR. The resultant PCR fragments were inserted into the pPD117.01. For the construction of *Pdpy-30::F42G9.6::gfp*, a full-length *F42G9.6b* cDNA fused with GFP (from pPD95.77) was inserted into the ps235 vector, which already contains a *dpy-30* promoter. A *Pdpy-30::gfp* plasmid based on ps235 vector （Ref. 37）was also constructed without the *F42G9.6b* insertion. All plasmids were sequenced for confirmation. Primers used and amplicon information are included in Supplementary Table S3.

Western blot. Five hundred worms at L3 stage from the *Pche-2::che-2::gfp*;*rol-6* transgenic line were handpicked for each sample (worms feeding on K12, OxyR, and OxyS *E. coli* respectively). Whole cell lysates were separated on SDS-PAGE gels and then transferred to PVDF membranes (Millipore). Membranes were processed following the ECL Western blotting protocol (GE Healthcare). anti-GFP antibody (Millipore) was used at 0.1 microgram/ml. HRP-conjugated secondary antibodies were obtained from Sigma-Aldrich. For loading controls, membranes were stripped and reprobed with the antibody against β-Actin (Abcam) at 0.1 microgram/ml. Quantification was based on three repeats.

Imaging of *C. elegans*. Preparations were viewed on a Zeiss LSM 510 laser scanning confocal microscope and image stacks were collected using Zeiss 510 software (version 2.8). For preparations in which GFP expression was viewed directly, living animals were immobilized on 4% agar pads containing 1 mM sodium azide. Micrographs composed of visible and fluorescent images were made by collapsing

fluorescent image stacks. Images were exported into Adobe Photoshop for final modifications.

Quantification of GFP intensity. Images for analyzing the expression intensity were taken under the same microscopy parameters. GFP intensities from twenty worms for each RNA injected were quantified with the ImageJ software. Data were presented with relative GFP intensity when the readings from negative controls were normalized to 1.0.

C. elegans **Transformation.** Transgenic lines were constructed by injecting plasmids at a concentration of 20 ng/µl with carrier DNA. All experiments were performed with at least two lines, although for some experiments, data from one line were presented as variations between lines were minimal.

Supplementary References:

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