Supplemental Files

Balanced cell division is secured by two different regulatory sites in OxyS RNA

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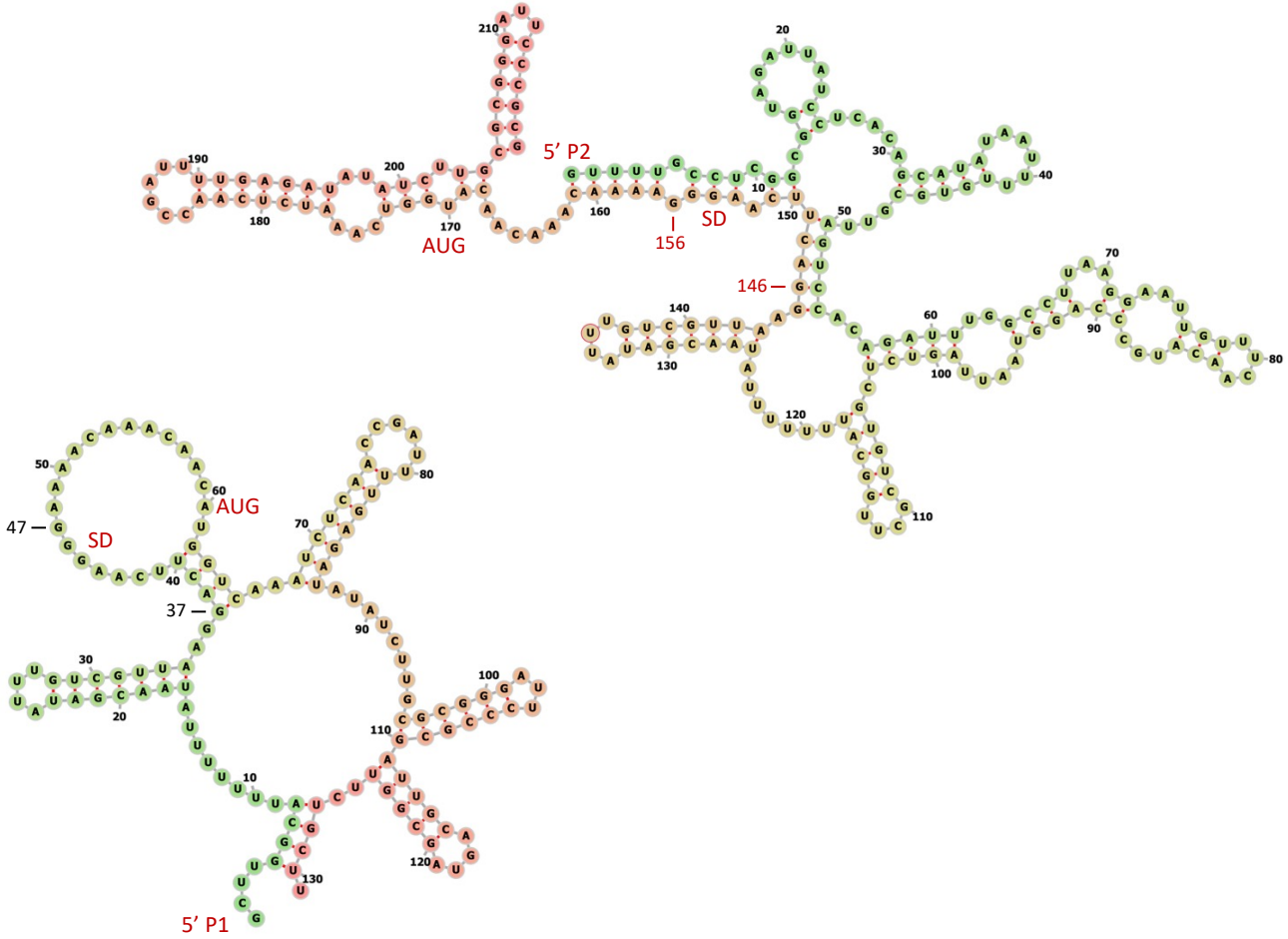
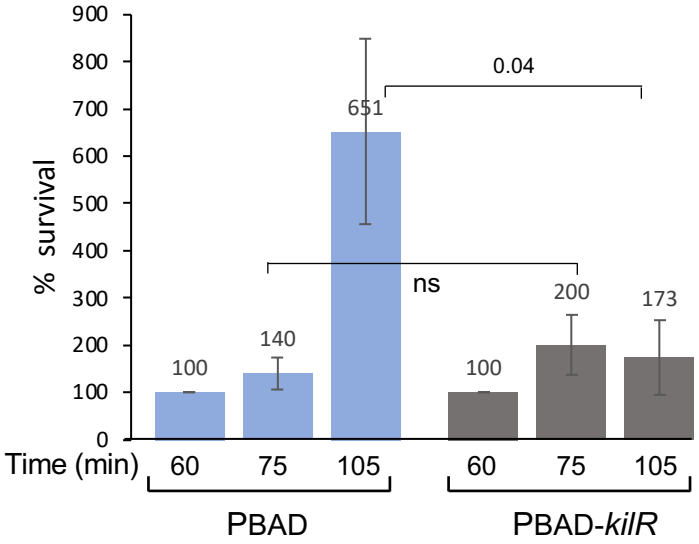
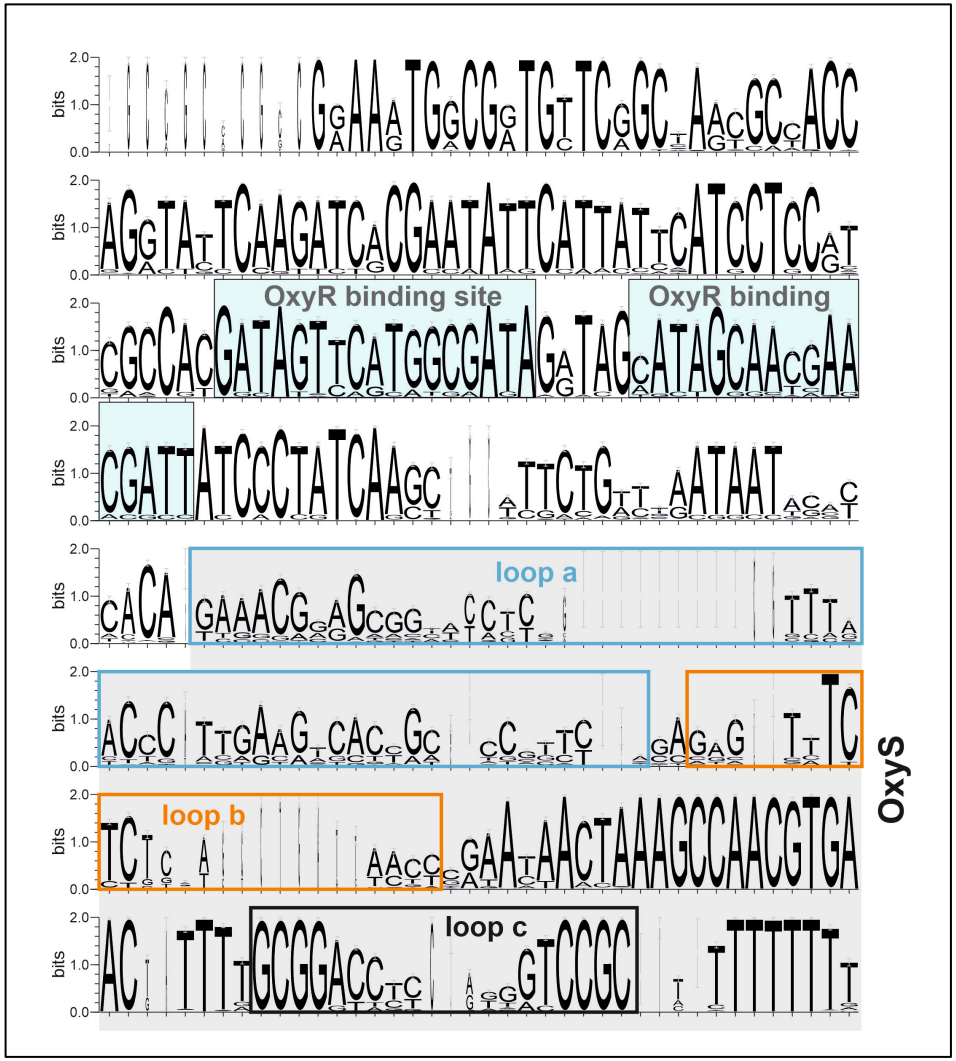


Supplemental Fig. S1 Predicted secondary structures of short and long *mepS* transcripts using RNA fold program (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi.) For transcripts details see Figure 2

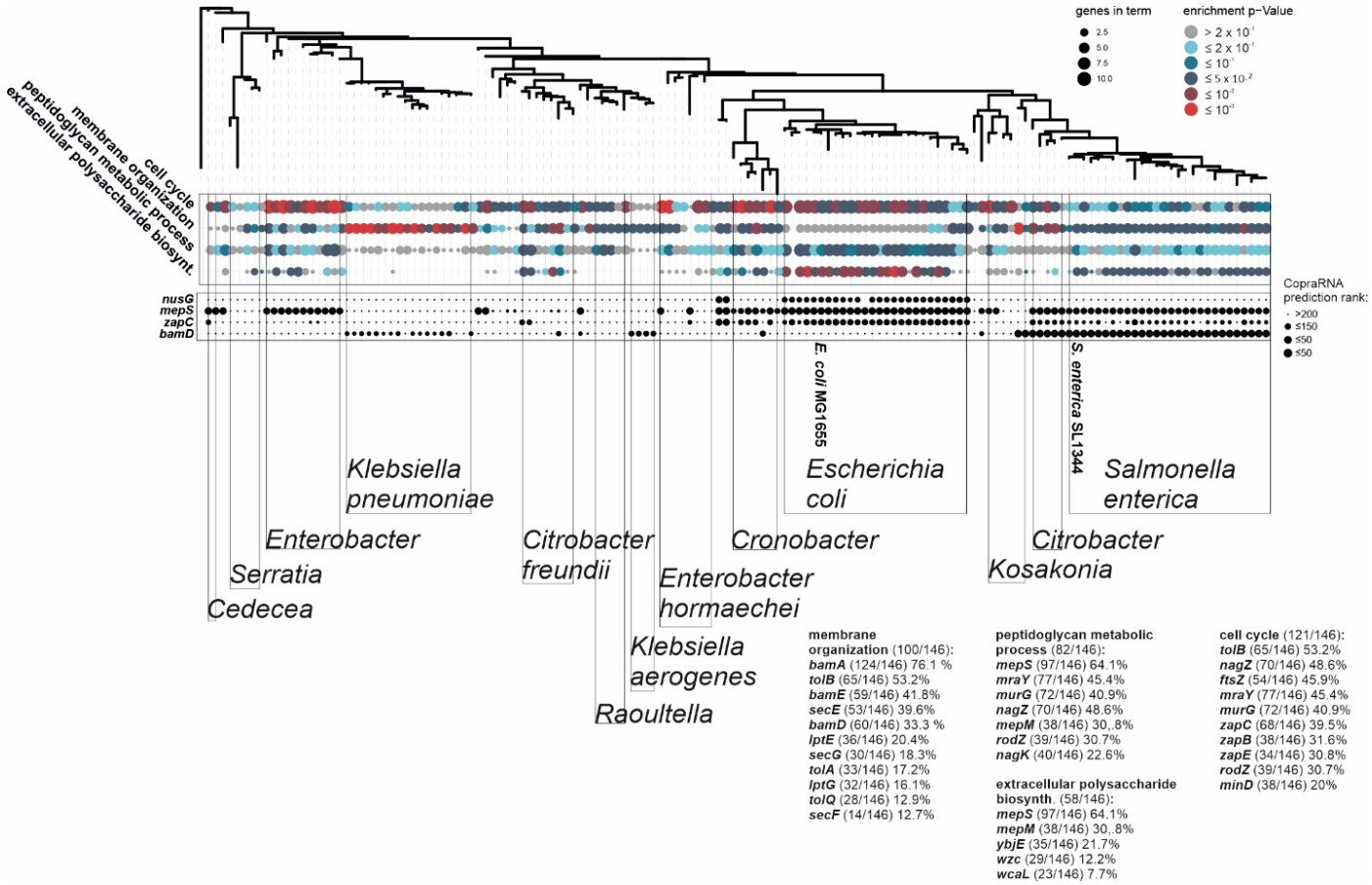


Supplemental Fig. S2. Cultures (MG1655 *mal*::*lacI*^{q} Δ*kil*::FRT) carrying P*tac* as well as PBAD or PBAD-*kilR* were treated with arabinose 0.2% and IPTG (1mM) at dilution, CFU were determined as indicated in the figure. Results are displayed as mean of 3 biological experiments ± standard deviation.

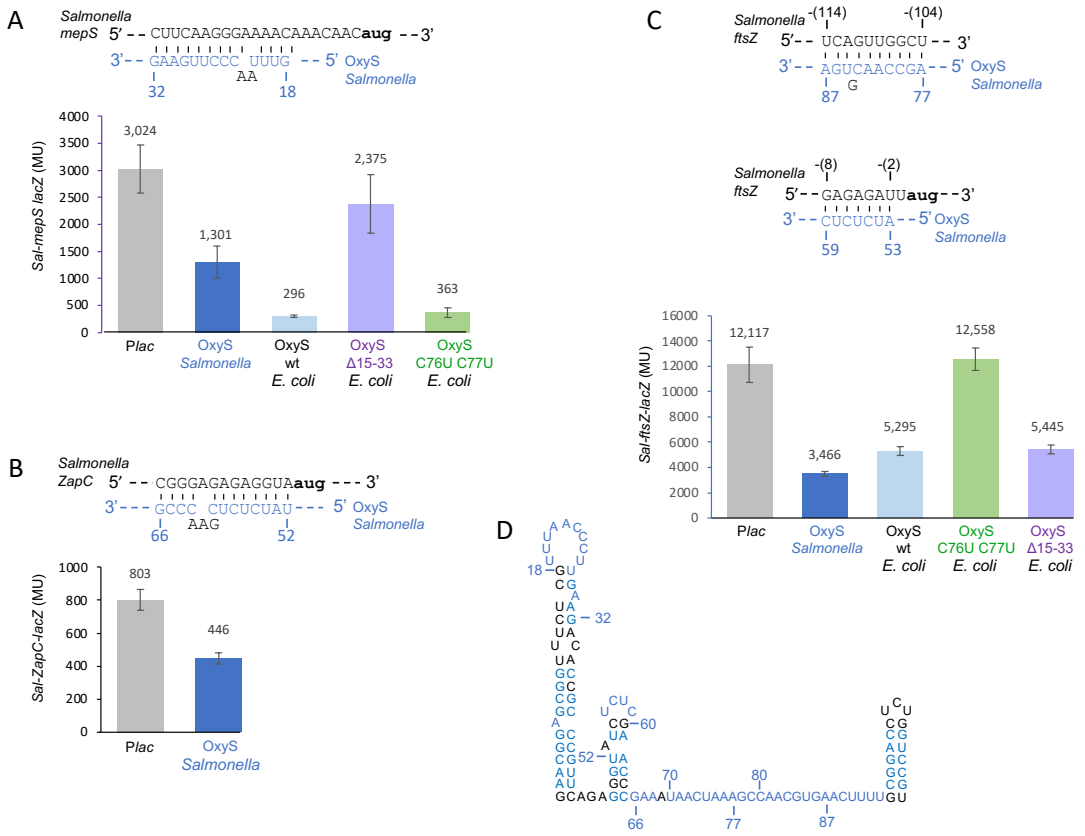


Supplemental Fig. S3. OxyS consensus motif based on an alignment of 1340 homologs. The actual sRNA sequence is highlighted in grey. The regions equivalent to loop a, loop b and loop c as defined in *E. co*li OxyS are boxed.

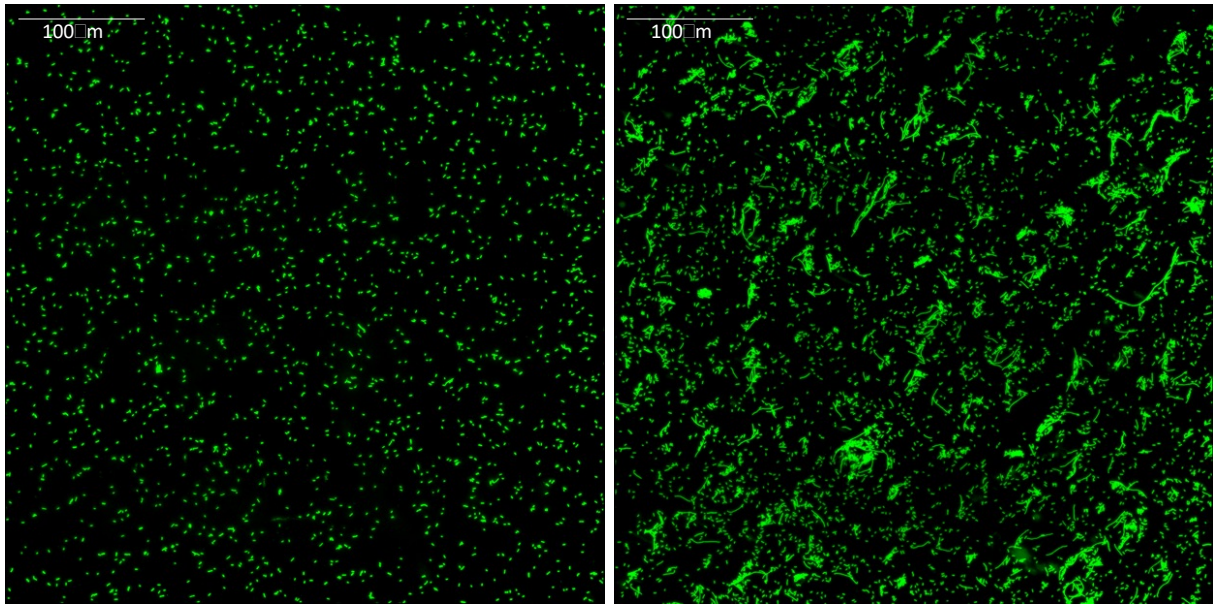
Supplemental Fig. S4. Rooted ML-tree based on the 16s rDNA sequences of the 146 investigated organisms. *Y. pseudotuberculosis* YPIII was used as an outgroup.



Supplemental Fig. S5. Phylogenetic conservation of predicted OxyS functions and targets. Rooted ML-tree based on the 16s rDNA sequences of the 146 investigated organisms. The position of *S. enterica* SL1344, *E. coli* MG1655 and other species groups is indicated at the right side. A reference tree with all species names is given in Supplemental Fig. S4. The middle dot-plot maps the gene set enrichment results for 4 selected terms (cell cycle GO:0007049, membrane organization GO:0061024, peptidoglycan metabolic process GO:0000270, extracellular polysaccharide biosynthetic process GO:0045226) to the tree. The dots are color-coded for the respective enrichment p-Values from red ($\leq 10^{-3}$) to light blue ($\leq 2*10^{-1}$) and grey ($> 2*10^{-1}$). The size of the dots increases with the number of genes belonging to the term in the top 200 target predictions of the respective organism. The right dot-plot shows the predicted conservation of specific targets (*nusG*, *mepS*, *zapC*, *bamD*). The dot size scales with the CopraRNA prediction ranks. The most frequent predicted targets belonging to the 4 displayed GO-terms are shown with the number of organisms where the target was predicted (XX/146) and the phylogenetically weighted abundance in % (See also Table 1 below).



Supplemental Fig. S6. *Salmonella* OxyS and cell division and cell elongation targets. (A,B) The position of the predicted base-pairing between *Salmonella mepS, zapC* and OxyS is close to the AUG of the targets (indicated in bold). (C) Two possible interaction sites between *ftsZ* and OxyS are presented, a non-canonical site positioned at 114 to 104 nt upstream of the AUG (top) and a more canonical site located at 8-2 nt upstream of the AUG (bottom). As OxyS C76U C77U is unable to repress *ftsZ-lacZ* indicates that repression is mediated via the top site (D) *Salmonella* OxyS sequence. Nucleotides conserved between *Salmonella* and *E. coli* are in blue. *Salmonella* cultures (SL1344 Δ *hisG*::*lacI*^{q}:cm) carrying translational fusions of *mepS, zapC and ftsZ* to *lacZ* (pSC101*) and P*lac*-OxyS as indicated were treated with IPTG (1mM) at dilution. β-galactosidase activity was measured 150 min after treatment. Results are displayed as mean of 5 biological experiments ± standard deviation).



Supplemental Fig. S7. Fluorescence microscopy images of *Salmonella* expressing OxyS. Cultures of *Salmonella* (A-834: SL1344 Δ*hisG*::*lacI*^{q} :cm) carrying control and *Salmonella* OxyS (P*lac-St-oxyS*) expressing plasmid were grown for 20 hours. OxyS was induced by 1 mM of IPTG from dilution. Scale bar 100□□m.

Supplemental Table S1. Top 10/5 most frequent BP/CC GO-terms in the predictions of the 146 selected organisms after deletion of terms with semantic overlaps.

GO class	ID	term	median pVAL	count	IC
Biological process	GO:0007049	cell cycle	0.02681	121	4.256431
Biological process	GO:0000097	sulfur amino acid biosynthetic process	0.047725	110	5.604985
Biological process	GO:0061024	membrane organization	0.0193	100	5.499624
Biological process	GO:0051128	regulation of cellular component organization	0.07619	87	4.757687
Biological process	GO:0010033	response to organic substance	0.0127	83	5.029621
Biological process	GO:0000270	peptidoglycan metabolic process	0.10079	82	4.389962
Biological process	GO:0006073	cellular glucan metabolic process	0.11508	71	5.604985
Biological process	GO:0010629	negative regulation of gene expression	0.059	63	3.782828
Biological process	GO:0045226	extracellular polysaccharide biosynthesis.	0.03595	58	5.722768
Biological process	GO:0000003	reproduction	0.034425	58	5.466834
Cellular compartment	GO:0009279	cell outer membrane	0.0794	73	3.553163
Cellular compartment	GO:0032153	cell division site	0.031	68	4.996616
Cellular compartment	GO:0098796	membrane protein complex	0.102	68	3.341193
Cellular compartment	GO:0005667	transcription regulator complex	0.0775	28	7.136682
Cellular compartment	GO:0032993	protein-DNA complex	0.0791	22	4.395842

Supplemental Table S2. Prediction of interactions between OxyS and possible target genes.
The numbers (from TSS and on) denote the first and the last nucleotide of the complementary region

CopraRNA *E.coli* prediciton rank	Gene	First nt in OxyS	Last nt in OxyS
3	b4365 - yjjQ	14	28
5	b2963 - mltC	12	32
8	b2441 - eutB	12	32
12	b2175 - mepS	25	35
15	b4529 - ydbJ	3	81
22	b3721 - bglB	11	17
23	b0784 - moaD	15	38
24	b1460 - ydcC	15	35
29	b4747 - yneP	16	35
41	b0039 - caiA	15	31
42	b1823 - cspC	3	27
54	b1824 - yobF	15	35
55	b3821 - pldA	12	26
59	b0177 - bamA	23	29
65	b0979 - appB	16	34
67	b0946 - zapC	15	35
79	b4595 - yciY	14	34
80	b1093 - fabG	15	41

Supplemental Table S3. Strains

Lab stock	Relevant genotype	Source or reference
A1063	XTL634 (W3110 araD<>tetA-sacB-amp)	(Li et al. 2013)
A411	DY378 (W3110 λcI857Δ(cro-bioA))	(Yu et al. 2000)
A708	MG1655 *mal*::*lacI*^{q}	(Guillier and Gottesman 2006)
A817	(A708) *mal*::*lacI*^{q} Δ*lacZ*::*Tn*10	(Barshishat et al. 2018)
A1086	(A708) *mal*::*lacI*^{q} Δ*oxySF*::*kan*	This study
A1120	(A708) *mal*::*lacI*^{q} *mepS*-SPA *kan*	This study
A1081	(A708) *mal*::*lacI*^{q} $oxyS_{\Delta 15\text{-}33}$	This study
A1085	(A708) *mal*::*lacI*^{q} $oxyS_{C76U\text{-}C77U}$	This study
A1104	(A708) *mal*::*lacI*^{q} Δ*P2-mepS*	This study
A1105	(A708) *mal*::*lacI*^{q} Δ*P1-mepS*	This study
A834	SL1344 *Salmonella Typhimurium* Δ*hisG*::*lacI*^{q}*:Cm*	This study

Supplemental Table S4. Plasmids

Plasmids	Genetic elements	Origin	Marker	Source or reference
pKD46		ColE1	Amp^r	(Datsenko and Wanner 2000)
pKD4		ColE1	Kan^r	(Datsenko and Wanner 2000)
pJL148			Kan^r	(Zeghouf et al. 2004)
pEF21	PBAD	p15A	Cm^r	(Guzman et al. 1995)
pBR-P*lac*	P*lacO*	ColE1	Amp^r	(Guillier and Gottesman 2006)
pSA84	MG1655 P*lacO-oxyS*	ColE1	Amp^r	(Barshishat et al. 2018)
pSA98	MG1655 P*lacO-oxyS* $_{\Delta 15\text{-}33}$	ColE1	Amp^r	This study
pSA99	MG1655 P*lacO-oxyS* $_{C18G,\ G30C}$	ColE1	Amp^r	This study
pSA88	MG1655 P*lacO-oxyS*$_{C76U\text{-}C77U}$	ColE1	Amp^r	(Barshishat et al. 2018)
pSA100	SL1344 P*lacO-oxyS*	ColE1	Amp^r	This study
pSA97	MG1655 P*lacO-kilR*	ColE1	Amp^r	(Barshishat et al. 2018)
pSA101	MG1655 PBAD-*kilR*	p15A	Cm^r	This study
pSA68	*'lacZ* (translation fusion)	pSC101*	Kan^r	(Hershko-Shalev et al. 2016)
pSA102	*MCS *'lacZ* (translation fusion)	pSC101*	Kan^r	This study
pSA103	MG1655 *mepS'-'lacZ*	pSC101*	Kan^r	This study
pSA104	MG1655 (ΔP2)P1-*mepS'-'lacZ*	pSC101*	Kan^r	This study
pSA105	MG1655 P2(ΔP1)-*mepS'-'lacZ*	pSC101*	Kan^r	This study
pSA106	MG1655 *mepS'*$_{C(-18)G}$*-'lacZ*	pSC101*	Kan^r	This study
pSA107	SL1344 *mepS'-'lacZ*	pSC101*	Kan^r	This study
pSA108	SL1344 *zapC'-'lacZ*	pSC101*	Kan^r	This study
pSA109	SL1344 *Plac-ftsZ'-'lacZ*	pSC101*	Kan^r	This study

*MCS multiple cloning site caries EcoRI, KpnI, SmaI, BamHI

Supplemental Table S5. Oligonucleotides

Oligonucleotides used for cloning

Primer	Primer sequence (5'-3')[a]	Use[b]
3319	CC**GAATTC**ATAAATGTGAGCGGATAACATTG	P*lacO* (EcoRI +)
3320	CC**GGTACC**AGTATCTTGTTATCCGCTCAC	P*lacO* (KpnI -)
2026	CCC**GACGTC**GAAACGGAGCGGCACCTC	MG1655 *oxyS* (AatII +)
2027	CCC**AAGCTT**ATCGCCGGCTTTTTATGG	MG1655 *oxyS* (HindIII -)
3419	TGCCGCTCCGTTTCGACG	MG1655 *oxyS* Δ15-33 (-)
3420	TCACTGCCCGTTTCGAGAG	MG1655 *oxyS* Δ15-33 (+)
3745	CCCTT**C**AAGTCACTGCCCGTTTCG	MG1655 *oxyS* $_{C18G,\ G30C}$ (+)
3746	TTAAAA**C**AGGTGCCGCTCCGTTTC	MG1655 *oxyS* $_{C18G,\ G30C}$ (-)
3411	CC**GAATTC**CGTGCTTTATC TATCGAACGC	MG1655 *mepS* (EcoRI +)
3412	CC**GGATCC**CGCAAGATATATCTCAAAATCGGTTG	MG1655 *mepS* (BamHI -)
3754	**G**AAGGGAAAACAAACAACATGG	MG1655 *mepS* $_{C(-18)G}$ (+)
3755	AAGTCCTTAACGACAAATATCG	MG1655 *mepS* $_{C(-18)G}$ (-)
3922	CC**CTGCAG**TTGTAAAAATGGAGATAATTATGATTGC	MG1655 *kilR* (PstI +)
2701	CCC**AAGCTT**TGCAAAGGTGGTAAGCAC	MG1655 *kilR* (HindIII -)
2805	GGG**GACGTC**GAACGGAGCGGTTTCTCGTTTAACC	SL1344 *oxyS* (AatII +)
2561	GGG**AAGCTT**ACGCCGGGTTTTTTTAC	SL1344 *oxyS* (HindIII -)
3940	AG**GAATTC**GCAACAATCGGTAA TCTC	SL1344 *mepS* (EcoRI+)
3941	CG**GGATCC**AGAACCGCAACTGCAATC	SL1344 *mepS* (BamHI -)
3942	AG**GAATTC**GGCAGGAGCTACGCTGG	SL1344 *zapC* (EcoRI +)
3943	CC**GGATCC**GCGAGATCGAGCATCATACG	SL1344 *zapC* (BamHI -)
3952	GTC**GGTACC**GGGATTGCTTCACTACGGG	SL1344 *ftsZ* (KpnI +)
3953	TCT**GGATCC**CCACCGACGCCGATGAC	SL1344 *ftsZ* (BamHI -)

[a]Nucleotides in bold indicate restriction sites mutations or cloning sequences. [b]Plus (+) and minus (-) strands are indicated.

Oligonucleotides used for construction of *E. coli* strains

Primer	Primer sequence (5'-3')[a]	Use[b]
3651	ATTATCCCTATCAAGCATTCTGACTGATAATTGCTCACA**TGTAGGCTGGAGCTGCTT C**	Δ*oxySF* (*kan* +)
3652	TGTATAAATTTGAGCCTGCT TATCGCCGGGCTTTTTTATGGCAAAAAAAA**ATGGGAATTAGCCATGGTCC**	Δ*oxySF* (*kan* -)
2156	**CAGGCCGTTTAAGCGATGATTCACGAAAT TGCTGGCCCG**GATGTCAATCTCTATCACTG	*hisG*::*lacI*^{q}*:Cm*(+)
2157	**TCGGCAGTACCAGAATCGAGCTGGCGCCAAGCGCTTTCAG**CAAGCGAGCTCGATATC	*hisG*::*lacI*^{q}*:Cm*(-)
3425	ATCCCTATCAAGCATTCTGACTGATAATTGCTCACAGAAACGGAGCGGCA**TCCTAATTTTTGTTGACACTCTATC**	*oxyS::tet-sacB* Δ15-33 (+)
3426	CACGTTGGCTTTAGTTATTCGAGTTGAGAAACTCTCGAAACGGGCAGTGA**ATCAAAGGGAAAACTGTCCATA**	*oxyS::tet-sacB* Δ15-33 (-)
3427	CGCTGTCCAGTT GCGCCAG	Scarless *oxyS* Δ15-33, C76U-C77U (+)
3428	TGCCGCTCCGTTTCTGTGAG	Scarless *oxyS* Δ15-33 (+)
3429	CTCACAGAAACGGAGCGCA TCCTG CCCGTTTCGAGAGTTTC	Scarless *oxyS* Δ15-33 (-)
3430	TCGACGCGCTCGATACCTGG	Scarless *oxyS* Δ15-33, C76U-C77U (-)
3640	CCTTGAAGTCACTGCCCGTTTCGAGAGTTTCTCAACTCGAATAACTAAAG**TCCTAATTTTTGTTGACACTCTATC**	*oxyS::tet-sacB* C76U-C77U (+)
3641	GCTTTTTTATGGCAAAAAAAAGCGGATCCTGGAGATCCGCAAAAGTTCACGTT**ATCAAAGGGAAAACTGTCCATA**	*oxyS::tet-sacB* C76U-C77U (-)
3642	CTTTAGTTATTCGAGTTGAGAAACTCTCG	Scarless *oxyS* C76U-C77U (+)
3643	CCGTTTCGAGAGTTTCTCAACTCGAATAACTAAAG**TT**AACGTGAACTTTT	Scarless *oxyS* C76U-C77U (-)
3722	TTGACCAACCCCGCTTATTAACTTTCTGTATCACTTTTTCTTATAAAAAA**TCCTAATTTTTGTTGACACTCTATC**	*mepS::tet-sacB* ΔP2 (+)
3723	TAACGCACAAAATTATATGCTGTGAGGATAATCTACCGCCGAGGCAAAAC**ATCAAAGGGAAAACTGTCCATA**	*mepS::tet-sacB* ΔP2 (-)
3726	ACGAGATAGCTTTCCCGG	Scarless *oxyS* ΔP2, ΔP1 (+)
3724	TTTTTTATAAGAAAAAGTGATACAGAAAGTTAATAAGC	Scarless *oxyS* ΔP2 (+)
3725	GCTTATTAACTTTCTGTATCACTTTTTCTTATAAAAAAGTTTTGCCTCGGCGGTAG	Scarless *oxyS* ΔP2 (-)
3727	GCTGGTGGAAGCATGGAC	Scarless *oxyS* ΔP2, ΔP1 (-)
3728	GTGCGTTAGTCCACAGTTTGGCCTTAAGGAA**CC**GTTTCAACATGCCCAGG**TCCTAATTTTTGTTGACACTCTATC**	*mepS::tet-sacB* ΔP1 (+)
3729	TTCCCTTGAAGTCCTTAACGACAAATATCGTTATAAAAAAATGCCAAGCGACACG**ATCAAAGGGAAAACTGTCCATA**	*mepS::tet-sacB* ΔP1 (-)
3730	CCTGGGCATGTTGAAAC**GG**TT C	Scarless *oxyS* ΔP1 (-)
3731	GAA**CC**GTTTCAACATGCCCAGG**CGCGG CGCCG**CGTGTCGCTTGGCATTTTTTTATAACG	Scarless *oxyS* ΔP1 (+)
3751	GAAGCG TTACAACGAAGCACGCCGGG TTCTCAGCCG CAGC**TCCATGGAAAAGAGAAG**	*mepS*-SPA (+)
3752	CGTCAGGATAGCCAAGGGATTGCATCCAAACGGTTTAtt**AGTTCCTATTCCGAAGTTC**	*mepS* -SPA (-)
3753	CAGCAGAA TGAACCGTACTGG	*mepS* -SPA insertion (+)

3691	CG**CCTAGG**ATAGCCTGGATCAAGACGCG	*mepS* -SPA insertion (-)
2227	GGATGAGATTTTCTTAAAGCGG	SPA sequence

[a]Nucleotides in bold indicate restriction sites, mutations or cloning sequences. [b]Plus (+) and minus (-) strands are indicated.

Oligonucleotides used for *in vitro* RNA synthesis

Primer	Primer sequence (5'-3')[a]	fragment[b]
3719	**CGAAATTAATACGACTCACTATAGGGACAGG**GTTTTGCCTCGGCGGTAG	**PT7**- *mepS* P2 long (+)
3720	**CGAAATTAATACGACTCACTATAGGGACAGG**GCTTGGCATTTTTTTATAACGATATTTGTCG	**PT7**- *mepS* P1 short (+)
3721	CATGCAGAAAGCAGAACCGC	*mepS* (-)
2238	**CGAAATTAATACGACTCACTATAGGGACAGG**GAAACGGAGCGGCACC	**PT7**- *OxyS* (+)
2027	CCC**AAGCTT**ATCGCCGGCTT TTTATGG	*oxyS* (-)

[a]Nucleotides in bold indicate restriction sites, mutations or cloning sequences. [b]Plus (+) and minus (-) strands are indicated.

Oligonucleotides used for RNA detection

Primer	Primer sequence (5'-3')[a]	Use
3708	GGATCCTGGAGATCCGCAAAAGTTCACG	*oxyS*
3740	CCTTGAGCTTCGGTATTC	*mepS* (in vivo)
3412	CC**GGATCC**CGCAAGATATATCTCAAAATCGGTTG	*mepS* (in vitro)
1912	CCGCGTCCGAAATTCCTA	tm RNA

[a]Nucleotides in bold indicate restriction sites, mutations or cloning sequences.

Supplemental references

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