

Table S1: Sequences of oligonucleotide primers used in this work

Gene Name	Primer Sequence
<i>Hisxy</i> F	GGCCCTGACGGCCAATATAAAGGATGAGCAT
<i>Hisxy</i> R	GGCCGCATAGGCCTTCATCATCTAATTTTCT
<i>Hicrp</i> F	CGGGATCCATCGAGCCACTTTTTGCC
<i>Hicrp</i> R	CGGAATTCCCCAAATTAATTTTCGGATGG
<i>Eccrp</i> F	CGGGATCCCCACTGCGTCAATTTTCCTG
<i>Eccrp</i> R	CGGAATTCAAATCAGTCTGCGCCACATC
b0108 <i>ppdD</i> F	GCCTACCGCATCCGCTT
b0108 <i>ppdD</i> R	CCAGGTTTCCCAGCACTTTTAAT
b0913 <i>ycal</i> F	GTGGTTTCCCTGTCCTGAGT
b0913 <i>ycal</i> R	TGTGATTCCCAGTTGCAGAT
b1188 <i>ycgB</i> F	ATCTTTGAAATCACGCATCG
b1188 <i>ycgB</i> R	GCCAACGGAAGAAGACAAAT
b2952 <i>yggT</i> F	TATCAAAGCCATCGTGCTGT
b2952 <i>yggT</i> R	AATCAGTAAACCGGCAATCC
b2972 <i>pppA</i> F	TCCACAATACGCCCTGAGTA
b2972 <i>pppA</i> R	ATGATATTATCCGCCTGGCT
b3189 <i>murA</i> F	CACCAGGAAAGTACCGGTTT
b3189 <i>murA</i> R	AAGGTGTGGAACGTTTAGGC
b3391 <i>hofQ</i> F	GCAATGGAATGCACTGAGAG
b3391 <i>hofQ</i> R	CTGGAAGCAGGCACTACAAA
b3392 <i>hofP</i> F	TTTGTATCTCCTTGCCGTTG
b3392 <i>hofP</i> R	GCTGACGCCAGACGTACTAA
b3393 <i>hofO</i> F	TCTACCAGGCGATACAGTGC
b3393 <i>hofO</i> R	CTGTCATCGACACACCATGA
b3394 <i>hofN</i> F	CAGTCGCGGGTAAATTGTC
b3394 <i>hofN</i> R	CCGCAGCTTACAGATAACGA
b3395 <i>hofM</i> F	TGGCTGTCAGGGACGATG
b3395 <i>hofM</i> R	ACTGAGTGAGTCTTCGCTGTAATCG

b3413 <i>gntX</i> F	TTAAGTCCGCTTATCCACCA
b3413 <i>gntX</i> R	CGACGAGCGTGTAAGACTTC
b3414 <i>gntY</i> F	CCTGAAATTTGACCTGCTGA
b3414 <i>gntY</i> R	TCGATCTCTGCATCTTCCAG
b3499 <i>yhiR</i> F	GCGGGATAGCAGAAGGTTAC
b3499 <i>yhiR</i> R	ATCATGCGCTTAATTTGCTG
b4473 <i>smf</i> F	TGTCACCAGCGAACCACTAC
b4473 <i>smf</i> R	AATTTCCCACGAAGAAATCG

Table S2: Transcriptional Units (TUs) differentially regulated by *E. coli* *sxy* expression.

TU numbers correspond to those in Fig. 4. Functional categories: C: Energy production and conversion, E: Amino acid transport and metabolism, F: Nucleotide transport and metabolism, H: Coenzyme transport and metabolism, G: Carbohydrate transport and metabolism, J: Translation, ribosomal structure and biogenesis, K: transcription, L: Replication, recombination and repair, M: Cell wall/membrane/envelope biogenesis, N: Cell motility, O: Posttranslational modification, protein turnover, chaperones, R: General function prediction only, S: function unknown, T: Signal transduction mechanisms, U: Intracellular trafficking, secretion, and vesicular transport. *: replaces b1437.

competence gene homologues:

TU	Gene name	Predicted Function	COG	Functional Category
16	b0913 <i>ycal</i>	conserved inner membrane protein, <i>rec-2</i> homologue	COG0658	R
18	b0106 <i>hofC</i>	fimbriae biogenesis	COG1459	NU
18	b0107 <i>hofB</i>	fimbriae biogenesis	COG2804	NU
18	b0108 <i>ppdD</i>	pilus subunit (pilin)	COG2165	NU
20	b3391 <i>hofQ</i>	predicted fimbrial transporter	COG1450	NU
20	b3392 <i>hofP</i>	predicted inner membrane hypothetical protein	not available	n/a
20	b3393 <i>hofO</i>	predicted inner membrane hypothetical protein	not available	n/a
20	b3394 <i>hofN</i>	predicted inner membrane fimbrial assembly protein	COG3166	NU
20	b3395 <i>hofM</i>	predicted periplasmic pilus assembly protein	COG1555	L
21	b2950 <i>yggR</i>	putative ATP-binding protein, pilus retraction ATPase	COG2805	NU
28	b3765 <i>yifB</i>	predicted ATP-dependent protease, <i>comM</i> homologue	COG0606	O
30	b2823 <i>ppdC</i>	prepilin peptidase dependent protein C	COG2165	NU
30	b2824 <i>ygdB</i>	hypothetical protein	not available	n/a
30	b2825 <i>ppdB</i>	prepilin peptidase dependent protein B	COG4795	U
30	b2826 <i>ppdA</i>	prepilin peptidase dependent protein A	COG2165	NU

33	b0442	<i>ybaV</i>	competence protein ComEA homolog, function unknown	COG1555	L
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replication, recombination and repair:

TU	Gene name	Predicted Function	COG	Functional Category	
1	b3645	<i>dinD</i>	DNA damage inducible protein	COG0214	L
4	b0799	<i>dinG</i>	probably ATP-dependent helicase	COG1199	KL
8	b4059	<i>ssb</i>	cytoplasmic ssDNA-binding protein	COG0629	L
11	b2733	<i>mutS</i>	methyl-directed mismatch repair	COG0249	L
15	b0228	<i>yafM</i>	putative transposase	COG1943	L
27	b2798	<i>xni</i>	ExoIX, 5'-3' exonuclease	COG0258	L
32	b3647	<i>ligB</i>	putative enzyme involved in DNA metabolic process	COG0272	L
68	b3863	<i>polA</i>	DNA polymerase I	COG0749	L
72	b4326	<i>yjiD</i>	DNA replication/recombination/repair protein	not available	n/a
79	b2819	<i>recD</i>	ATP-dependent dsDNA/ssDNA exonuclease V subunit	COG0507	L
79	b2820	<i>recB</i>	ATP-dependent dsDNA/ssDNA exonuclease V subunit	COG1074	L
	b3638	<i>yicR</i>	DNA repair protein RadC	COG2003	L

transcription:

TU	Gene name	Predicted Function	COG	Functional Category	
2	b3934	<i>cytR</i>	transcriptional regulator	COG1609	K
6	b2898	<i>ygfZ</i>	tRNA-modifying protein; folate-dependent	COG0354	R
7	b2897	<i>ygfY</i>	transcriptional regulation	COG2938	S
24	b1438	<i>ydcQ</i>	predicted DNA-binding transcriptional regulator	COG1598	S
26	b3890	<i>yiiF</i>	conserved protein, unknown function	not available	n/a
69	b0294	<i>matA</i>	putative transcriptional regulator, lux regulon	COG2771	K
77	b4327	<i>yjiE</i>	putative transcriptional regulator LYSR-type	COG0583	K
80	b1439	<i>ydcR</i>	predicted transcriptional regulator / aminotransferase	COG1167	KE

transposases:

TU	Gene name	Predicted Function	COG	Functional Category	
3	b3411	<i>yhgA</i>	predicted transposase	COG5464	S
13	b2305	<i>yfcI</i>	predicted transposase	COG5464	S
29	b4338	<i>yjiP</i>	predicted transposase, PSEUDOGENE	not available	n/a

29	b4339	<i>yjiP</i>	predicted transposase, PSEUDOGENE	not available	n/a
34	b2244	<i>yfaD</i>	predicted transposase	COG5464	S

hypothetical proteins:

TU	Gene name	Predicted Function	COG	Functional Category	
7	b2896	<i>ygfX</i>	hypothetical protein	not available	n/a
17	b1289	<i>ycjD</i>	hypothetical protein	COG2852	S
23	b4532*	<i>yncN</i>	YcfA-like protein	not available	n/a
31	b2272	<i>yfbM</i>	hypothetical protein	not available	n/a
74	b0235	<i>ykfJ</i>	conserved protein, PSEUDOGENE	COG1690	S
76	b3554	<i>yiaF</i>	hypothetical protein	not available	n/a

toxin-antitoxin:

TU	Gene name	Predicted Function	COG	Functional Category	
9	b3129	<i>sohA</i>	predicted antitoxin of the SohA-YhaV toxin-antitoxin system	COG2002	K
9	b3130	<i>yhaV</i>	predicted toxin of the SohA-YhaV toxin-antitoxin system	not available	n/a
12	b1420	<i>mokB</i>	regulatory peptide whose translation enables hokB expression	not available	n/a
19	b0018	<i>mokC</i>	regulatory peptide whose translation enables hokC expression	not available	n/a
24	b3082	<i>ygjM</i>	predicted DNA-binding transcriptional regulator	COG5499	K
24	b3083	<i>ygjN</i>	hypothetical protein	COG4680	S
25	b4224	<i>chpS</i>	antitoxin of the ChpB-ChpS toxin-antitoxin system	COG2336	T
25	b4225	<i>chpB</i>	toxin of the ChpB-ChpS toxin-antitoxin system	COG2337	T
71	b0245	<i>ykfI</i>	toxin of the YkfI-YafW toxin-antitoxin system	not available	n/a
73	b1562	<i>hokD</i>	polypeptide destructive to membrane potential hokD	not available	n/a

metabolism:

TU	Gene name	Predicted Function	COG	Functional Category	
10	b3648	<i>gmk</i>	guanylate kinase, GMP recycling	COG0194	E
14	b2936	<i>yggG</i>	Uncharacterized metalloprotease	COG0501	O
75	b0325	<i>yahK</i>	Zn-dependent alcohol dehydrogenase	COG1064	C
78	b0032	<i>carA</i>	glutamine amidotransferase	COG0505	EF
79	b2821	<i>ptrA</i>	periplasmic protease	COG1025	O
81	b0443	<i>ybaW</i>	Putative acyl-CoA thioester hydrolase	COG0824	R

translation:

TU	Gene name	Predicted Function	COG	Functional Category
5	b2569 <i>lepA</i>	GTP-binding elongation factor	COG0481	M

other:

TU	Gene name	Predicted Function	COG	Functional Category
70	b3334 <i>gspM</i>	general secretion pathway protein M	not available	n/a
22	b2700 <i>ygaD</i>	CinA-family protein	COG1546	R
82	b1015 <i>putP</i>	major sodium/proline symporter	COG0591	ER
83	b2752 <i>cysD</i>	ATP:sulfurylase, subunit 2	COG0175	EH
84	b2778 <i>ygcG</i>	hypothetical protein	COG1512	R
85	b4109 <i>yjda</i>	putative vimentin	COG0699	R
86	b3988 <i>rpoC</i>	RNA Polymerase subunit	COG0086	K

σ^{32} regulon:

TU	Gene name	Predicted Function	COG	Functional Category
35	b3931 <i>hslU</i>	heat shock protein	COG1220	O
36	b3498 <i>prlC</i>	trypsin-like proteinase	COG0339	E
37	b0439 <i>lon</i>	DNA-binding, ATP-dependent protease	COG0466	O
38	b0473 <i>htpG</i>	chaperone Hsp90, heat shock protein	COG0326	O
39	b0492 <i>ybbN</i>	putative thioredoxin-like protein	COG3118	O
40	b1060 <i>bssS</i>	biofilm regulator	not available	n/a
41	b1322 <i>ycjF</i>	putative membrane protein	COG3768	S
42	b2592 <i>clpB</i>	heat shock protein, protease	COG0542	O
43	b2614 <i>grpE</i>	heat shock protein, protease	COG0576	O
44	b0014 <i>dnaK</i>	chaperone	COG0443	O
45	b2699 <i>recA</i>	DNA-dependent ATPase	COG0468	L
46	b0966 <i>hspQ</i>	heat shock protein	COG3785	S
47	b3686 <i>ibpB</i>	16 kDa heat shock protein	COG0071	O
47	b3687 <i>ibpA</i>	heat shock protein	COG0071	O
48	b3635 <i>mutM</i>	DNA repair protein	COG0266	L
49	b4140 <i>fxsA</i>	inner membrane protein of unknown function	COG3030	R

66	b1073	<i>flgB</i>	flagellar biosynthesis cell-proximal portion of basal-body rod	COG1815	N
66	b1074	<i>flgC</i>	flagellar biosynthesis, cell-proximal portion of basal-body rod	COG1558	N
66	b1075	<i>flgD</i>	flagellar biosynthesis, initiation of hook assembly	COG1843	N
66	b1076	<i>flgE</i>	flagellar biosynthesis, hook protein	COG1749	N
66	b1077	<i>flgF</i>	flagellar biosynthesis, cell-proximal portion of basal-body rod	COG4787	N
66	b1078	<i>flgG</i>	flagellar biosynthesis, cell-distal portion of basal-body rod	COG4786	N
67	b4034	<i>malE</i>	periplasmic maltose-binding protein	COG2182	G

Fig. S1: Effect of inducing *E. coli* *sxy* expression with IPTG. **A.** SDS-PAGE confirming *E. coli* Sxy expression. Whole cell extracts of *E. coli* *sxy::kan* carrying *pEcsxy* in the absence (lane 1) and presence (lane 2) of 1 mM IPTG. His-tagged *E. coli* Sxy size is shown with an arrow. **B.** Growth of *E. coli* *sxy::kan* carrying *pEcsxy* in presence of 0 (squares), 0.02 (diamonds), 0.1 (triangles) and 1 (circles) mM IPTG added at OD₆₀₀ 0.2.

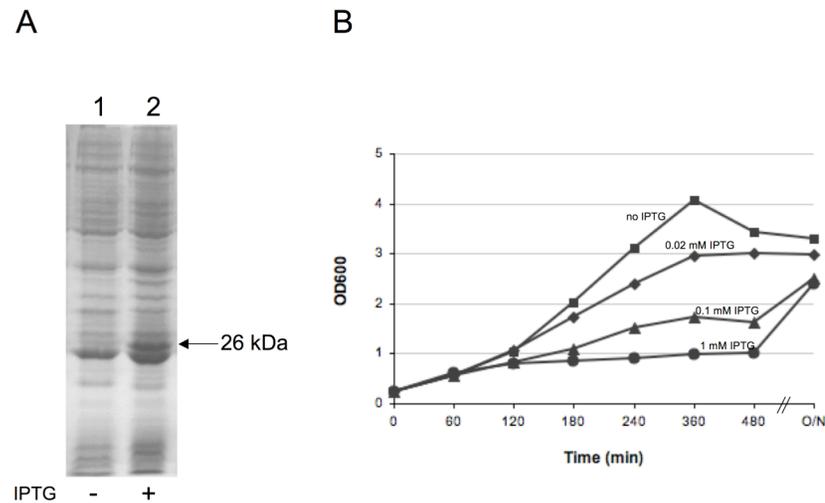


Fig. S2: CRP-S motifs in the genes that were induced equally well by both Sxy proteins (A) (n=18 sites) or genes that were poorly induced by *H. influenzae* Sxy (B) (n=25 sites). Sequence logos were generated using WebLogo (<http://weblogo.berkeley.edu/logo.cgi>) (16).

