Supplemental Material:

Identification of a target gene and activating stimulus for the YpdA/YpdB histidine kinase/response regulator system in *Escherichia coli*

Luitpold Fried[1#], Stefan Behr[1#], and Kirsten Jung[1*]

[1]Munich Center for Integrated Protein Science (CIPSM) at the Department of Microbiology,

Ludwig-Maximilians-Universität München, 82152 Martinsried, Germany

[#] These authors contributed equally to this work

Running title: The YpdA/YpdB-system in *E. coli*

*To whom correspondence should be addressed:
Dr. Kirsten Jung
Ludwig-Maximilians-Universität München
Department Biologie I, Bereich Mikrobiologie
Großhaderner Str. 2-4
82152 Martinsried
Germany
Phone: +49-89-2180-74500
Fax: +49-89-2180-74520
E-mail: jung@lmu.de

TABLE S1. Plasmids used in this study.

Plasmid	Description	Reference or source
pRed/ET	λ-RED recombinase in pBAD24; Amp^r	Gene Bridges
pCP20	FLP-recombinase, λcI 857^+, λpR Rep^{ts}; Amp^r, Cm^r	(1)
pBAD33-Cm	Arabinose-inducible P_{BAD} promoter, pBR322 ori; Kan^r	(2)
pBAD33-*ypdB*	*6his-ypdB* cloned in the AflII and XbaI sites of pBAD33-Cm; Cm^r	This work
pBAD24	Arabinose-inducible P_{BAD} promoter, pBR322 ori; Amp^r	(2)
pBAD24-*ypdB*	*6his-ypdB* cloned in the EcoRI and XbaI sites of pBAD24; Amp^r	This work
pBAD24-*ypdB* D53E	*ypdB* D54E cloned in the NdeI and XbaI sites of pBAD24-*ypdB*; Amp^r	This work
pBAD24-*ypdB* D53N	*ypdB* D54N cloned in the NdeI and XbaI sites of pBAD24-*ypdB*; Amp^r	This work
pBAD24-*yehS*	*yehS* cloned in the NdeI and XbaI sites of pBAD24-*kdpE*; Amp^r	This work
pBAD24-*ypdA*	*ypdA* cloned in the EcoRI and XbaI sites of pBAD24; Amp^r	This work
pBAD24-*ypdA* H371Q	*ypdA* H371Q cloned in the EcoRI and XbaI sites of pBAD24; Amp^r	This work
pBAD24-*yhjX*	*yhjX-6his* cloned in the EcoRI and XbaI sites of pBAD24; Amp^r	This work
pBAD24-*ypdAB*	*ypdAB* cloned in the EcoRI and XbaI sites of pBAD24; Amp^r	This work
pBAD24-*ypdABC*	*ypdABC* cloned in the EcoRI and XbaI sites of pBAD24; Amp^r	This work
pUC19	IPTG-inducible P_{Lac} promoter, pMB1 ori, Amp^r	(3)
pUC19 $P_{yjiY\ -212/+88}$	$P_{yjiY\ -212/+88}$ cloned in the EcoRI and BamHI sites of pUC19; Amp^r	(4)
pUC19 $P_{yhjX\ -264/+36}$	$P_{yhjX\ -264/+36}$ cloned in the EcoRI and BamHI sites of pUC19; Amp^r	This work
pUC19 $P_{yhjX\ -264/-165}$	$P_{yhjX\ -264/-165}$ cloned in the EcoRI and BamHI sites of pUC19; Amp^r	This work
pUC19 $P_{yhjX\ -164/-65}$	$P_{yhjX\ -164/-65}$ cloned in the EcoRI and BamHI sites of pUC19; Amp^r	This work
pUC19 $P_{yhjX\ -64/+36}$	$P_{yhjX\ -64/+36}$ cloned in the EcoRI and BamHI sites of pUC19; Amp^r	This work
pRS415	Operon fusion vector	(5)
pRS415 $P_{yhjX\ -264/+36}$	$P_{yhjX\ -264/+36}$ cloned in the EcoRI and BamHI sites of pRS415; Amp^r	This work
pRS415 $P_{yhjX\ up\_rplmt}$	$P_{yhjX\ up\_rplmt}$ (replacement of 15 bp upstream of M1) cloned in the EcoRI and BamHI sites of pRS415; Amp^r	This work
pRS415 $P_{yhjX\ M1}$	$P_{yhjX\ M1}$ (replacement of M1) cloned in the EcoRI and BamHI sites of pRS415; Amp^r	This work
pRS415 $P_{yhjX\ spacer}$	$P_{yhjX\ spacer}$ (replacement of spacer) cloned in the EcoRI and BamHI sites of pRS415; Amp^r	This work
pRS415 $P_{yhjX\ M2}$	$P_{yhjX\ M2}$ (replacement of M2) cloned in the EcoRI and BamHI sites of pRS415; Amp^r	This work
pRS415 $P_{yhjX\ down\text{-}rplmt}$	$P_{yhjX\ down\_rplmt}$ (replacement of 15 bp downstream of M2) cloned in the EcoRI and BamHI sites of pRS415; Amp^r	This work
pRS415 $P_{yhjX\ M2S1}$	$P_{yhjX\ M2S1}$(replacement of bp 1 and 10 in M2) cloned in the EcoRI and	This work

	BamHI sites of pRS415; Amp^r	
pRS415 $P_{yhjX\ M2S2}$	$P_{yhjX\ M2S2}$(replacement of bp 1,2,9 and 10 in M2) cloned in the EcoRI and BamHI sites of pRS415; Amp^r	This work
pRS415 $P_{yhjX\ M2S3}$	$P_{yhjX\ M2S3}$ (replacement of bp 1,2,3,8,9 and 10 in M2) cloned in the EcoRI and BamHI sites of pRS415; Amp^r	This work
pBBR1-MCS5-TT-RBS-*lux*	*luxCDABE* and terminators lambda *T0 rrnB1 T1* cloned into pBBR1-MCS5 for plasmid-based transcriptional fusions; Gm^r	(6)
pBBR *yhjX-lux*	$P_{yhjX\ -264/+36}$ cloned in the BamHI and EcoRI sites of pBBR1-MCS5-TT-RBS-*lux*; Gm^r	This work

TABLE S2. Oligonucleotides used in this study

Oligonucleotide	#	Oligonucleotide Sequence (5´-3´)	Description
Plasmid or strain construction			
YpdB NdeI sense		AACATATGGTGAAAGTCATCATTGTTGAA	pBAD24-*ypdB*
YpdB XbaI antisense		CCTCTAGATTAAAGATGCATTAACTGGCG	pBAD24-*ypdB*, pBAD24-*ypdAB*
ypdB B53E sense		GCCATTTTTCTGGAAATCAATATTCCG	pBAD24-*ypdB*-D53E
ypdB B53E antisense		CGGAATATTGATTTCCAGAAA AATGGC	pBAD24-*ypdB*-D53E
ypdB D53N sense		ATAACCGCGTCGACGCCATTT TTCTGAATATCA ATATTCCGTCGCTGG ATGGCG T	pBAD24-*ypdB*-D53N
ypdB D53N anti		ACGCCATCCAGCGACGGAATATTGATATTCAGAAAAATGGCGTCGAC GCGGTTAT	pBAD24-*ypdB*-D53N
yehS NdeI sense		ATGCGCCATATGCTAAGTAACGATATTCTGC	pBAD24-*yehS*
yehS XbaI antisense		CTCTCTAGATTAGCCTTTTTTCACATGCT	pBAD24-*yehS*
yhjX EcoRI sense		CAGGAGGAATTCATGACACCTTCAAATTATCAGC	pBAD24-*yhjX*
yhjX NdeI anti		GGAATTCCATATGAAGGGAGCCATGCGCCTCACGCAAC	pBAD24-*yhjX*
YpdA EcoRI sense		CCGAATTCGTGCACGAAATATTCAACATG	pBAD24-*ypdA*, pBAD24-*ypdAB*, pBAD24-*ypdABC*, pBAD24-*ypdA* H371Q
YpdA NdeI antisense		AACATATGAAGCAATAACGTAGCCTGTGA	pBAD24-*ypdA*, pBAD24-*ypdA* H371Q
YpdC XbaI antisense		CCTCTAGATTAGCCCTGAAAACGGGCGCT	pBAD24-*ypdABC*
ypdA H371Q sense		TCGCGCCCTGCAAAGCAAAATTAATCCCCAGTTTCTGTTTAACGCTCT GAACGCTATTTCA	pBAD24-*ypdA* H371Q
ypdA H371Q anti		TGAAATAGCGTTCAGAGCGTTAAACAGAAACTGGGGATTAATTTTGCT TTGCAGGGCGCGA	pBAD24-*ypdA* H371Q
pBAD24 anti		CAAATTCTGTTTTATCAGACCGCTTCTGCG	pBAD24 sequencing
pBAD24 sense		TCGCAACTCTCTACTGTTTCTCCATA	pBAD24 sequencing
rev24		TTCACACAGGAAACAGCTATGACC	pUC19 sequencing, labeling EMSA
uni24		ACGACGTTGTAAAACGACG	pUC19 sequencing
up yhjX 300bp BamHI sense		AATCCGGATCCCTAACTCAGGCAGAAAATACCA	pBBR *yhjX-lux*
up yhjX EcoRI anti		ATACCGGAATTCGGCAGTATTCCTGCAGTAATAAAAAG	pBBR *yhjX-lux*
Up YpdA		AGCCTTCAGGTTACCTATCATAGAGGTTTAATCCTTATTCAGAGTCAC CCAATTAACCCTCACTAAAGGGCGG	*E. coli* MG20 construction
Low YpdC		GATGCACAAAGTATCCTGACGCTGCTGGAAACAGAATTAACCTTCTGA CGTAATACGACTCACTATAGGGCTCG	*E. coli* MG20 construction
YpdBC-rpsL-neo-up		AACAGGAACTGAGCTGGCTAATTAAAGAGCACAGCCAGATGGAGATT GTCGGCACCTTTGGGCCTGGTGATGATGGCGGGATCG	*E. coli* MG21 construction
YpdBC-rpsL-neo-down		GCAAGATGCACAAAGTATCCTGACGCTGCTGGAAACAGAATTAACCT TCTGACGTCAGAAGAACTCGTCAAGAAGGCG	*E. coli* MG21 construction
RED-Kan anti		CGAGACTAGTGAGACGTGCTAC	control primer
RED-Kan sense		TATCAGGACATAGCGTTGGCTACC	control primer
ypdB sense		CGTTACTTAGCATGAGGCCTT	control primer
ypdB +84 sense		TGTGAGCCTGATAGTTACACC	control primer
ypdA +350 s		CCGGACCGTCCGAGCGACGCT	control primer
ypdA +50 s		AGCCTTCAGGTTACCTATCAT	control primer
ypdC + 50 a		GATGCACAAAGTATCCTGACG	control primer
ypdC + 350 a		CGCACTGAACATCCGTTTGAG	control primer

down-ypdB-rpsl -D53	CACGCGGTGATGAACACAATAAACGGTTTATGGGCGAACTGGCTGATGTTTCAGAAGAACTCGTCAAGAAGGCG	*E. coli* MG 24 / MG 25 construction
up ypdA rpsl neo	AATGCTTATCTGCCTGTTCTTTCTCATCCGTATCCGCCTGTTTCGCGAACGGCCTGGTGATGATGGCGGGATCG	*E. coli* MG23 construction
down ypdA rspl neo	AATGTAAAACGCAATTTCCGTCCCCGGCTCCAGGCGGCGGATATGCAGCCTCAGAAGAACTCGTCAAGAAGGCG	control primer
ypdA sense	GTGCACGAAATATTCAACATG	control primer
ypdA anti	TCAAAGCAATAACGTAGCCT	control primer
YpdA+up50bp sense	AGCCTTCAGGTTACCTATCATAGAGGTTTAATCCTTATTCAGAGTCACCCGTGCACGAAATATTCAACATG	*E. coli* MG 23 construction
YpdA-down50bp anti	TGCCAGGAATTCGTCTTCAACAATGATGACTTTCACAATATCACTCCGGCTCAAAGCAATAACGTAGCCTGT	*E. coli* MG 23 construction
YpdB+up50bp sense	ACCCCAGTCGCCTCACAGGCTACGTTATTGCTTTGAGCCGGAGTGATATTGTGAAAGTCATCATTGTTGAAGA	*E. coli* MG 24 / MG 25 construction
YpdB-down50bp anti	AAAAATTGTTGATCGGCGGGCAAGCCTGGTGCTTTCATGAAAGTTCCCGATTAAAGATGCATTAACTGGCGAAAT	*E. coli* MG 24 / MG 25 construction
up yhjX	TATGGTTGTCGGCAGAGATTTTTCCTTTTTATTACTGCAGGAATACTGCCAATTAACCCTCACTAAAGGGCG	*E. coli* MG26 construction
down yhjX	ATGCGTTTGATGCACACGGAAGCTGAAGCCCAGTAGCTCGCGGCTGAGCATAATACGACTCACTATAGGGCTC	*E. coli* MG26 construction
yhjX-200	GCAAAGGGAAAAAGTGTGGGGA	control primer

Northern Blot DNA probes

cpxP anti	CTACTGGGAACGTGAGTTGCT	*cpxP* probe
cpxP sense	ATGCGCATAGTTACCGCTGCC	*cpxP* probe
entC anti	TTAATGCAATCCAAAAACGTT	*entC* probe
entC sense	ATGGATACGTCACTGGCTGAG	*entC* probe
entE anti	TGCCAAACACCTGCTGCAACT	*entE* probe
entE sense	ATGAGCATTCCATTCACCCGC	*entE* probe
fecA anti	GCAGGCTGTTGAAGGTGTGCA	*fecA* probe
fecA sense	ATGACGCCGTTACGCGTTTTT	*fecA* probe
fecB anti	TCATTTCACAACGGTAAGCGG	*fecB* probe
fecB sense	ATGTTGGCATTTATCCGTTTT	*fecB* probe
fhuA anti	GCAGGTTCTGACGCACAGTAA	*fhuA*probe
fhuA sense	ATGGCGCGTTCCAAAACTGCT	*fhuA*probe
fhuF anti	TCATTTCAGCGTACAATCGCC	*fhuF* probe
fhuF sense	ATGGCCTATCGTTCCGCACCG	*fhuF* probe
guaC anti	TTACAGGTTGTTGAAGATGCG	*guaC* probe
guaC sense	ATGCGTATTGAAGAAGATCTG	*guaC* probe
iraP anti	TTACTGACGAGGATGCTTCAA	*iraP* probe
iraP sense	ATGAAAAATCTCATTGCTGAG	*iraP* probe
rpoD anti	AATCGTCCAGGAAGCTACGCAGC	*rpoD* probe
rpoD sense	ATGGAGCAAAACCCGCAGTCAC	*rpoD* probe
yahM anti	CTACGTAATCAACCTGATTTG	*yahM* probe
yahM sense	ATGGCGGTCCAACTTTTCAAA	*yahM* probe
yehS anti	TTAGCCTTTTTTCACATGCTG	*yehS* probe
yehS sense	ATGCTAAGTAACGATATTCTG	*yehS* probe
ygbK anti	TTACCCACGGCACGCCGGGGAAAT	*ygbK* probe
ygbK sense	ATGATCAAGATTGGCGTTATC	*ygbK* probe
ygbL anti	TTAACTCCTTAATTCCGCAAT	*ygbL* probe
ygbL sense	ATGAGCGATTTCGCAAAAGTA	*ygbL* probe
yhjX anti	CAAAGAACTCACTGACCAGTG	*yhjX* probe
yhjX sense	ATGACACCTTCAAATTATCAG	*yhjX* probe
yjiY anti	TGATGAACAGGAACGGGAACA	*yjiY* probe
yjiY sense	ATGGATACTAAAAAGATATTC	*yjiY* probe
ynjH anti	TTATGGCTTTACGCGCCGCCA	*ynjH* probe
ynjH sense	GTGAGTCGAGCATTGTTCGCC	*ynjH* probe
ypdB anti	TTAAAGATGCATTAACTGGCG	*ypdB* probe

ypdB sense	GTGAAAGTCATCATTGTTGAA	*ypdB* probe
EMSA/footprint		
6-FAM uni24	[6-FAM]ACGACGTTGTAAAACGACGGCCAG	EMSA labeling DNA-fragments
yhjX 1 sense	TTGAATTCTTCTGATGGCATTTCATG	pUC19 $P_{yhjX\ -64/+36}$
yhjX 1 anti	TTGGATCCGGCAGTATTCCTGCAGTA	pUC19 $P_{yhjX\ -264/+36}$, pRS415 $P_{yhjX\ -264/+36}$ + derivates
yhjX 2 sense	TTGAATTCTAACAATAGTTGTGGCGA	pUC19 $P_{yhjX\ -164/-65}$
yhjX 2 anti	TTGGATCCCGGAATGAAATGCCTTAG	pUC19 $P_{yhjX\ -164/-65}$
yhjX 3 sense	TTGAATTCCTAACTCAGGCAGAAAAT	pUC19 $P_{yhjX\ -264/-165}$, pUC19 $P_{yhjX\ -264/+36}$
yhjX 3 anti	TTGGATCC TTTAATGGTTTCAATTGT	pUC19 $P_{yhjX\ -264/-165}$
yjiY-5P-1 anti	TTTTTTGGATCCAGTAAAACCTGGCATGTA	pUC19 $P_{yjiY\ -212/+88}$
yjiY-5P-3 sense	TTTTTTGAATTCCGCCGAGTGAATTTTATTCA	pUC19 $P_{yjiY\ -212/+88}$
In vivo reporter		
upstream-replacement as	GGCTGGACTTCCGTCATGACGCGACAATTATTC	pRS415 $P_{yhjX\ up\_rplmt}$
upstream-replacement s	GACGGAAGTCCAGCCGGCATTTCATTCCGTTCT	pRS415 $P_{yhjX\ up\_rplmt}$
motif 1 replacement as	CGTCCCGTAATTAGTTCAGGAATGAATG	pRS415 $P_{yhjX\ M1}$
motif 1 replacement s	TTACGGGACGTCCGTTCTGATGGCATTT	pRS415 $P_{yhjX\ M1}$
spacer replacement as	CGACTCCATTCATGAAATGCCTTAGTTCA	pRS415 $P_{yhjX\ spacer}$
spacer replacement s	GAATGGAGTCGGGCATTTCATGCCGTTTT	pRS415 $P_{yhjX\ spacer}$
motif 2 replacement as	CGTCCCGTAAATCAGAACGGAATGAAAT	pRS415 $P_{yhjX\ M2}$
motif 2 replacement s	TTACGGGACGGCCGTTTTTCCCCAGGCA	pRS415 $P_{yhjX\ M2}$
downstream-replacement as	AGTTTTCCCCCATTAATGAAATGCCATCAGAAC	pRS415 $P_{yhjX\ down\_rplmt}$
downstream-replacement s	TAATGGGGGAAAACTGCATAAAGTGCACTTCGT	pRS415 $P_{yhjX\ down\_rplmt}$
motif shortening 1 as	ATCAGAACGGACTGAAATGCATTAGTTCAGGAATGAATG	pRS415 $P_{yhjX\ M2\ G/T}$
motif shortening 2 as	ATCAGAACGGACGGAAATGAATTAGTTCAGGAATGAATG	pRS415 $P_{yhjX\ M2\ GG/AT}$
motif shortening 3 as	ATCAGAACGGACGTAAATTAATTAGTTCAGGAATGAATG	pRS415 $P_{yhjX\ M2\ GGC/CAT}$
motif shortening 1 s	TCCGTTCTGATTGCATTTCAGGCCGTTTTTCCCCAGGCA	pRS415 $P_{yhjX\ M2\ G/T}$
motif shortening 2 s	TCCGTTCTGATTTCATTTCCGGCCGTTTTTCCCCAGGCA	pRS415 $P_{yhjX\ M2\ GG/AT}$
motif shortening 3 s	TCCGTTCTGATTTAATTTACGGCCGTTTTTCCCCAGGCA	pRS415 $P_{yhjX\ M2\ GGC/CAT}$

TABLE S3: Influence of C-sources and additives on *yhjX* expression. Strain and cultivation conditions were the same as described in Figure 4B.

Medium	Additional C-source	Concentration C-source	Additive	Concentration additive	Average of max. *yhjX* expression [RLU/OD_{600}]	Standard deviation of max. *yhjX* expression [RLU/OD_{600}]
LB medium	Glucose	0.4%	Pyruvate	20 mM	41,710	3,020
LB medium	Pyruvate	20 mM			38,810	1,270
LB medium	-	-	-	-	35,450	3,670
LB medium	Glucose	0.4%	-	-	10,820	1,910
M9 medium	Pyruvate	20 mM	-	-	479,070	46,060
M9 medium	Yeast extract	0.5%	-	-	38,240	4,460
M9 medium	Gluconic acid	0.4%	-	-	7,260	620
M9 medium	Glucuronic acid	0.4%	-	-	4,120	860
M9 medium	Lactate	20 mM	-	-	1,000	160
M9 medium	Phosphoenol pyruvate	20 mM	-	-	370	80
M9 medium	Glycerol	0.4%	-	-	360	70
M9 medium	L-Serine	20 mM	-	-	360	50
M9 medium	Acetate	0.4%	-	-	320	30
M9 medium	Lactose	0.4%	-	-	300	20
M9 medium	Casamino acids	0.4%	-	-	260	30
M9 medium	L-Proline	20 mM	-	-	260	40
M9 medium	Fumarate	20 mM	-	-	250	50
M9 medium	Mannose	0.4%	-	-	250	50
M9 medium	Succinate	0.4%	-	-	250	40
M9 medium	Galactose	0.4%	-	-	230	30
M9 medium	Oxaloacetate	20 mM	-	-	230	30
M9 medium	Peptone	0.4%	-	-	220	60
M9 medium	L-Asparte	20 mM	-	-	210	40
M9 medium	Mannitol	0.4%	-	-	210	40
M9 medium	Fructose	0.4%	-	-	200	40
M9 medium	Xylose	0.4%	-	-	200	50
M9 medium	Maltose	0.4%	-	-	170	40
M9 medium	Glucose	0.4%	-	-	160	150
M9 medium	L-Glutamate	0.4%	-	-	130	40
M9 medium	Tryptone	0.4%	-	-	10	10
M9 medium	Cas amino acids	0.4%	Glucose	0.4%	22,640	520
M9 medium	Cas amino acids	1.5%	PIPES (pH 5.5) + Glycerol	20 mM + 0.8%	9,910	720
M9 medium	Cas amino acids	1.5%	PIPES (pH 7.0) + Glycerol	20 mM + 0.8%	7,620	240
M9 medium	Pyruvate	20 mM	Fumarate	20 mM	528,780	66,840
M9 medium	Pyruvate	20 mM	Glucose	20 mM	281,270	28,830
M9 medium	Pyruvate	20 mM	Glucose	1 mM	270,880	41,130

M9 medium	Pyruvate	20 mM	Lactate + PIPES (pH 7.0)	20 mM + 20 mM	240,000	16,200
M9 medium	Pyruvate	20 mM	Glucose	5 mM	201,370	6,160
M9 medium	Pyruvate	20 mM	Acetate	20 mM	165,180	20,310
M9 medium	Succinate	0.4%	Pyruvate	20 mM	274,060	17,380
M9 medium	Succinate	0.4%	Fumarate	20 mM	9,940	1,130
M9 medium	Succinate	0.4%	Lactose	0.4%	3,350	790
M9 medium	Succinate	0.4%	Guanidine hydrochloride	1 mg/ml	660	390
M9 medium	Succinate	0.4%	Methanol	1%	600	480
M9 medium	Succinate	0.4%	Fosfomycin	1 μg/ml	570	480
M9 medium	Succinate	0.4%	Crystal violet	0.05 μg/μl	560	450
M9 medium	Succinate	0.4%	D-Leucine	20 mM	540	40
M9 medium	Succinate	0.4%	L-Histidine	20 mM	530	380
M9 medium	Succinate	0.4%	Sulfamethazine	0.5 μg/ml	510	30
M9 medium	Succinate	0.4%	Arsenate	20 μg/ml	500	190
M9 medium	Succinate	0.4%	Imipenem	0.05 μg/ml	480	210
M9 medium	Succinate	0.4%	Plumbagin	8 μg/ml	470	240
M9 medium	Succinate	0.4%	D-Argine	20 mM	410	430
M9 medium	Succinate	0.4%	D-Proline	20 mM	410	180
M9 medium	Succinate	0.4%	Maltose	0.4%	390	60
M9 medium	Succinate	0.4%	Xylose	0.4%	380	50
M9 medium	Succinate	0.4%	Antimony(III)chloride	5 μg/ml	370	140
M9 medium	Succinate	0.4%	Hdroxyurea	100 μg/ml	350	200
M9 medium	Succinate	0.4%	Paromomycin	0.01 μg/ml	350	150
M9 medium	Succinate	0.4%	Paromomycin	0.005 μg/ml	340	130
M9 medium	Succinate	0.4%	5,7-Dichloro-8-hydroxyquinaldine	1 μg/ml	330	360
M9 medium	Succinate	0.4%	D-Tyrosine	20 mM	330	110
M9 medium	Succinate	0.4%	Oxalate	30 mM	330	220
M9 medium	Succinate	0.4%	Propanol	1%	330	330
M9 medium	Succinate	0.4%	Thiamphenicol	1 μg/ml	300	160
M9 medium	Succinate	0.4%	Polymyxin B	0.005μg/ml	290	130
M9 medium	Succinate	0.4%	Deoxycholate	100 μg/ml	280	340
M9 medium	Succinate	0.4%	Lactulose	30 mM	280	250
M9 medium	Succinate	0.4%	Natriumphosphat (pH 7)	200 mM	280	250
M9 medium	Succinate	0.4%	7-Hydoxycoumarin	10μg/ml	270	220
M9 medium	Succinate	0.4%	Tobramycin	0.01 μg/ml	270	460
M9 medium	Succinate	0.4%	Arsenite	10 μg/ml	260	10
M9 medium	Succinate	0.4%	L-Alanine	20 mM	260	60
M9 medium	Succinate	0.4%	Iodacetic acid	10 μg/ml	250	240
M9 medium	Succinate	0.4%	L-Leucine	20 mM	240	230
M9 medium	Succinate	0.4%	Lactate	20 mM	230	90
M9 medium	Succinate	0.4%	Deoxycholate	50 μg/ml	210	220
M9 medium	Succinate	0.4%	L-Arginine	20 mM	210	180
M9 medium	Succinate	0.4%	L-Isoleucine	20 mM	200	40
M9 medium	Succinate	0.4%	L-Tyrosine	20 mM	200	190

M9 medium	Succinate	0.4%	N-Acetyl-Glucosamine	0.4%	170	40
M9 medium	Succinate	0.4%	Deoxycholate	500 µg/ml	160	280
M9 medium	Succinate	0.4%	NaCl	2%	160	40
M9 medium	Succinate	0.4%	PIPES (pH 7.0) + Oxalate	20 mM + 30 mM	160	40
M9 medium	Succinate	0.4%	Apramycin	0.005 µg/ml	150	140
M9 medium	Succinate	0.4%	Benzoate	30 mM	140	120
M9 medium	Succinate	0.4%	Ethanol	5%	130	230
M9 medium	Succinate	0.4%	Peptidoglycan E. coli	1/20 fold dilution	130	100
M9 medium	Succinate	0.4%	PIPES (pH 7.0) + Mitomycin	20 mM + 0.3 µg/ml	130	30
M9 medium	Succinate	0.4%	D-Cycloserine	0,5 µg/ml	120	190
M9 medium	Succinate	0.4%	D-Serine	20 mM	120	110
M9 medium	Succinate	0.4%	Ethanol	1%	120	200
M9 medium	Succinate	0.4%	Gly-Gly	20 mM	100	300
M9 medium	Succinate	0.4%	L-Asparagine	20 mM	100	140
M9 medium	Succinate	0.4%	L-Threonine	20 mM	100	180
M9 medium	Succinate	0.4%	PIPES (pH 7.0)	20 mM	90	20
M9 medium	Succinate	0.4%	D-Alanine	20 mM	80	130
M9 medium	Succinate	0.4%	L-Glutamate	20 mM	80	130
M9 medium	Succinate	0.4%	L-Proline	20 mM	80	140
M9 medium	Succinate	0.4%	Chlorambucil	50 µg/ml	70	120
M9 medium	Succinate	0.4%	Ethanol	2%	70	110
M9 medium	Succinate	0.4%	L-Glycine	20 mM	70	120
M9 medium	Succinate	0.4%	Phenyl-methylsufonyl-fluorid	200 µg/ml	60	110
M9 medium	Succinate	0.4%	PIPES (pH 7.0) + Formate	20 mM + 30 mM	60	20
M9 medium	Succinate	0.4%	L-Serine	20 mM	50	80
M9 medium	Succinate	0.4%	Peptidoglycan Bacillus	1/20 fold dilution	50	30
M9 medium	Succinate	0.4%	Peptidoglycan Lactobacillus	1/20 fold dilution	50	90
M9 medium	Succinate	0.4%	Amitriptylin	10 µg/ml	40	40
M9 medium	Succinate	0.4%	Formate	30 mM	40	70
M9 medium	Succinate	0.4%	L-Aspartate	20 mM	40	30
M9 medium	Succinate	0.4%	Methylglyoxal	0,7 mM	40	10
M9 medium	Succinate	0.4%	Peptidoglycan Lactobacillus	1/2000 fold dilution	40	20
M9 medium	Succinate	0.4%	D-Ala-D-Ala	20 mM	30	20
M9 medium	Succinate	0.4%	Dulcitol	0.4%	30	50
M9 medium	Succinate	0.4%	Methylglyoxal	0,2 mM	30	10
M9 medium	Succinate	0.4%	Peptidoglycan Bacillus	1/2000 fold dilution	30	40
M9 medium	Succinate	0.4%	PIPES (pH 7.0) + Benzoate	20 mM + 30 mM	20	10

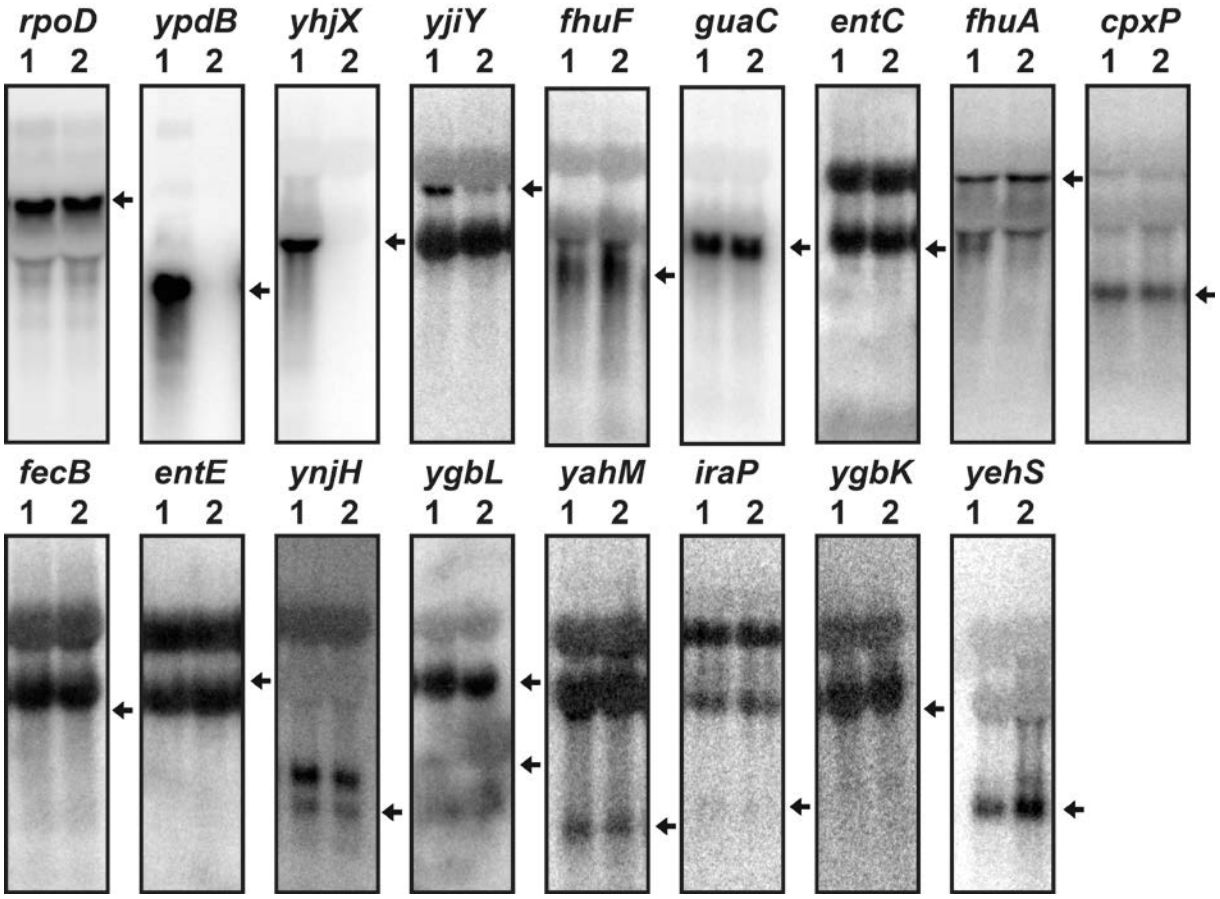

FIG. S1. Evaluation of potential YpdB target genes. A) Northern blot analysis was used to measure the effect of overproduction of YpdB on the expression of the genes identified by transcriptome analysis (see Table 1) and *rpoD* (control) in *E. coli* MG21 (Δ*ypdB*). The expression levels of these genes were also assessed in the *E. coli* strain MG21 (Δ*ypdB*) in the absence of YpdB (*E. coli* MG21 transformed with the empty pBAD24 vector) (lanes 2) or upon overproduction of YpdB (lanes 1). 20 µg of total RNA was loaded per lane, and the transcripts were detected with the corresponding gene-specific DNA probes. Transcripts of the corresponding genes are marked by an arrow.

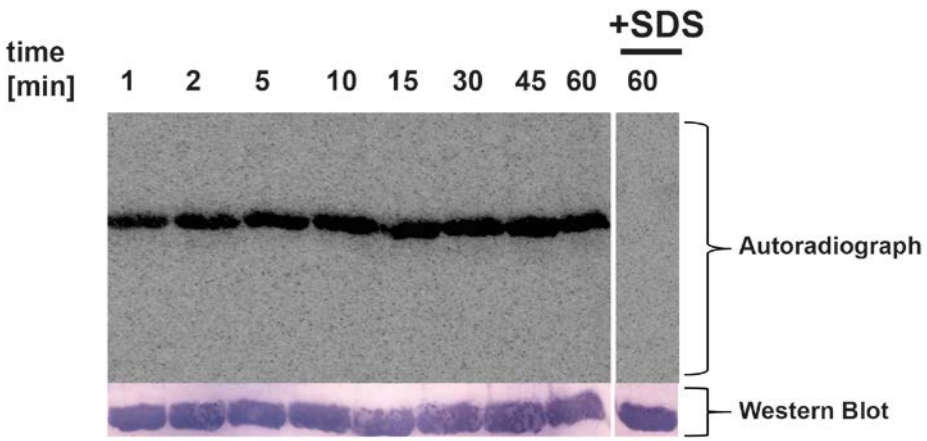

Fig. S2. In vitro phosphorylation of YpdB. Purified YpdB-6His was mixed with phosphorylation buffer. Phosphorylation was started by adding a mixture of [γ−^{32}P]acetyl phosphate and $MgCl_2$. At the indicated times, the reaction was stopped by adding SDS-sample buffer, the samples were subjected to SDS-PAGE and Semi Dry Western Blotting. As negative control, protein was denatured by adding SDS-sample before the reaction was started (+SDS). Phosphorylated YpdB was detected by autoradiography using a phosphor screen and a PhosphorImager Storm. The autoradiograph is representative of three independent experiments.

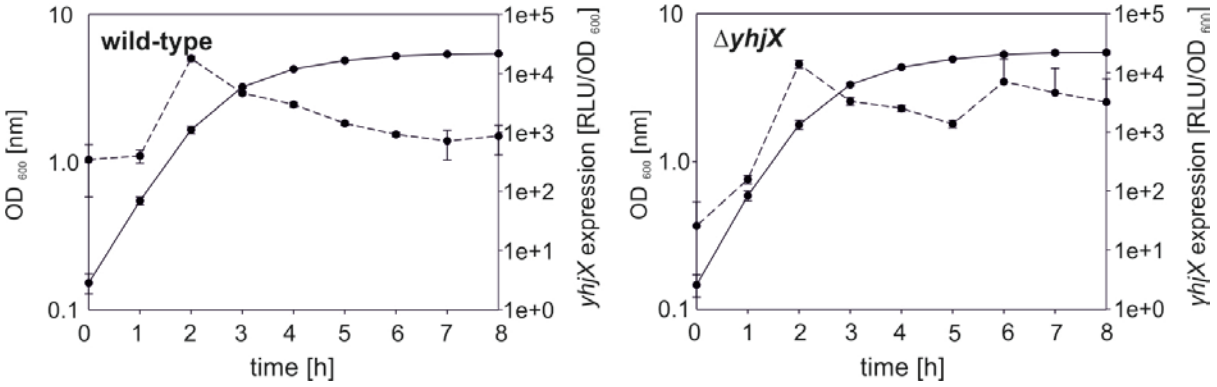

Fig. S3. *yhjX* induction is independent of YhjX feedback regulation. *Escherichia coli* MG1665 (wild-type) and MG26 (Δ*yhjX*) were transformed with pBBR *yhjX*-lux and grown aerobically in LB medium. Growth and luciferase activity were monitored continuously. The maximal luciferase activity normalized to an optical density of 1 (RLU/OD600) was used as a measure of the degree of induction of *yhjX*. Data were obtained from at least three independent experiments, and average values were used for calculations.

References:

1. **Cherepanov PP, Wackernagel W.** 1995. Gene disruption in *Escherichia coli*: TcR and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant. Gene **158:**9-14.
2. **Guzman L, Belin D, Carson M, Beckwith J.** 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. J. Bacteriol. **177:**4121-4130.
3. **Yanisch-Perron C, Vieira J, Messing J.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103 - 119.
4. **Kraxenberger T, Fried L, Behr S, Jung K.** 2012. First insights into the unexplored two-component system YehU/YehT in *Escherichia coli*. J. Bacteriol. **194:**4272-4284.
5. **Simons R, Houman F, Kleckner N.** 1987. Improved single and multicopy lac-based cloning vectors for protein and operon fusions. Gene **53:**85-96.
6. **Godeke J, Heun M, Bubendorfer S, Paul K, Thormann KM.** 2011. Roles of Two *Shewanella oneidensis* MR-1 Extracellular Endonucleases. Appl Environ Microbiol **77:**5342-5351.