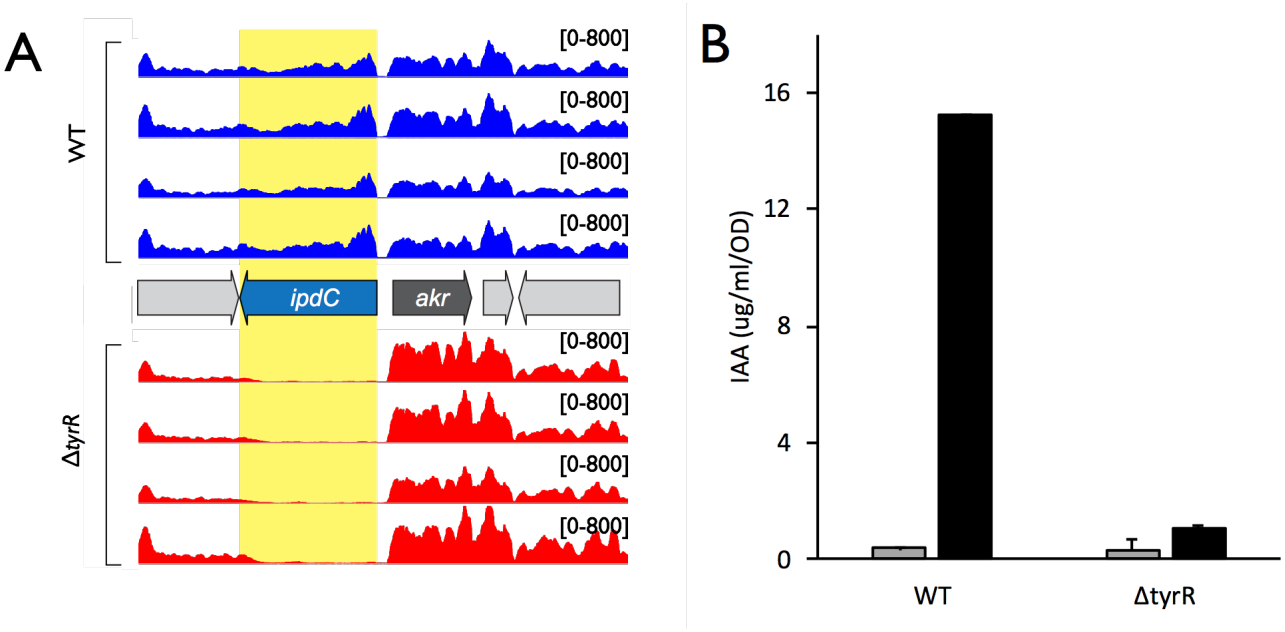
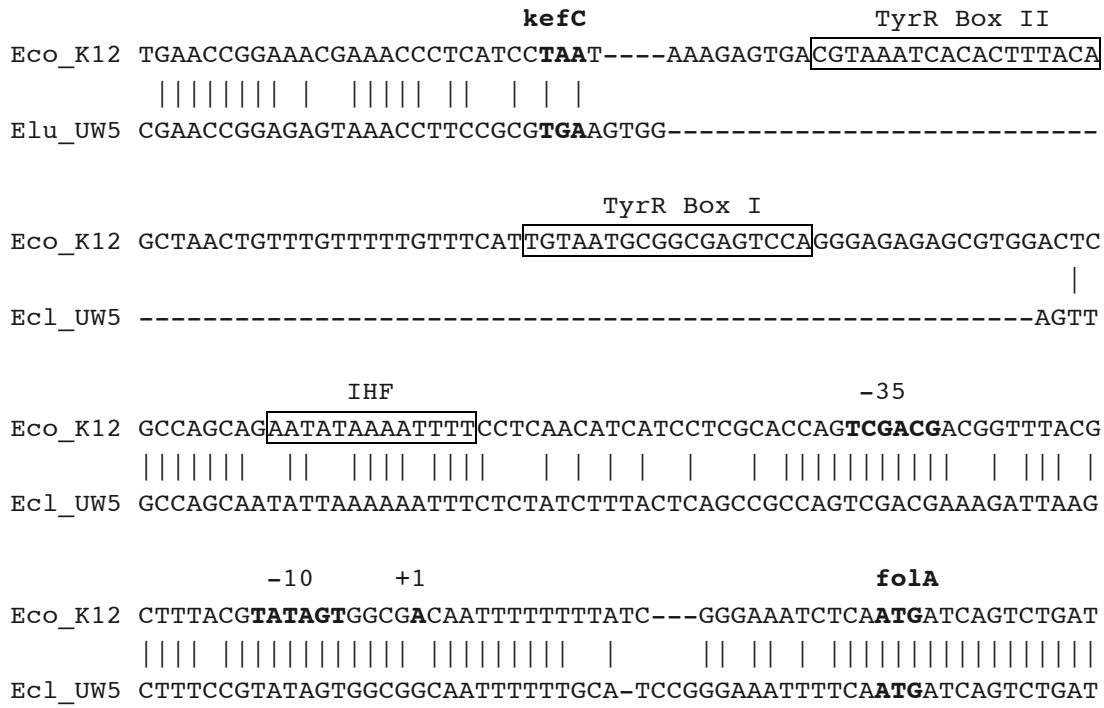
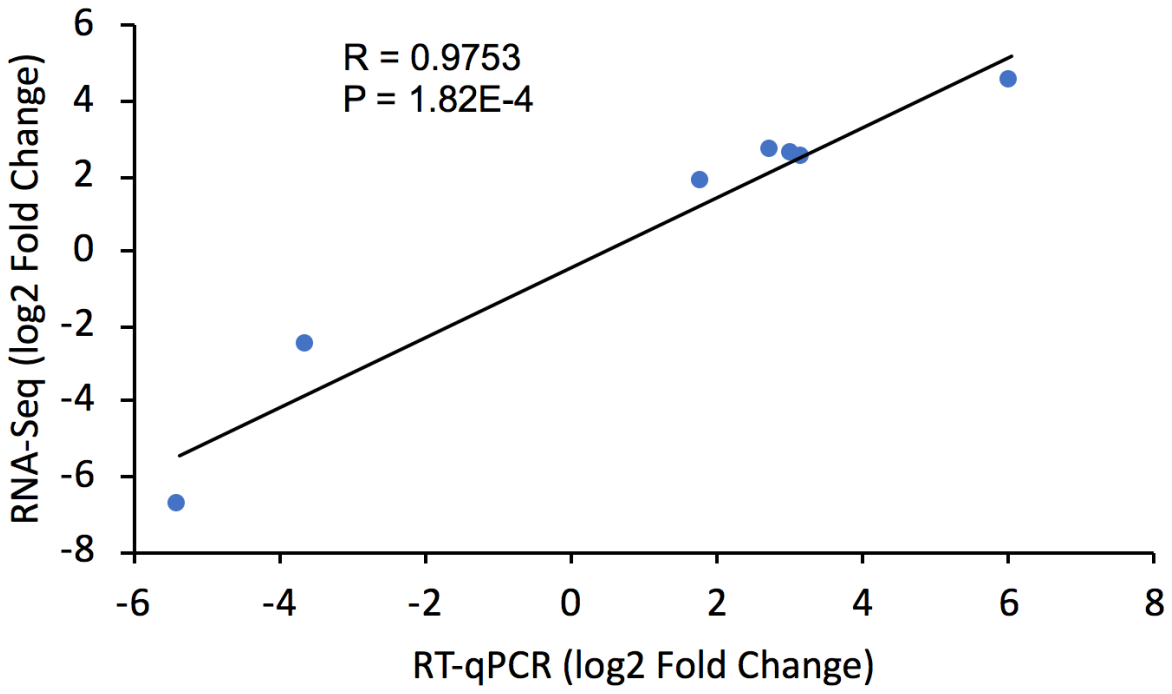
Supplementary Information



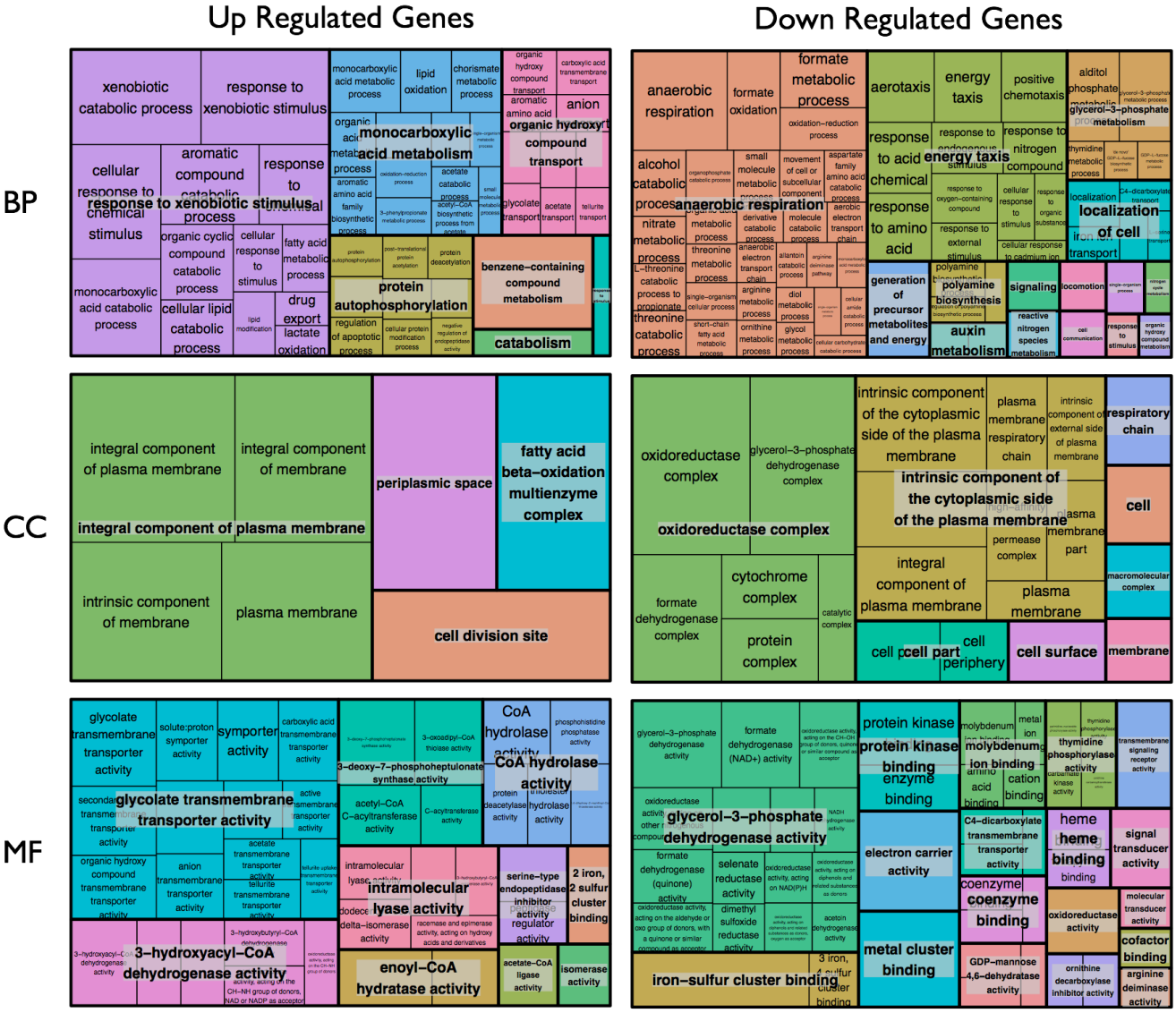
Supplementary Figure S1. TyrR is required for expression of *ipdC* and production of IAA. (A) Histogram of RNA-Seq read coverage per base of the *ipdC-akr* locus in the *E. ludwigii* genome. RNA-Seq reads in the wild-type strain (blue) indicate transcription beginning at 5' end of the *ipdC* gene and covering the length of the coding sequence, which is absent in the *tyrR* mutant (red). The results were similar for all biological replicates, highlighted in yellow. The maximal depth of read coverage was the same across all samples (noted in square brackets). (B) Effect of the *tyrR* deletion on IAA production in M9 minimal medium alone (grey bars) or supplemented with 1 mM L-tryptophan (black bars). Error bars indicate the standard error of the mean of three biological replicates.



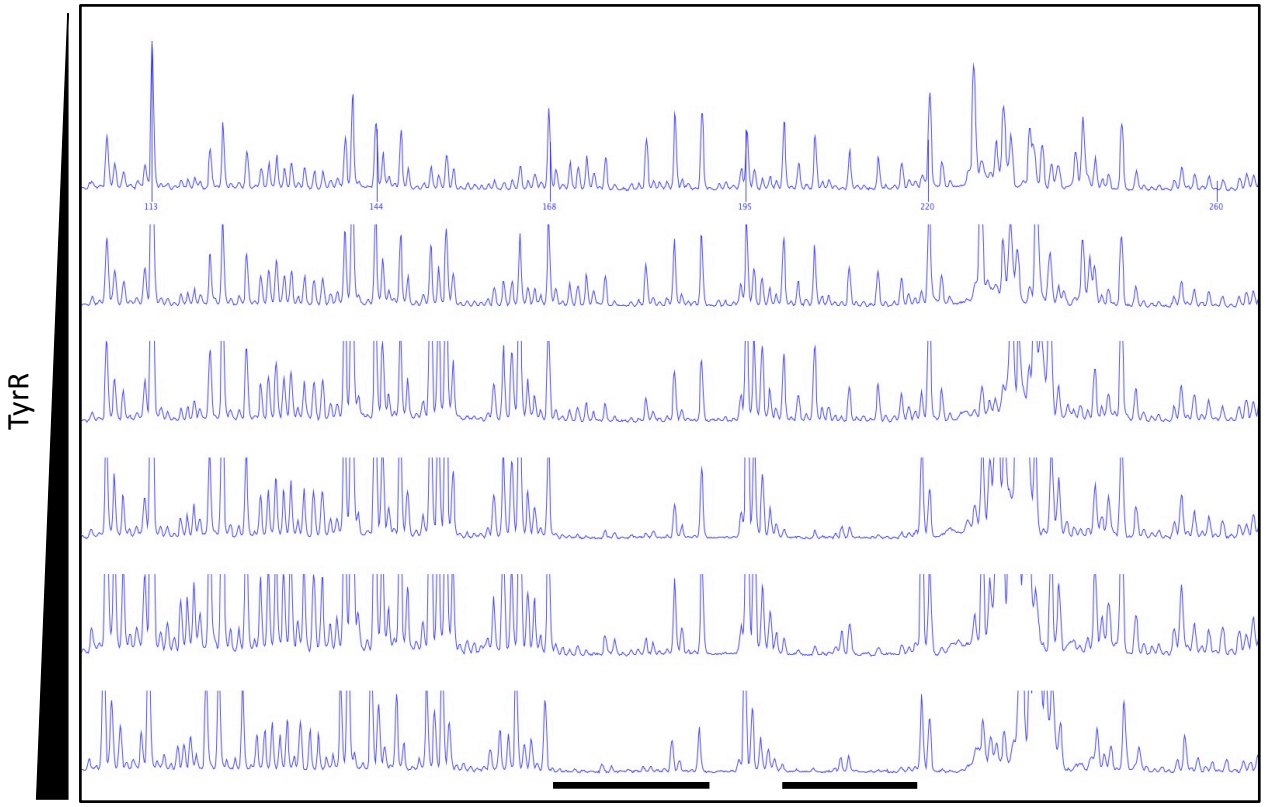
Supplementary Figure S2. Multiple sequence alignment of the *folA* promoter shows the absence of TyrR binding sites in *E. ludwgii*. The locations of TyrR and IHF binding sites in *E. coli* are boxed, promoter sequences, transcription start site, *E. coli folA* start codon and *kefC* stop codon are shown in bold. *E. coli* K12 (Eco K12), *E. ludwigii* UW5 (Elu UW5).

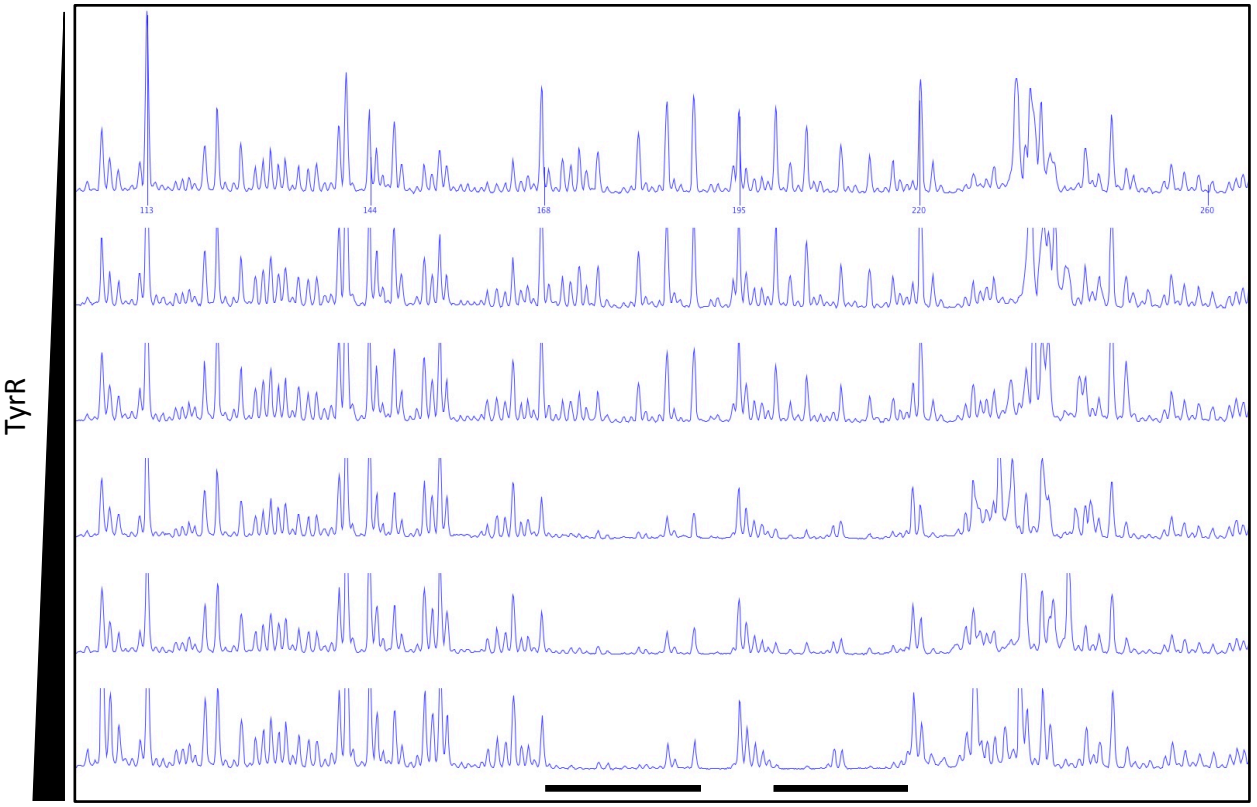


Supplementary Figure S3. Strong correlation of gene expression levels between quantitative RT-PCR and RNA-Seq data. Comparison of $\log_2$-fold changes for seven genes, each represented by a circle. The Pearson correlation coefficient (R), *p*-value (P), and the line of best fit from an ordinary least-squares linear regression are indicated.

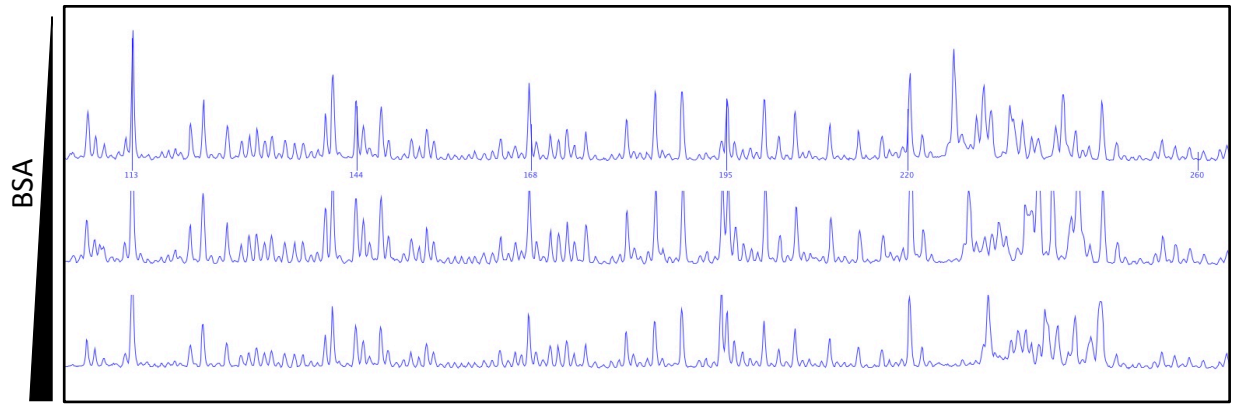


Supplementary Figure S4. Enriched GO terms in the genes differentially expressed in the *tyrR* mutant compared to wild type *E. ludwigii*. REVIGO TreeMap visualization of enriched GO terms at $p < 0.05$ determined by GOSeq for up- and down-regulated genes in the *E. ludwigii tyrR* mutant. GO terms are presented according to the three main classifications: biological processes (BP), cellular components (CC) and molecular function (MF). Semantically similar GO terms are grouped together by colour (SimRel = 0.7) (1) and box size correlates to the $-\log_{10}$ *p*-value calculated by GOSeq.

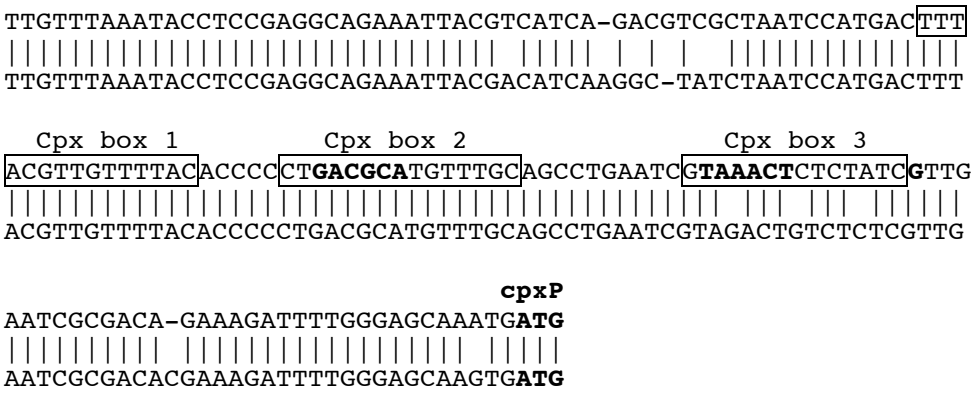

TyrR
113
144
168
195
220
260


TyrR
113
144
168
195
220
260

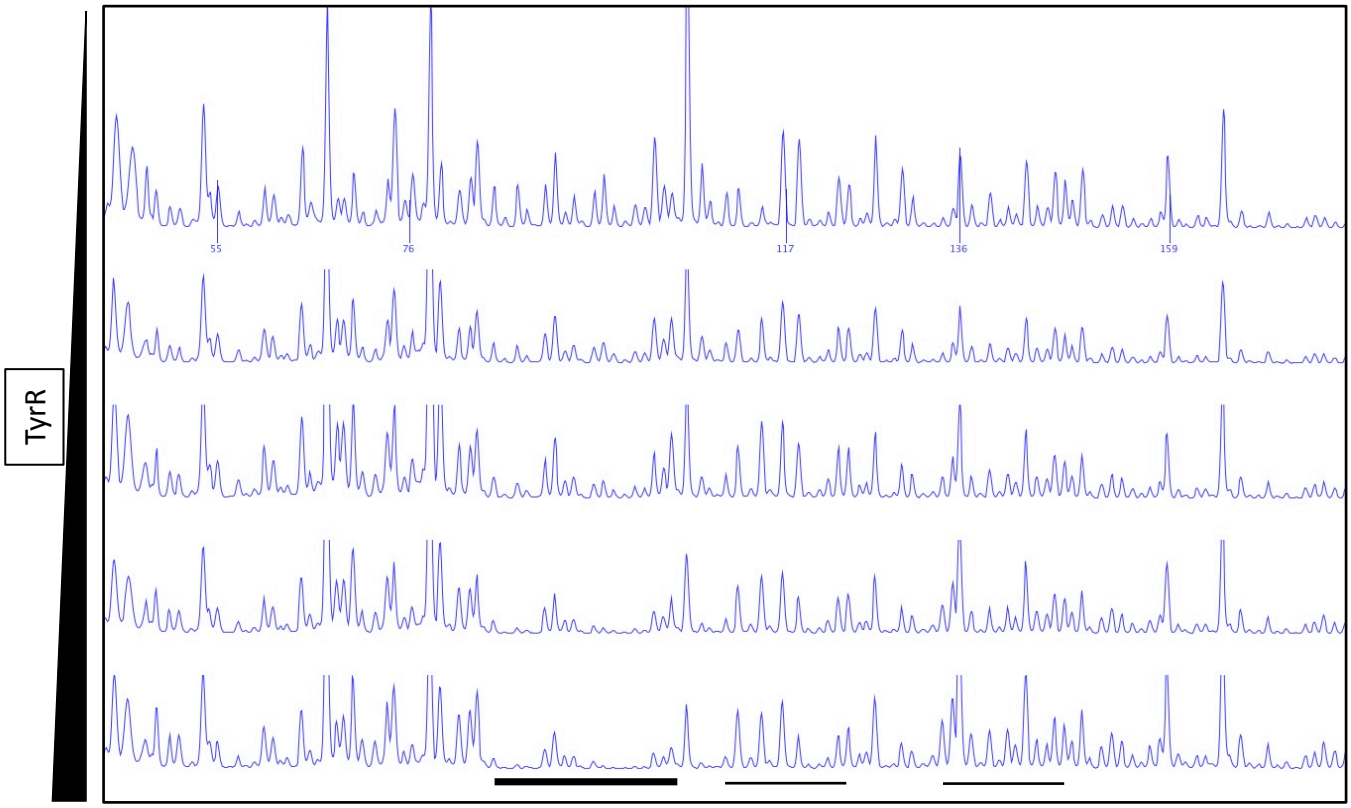
Supplementary Figure S5. DNase I footprinting assays indicate two TyrR binding sites in the *dmpM* promoter region. A 302 bp FAM-labeled PCR fragment containing the *dmpM* promoter region was incubated with increasing amounts (0, 0.18, 0.37, 0.75, 1.5, and 3.0 μg) of TyrR in the absence (top panel) and presence (bottom panel) of 1 mM tyrosine prior to digestion with DNase I. Larger regions of the same traces in Figure 5 are shown. Numbers on the upper trace correspond to the nucleotide position in the PCR fragment. Solid black bars highlight regions between nucleotides 169-188 and 199-218 that are protected from DNase I digestion as determined by capillary electrophoresis.

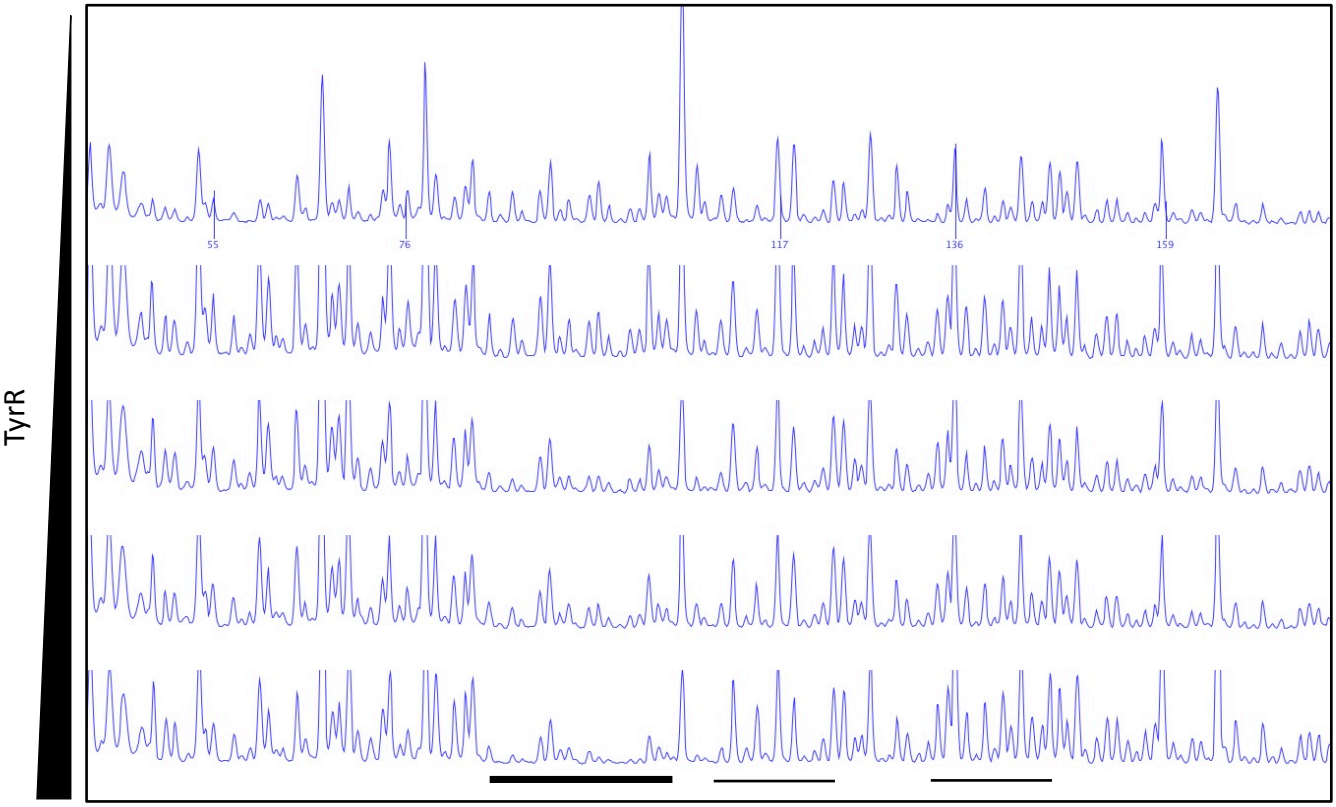


Supplementary Figure S6. Control DNase I footprinting assays show that bovine serum albumin (BSA) did not protect the *dmpM* promoter region from DNase I digestion. A FAM-labeled PCR fragment containing the *dmpM* promoter region was incubated with increasing amounts (0, 15, and 30 μg) of BSA prior to digestion with DNase I. Numbers shown on the upper trace correspond to the nucleotide position in the PCR fragment.

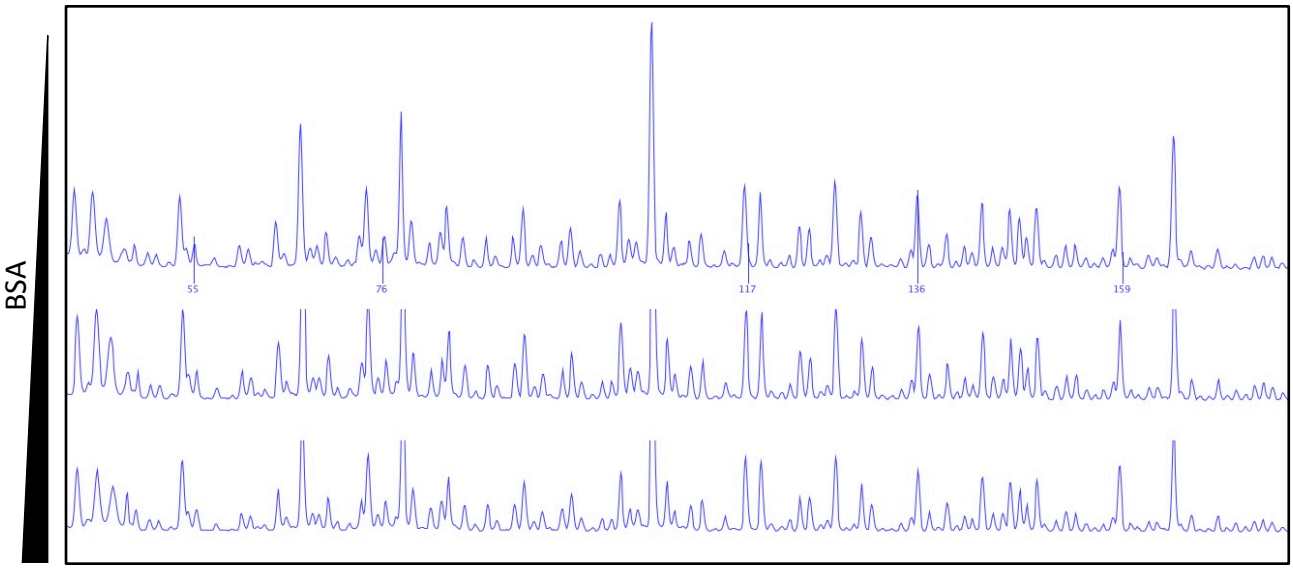


Supplementary Figure S7. Alignment of *cpxR-cpxP* intergenic regions of *E. coli* (top sequence) and *E. ludwigii* (bottom sequence) indicate that CpxR binding sites (boxed) determined for *E. coli* (2, 3) are conserved in *E. ludwigii*. Promoter sequences, transcription start site, and start codon for *E. coli cpxP* are shown in bold.

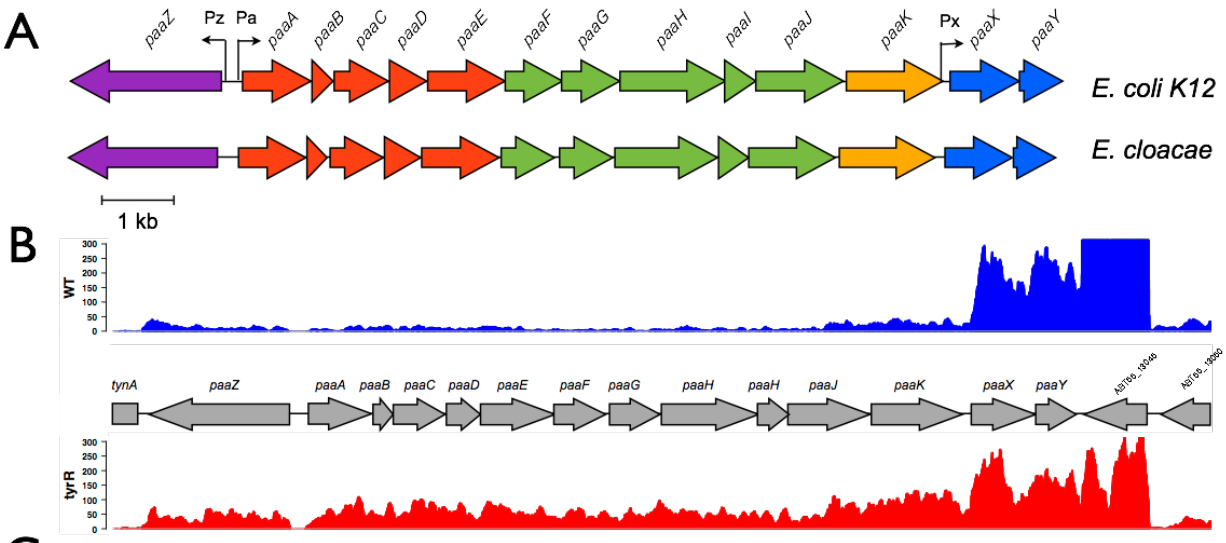

TyrR
55
76
117
136
159


TyrR
55
76
117
136
159

Supplementary Figure S8. DNase I footprinting assays indicate a single TyrR binding site in the *cpxR-cpxP* intergenic region. A 238 bp FAM-labeled PCR fragment containing the *cpxR-cpxP* intergenic region was incubated with increasing amounts (0, 0.75, 1.5, 3.0, and 6.0 μg) of TyrR in the absence (top panel) and presence (bottom panel) of 1 mM tyrosine prior to digestion with DNase I. Larger regions of the same traces in Figure 9 are shown. Numbers shown on the upper trace correspond to the nucleotide position in the PCR fragment. The thick black bars highlight regions between nucleotides 85-105, which encompass box 1, that are protected from DNase I digestion as determined by capillary electrophoresis. The thin black bars indicate the regions containing boxes 2 and 3 (see Figure 7), which are not protected.



Supplementary Figure S9. Control DNase I footprinting assays show that bovine serum albumin (BSA) did not protect the *cpxR-cpxP* intergenic region from DNase I digestion. A FAM-labeled PCR fragment containing the *cpxR-cpxP* intergenic region was incubated with increasing amounts (0, 15, and 30 μg) of BSA prior to digestion with DNase I. Numbers shown on the upper trace correspond to the nucleotide position in the PCR fragment.



C

```
                                                  MET
Eco_K12    CCAGGTACCGGATAAGAAACTGGCTAACTGCTGCATCGCTACTCTCCAGATGTTTCACAT      60
Ecl_WSU1   CCAGGCACCGGACAAGAAGCTGGCTAACTGCTGCATCGCTTATCTCCAAGTGTTACGTAA      60
Ecl_UW5    CCAGGTGCCGGACAAGAAGCTGGCTAACTGCTGCATCGCTTATCTCCAAGTGTTACGTAA      60
           *****  ***** ***** **********************  ******  **** *  *

             +1       Pz PaaX box
Eco_K12    TTCTGTTGCTAATAGTTAAATCGCGAATCATAAAAAGCAAAGGATCTTTTAACGAAATGT      120
Ecl_WSU1   TTTCACTATCAATAGTTTAATCATGAATCATAAAAAGCAAGCTGAGTTTTAATGAATTGT      120
Ecl_UW5    TTTCACTATCAATAGTTTAATCATGAATCATAAAAAGCAAGCTGAGTTTTAATGAATTGT      120
           **    *   ******* ****  ***************      ****** *** ***

                                IHF
Eco_K12    TAACTATGCGATCTGTATAGCAACTGCGGAAAACATTAATGCACTGATAAATAATGATTT      180
Ecl_WSU1   TAACATTGTGATCCAGCGTAATGAAGTGGAAGGCCGGAAGC-CCCGTTATGCAGGCCTTG      179
Ecl_UW5    TAACATTGTGATCCAGCGTAATGAAGTGGAAGGCCGGAAGC-CCCGTTGTGCAGGCCTTG      179
           ****  ** ****           * ****  *   **    * * *    *    **

                          CRP                -35
Eco_K12    ATAAAAATAGGGTGCGAAATCCGTCACAGTTCAAACATACAAAATTTGTGATTTTACTTA      240
Ecl_WSU1   TGCAAAAAGCCTTGCGAGCACCATCACAAAATAGGGAAACATTTCTTGGGGTTGTATTTA      239
Ecl_UW5    TGCAAAAAGCCTTGCGAGCACCATCACAAAATAGGGAAACATTTCTTGGGGTTATATTTA      239
             ****     *****   ** *****    *   * ***    *** * ** ** ***

             -10           +1                          Pa PaaX box
Eco_K12    ACTATTGTGTAACTTTCATAAAACAATGTGATTCGTGT-TTTTAATTAATTCACGAAAAC      299
Ecl_WSU1   ACCTTTGTGTAACTTTTAAGAAACGAAGTGATTCAGTATTTCACTTTAGGTGGTAAATAA      299
Ecl_UW5    ACCTTTGTGTAACTTTTAAGAAATGAAGTGATTCAGTATTTCACTTTAAGTGGTAAATAA      299
           **  ************ *  ***  * *******     **    ***  *    ** *

                                MET
Eco_K12    TGGAATCGTAAAGGTGATGACGTGACCCAAGAAGAACGCTTTGAGCAACGGATAGCCC   357
Ecl_WSU1   TCGAATCATAAAGGTGATGACGTGACGCAAGAACAACGCTTTGAGCAGCGCATAGCGC   357
Ecl_UW5    TCGAATCATAAAGGTGATGACGTGACGCAAGAACAACGCTTTGAGCAGCGCATAGCGC   357
           * ***** ****************** ****** ************ ** ***** *
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Supplementary Figure S10. T**he *paa* catabolic operon for phenylacetate degradation in *E. ludwigii*.** (A) Genome synteny of the *paa* genes of *E. ludwigii* and *E. coli* K12. Genes encoding the ring-hydroxylating complex are in red (*paaABCDE*), the beta-oxidation enzymes in green (*paaFGHIJ*), the phenylacetate-CoA ligase in orange (*paaK*), the transcriptional repressor and thioesterase in blue (*paaX*), and a ring opening enzyme in purple (*paaZ*). (B) RNA-Seq reads mapped to the *paa* operon in the wild-type (blue) and *tyrR* mutant (red) *E. ludwigii*. Read coverage is consistent across the length of the operon. (C) Multiple sequence alignment of the intergenic region of divergently transcribed *paaZ* and *paaA* genes of *E. coli* K12, *E. ludwigii* UW5, and *E. ludwigii Ec*WSU1. The locations of *E. coli* PaaX, IHF, and CRP binding sites are boxed and the promoter elements are in bold. Start codons for *paaZ* and *paaA* are in bold. Conserved nucleotides are indicated with asterisks.

Supplementary Table S1. Bacterial strains and plasmids used in this study

Strain or Plasmid	Relevant characteristics	Reference
E. coli		
JM109	Cloning host	Promega
S17-1λ*pir*	Cloning host; Tp^r Sm^r *recA thi pro hsdR*-M+ RP4-2-Tc::Mu-Km::Tn7λpir	(4)
A118	His_6-TyrR expression host; M15 strain (pREP4, pQEtyrR)	(5)
E. ludwigii		
UW5	Wild type strain; Amp^r	(5)
J224	UW5 Δ*tyrR*	This study
Plasmids		
pKD4	Source of FRT-flanked Km^r gene; rep R6K Amp^r FRT-Km^r-FRT	(6)
pJQ200SK	Low copy number suicide vector; Gm^r *sacB*	(7)
pCP20	Temperature-sensitive replication and induction of FLP-recombinase; rep $pSC101^{ts}$ Amp^r Cm^r *cI*857 λP_R	(6)
pGEM-T Easy	Cloning vector; Amp^r	Promega
pUCdmpM	pUCIDT-AMP cloning vector; Amp^r; 276 bp *dmpM* promoter	IDT
pUCdmpM-mut1	pUCdmpM with TyrR box 1 mutation	This study
pUCdmpM-mut2	pUCdmpM with TyrR box 2 mutation	This study
pUCdmpM-mut3	pUCdmpM with TyrR box 1 and 2 mutations	This study
pIDTcpx	pIDTSMART-AMP cloning vector; Amp^r; 151 bp *cpxR-cpxP* intergenic region	IDT
pIDTcpx-mut1	pIDTcpx with CpxR box 1 mutation	This study
pIDTcpx-mut2	pIDTcpx with CpxR box 2 mutation	This study
pIDTcpx-mut3	pIDTcpx with CpxR box 3 mutation	This study
pTUD	pGEM-T Easy; 1.5 kb insert of *tyrR* upstream and downstream flanking regions (TUD)	This study
pTUD-Km	pTUD::FRT-Km^r-FRT	This study
pJQTUD-Km	pJQ200SK; TUD::FRT-Km^r-FRT	This study

Supplementary Table S2. Oligonucleotides used in this study. Fluorescent modifications are in square brackets.

Primer	Sequence 5'-3'	Reference
TUF-PstI	AAAAAA**CTGCAG**GGGATCTGCTCCACAGTCAC	This study
TUR-XbaI	CGAAAAATGGC**TCTAGA**GGGAAATTCACCGTTTTAAG	This study
TDF-XbaI	GTGAATTTCCC**TCTAGA**CGGTAAAAAGCCTCTGTAAAC	This study
TDR-SacI	AAAAAA**GAGCTC**TGTATCGCAGGTTGAAGTGG	This study
PKDP1	GTGTAGGCTGGAGCTGCTTC	(6)
PKDP2	CATATGAATATCCTCCTTAG	(6)
TUF	GATCTGTTGCGACCGAGTG	This study
TUR	TTTCTACGTGTTCCGCTTCC	This study
TDF	CAAAAGCGCTCTGAAGTTCC	This study
TDR	CCAAATCTACACGCTTCACG	This study
ICRTIF	TCGAACTCAGCAAACAGCAC	(5)
ICRTIR	AGGTTTGCAACGTTCTCCAG	(5)
recAF2	GCTGGACCCTGTTTATGCTC	This study
recAR1	GCCTTCGATTTCAGCTTTTG	This study
proP35	ACTATAAAACCCTCACGCTCG	This study
proP160	GCAACCACTCCGGGATATC	This study
eco145	GTCATTCAGTTACCCGCTCAG	This study
eco269	TCCAGGGTTTTGCTTTCCAG	This study
aroG576	CATTAAGGTGGCAATTGACGC	This study
aroG670	TGGTATTCACAATGGCGGAG	This study
yagU384	ACTCTGGCAAGGTTTACTGG	This study
yagU518	AAATGCCCCACGATCTCTG	This study

Primer	Sequence 5’-3’	Reference
cpxP356	AGATGTTCCACCTGCTAACG	This study
cpxP494	CGGGTACTGCTATTGCTACTG	This study
cpxR209	ACCAGACGCCCGTAATTATG	This study
cpxR302	GGCTTTGGTAAATAGTCATCCG	This study
dmpM161	CCGATATGCTGAAACTTGACG	This study
dmpM294	GTGGTCCTTACAGAGAAAGTGG	This study
dmpM-F-FAM	[6FAM]GGTGATCAGCCTGTGCTCTA	This study
dmpM-R	TGTGAATTTCTTATTTACAGCTACCG	This study
cpx-F-FAM	[6FAM]CTGAGGCTCGTCCTGAATG	This study
cpx-R	TGATCGTGGACCGATCATAC	This study
dmpM-F1	CAACCGTTAATTTCGCATTC	This study
dmpM-R-FAM	[6FAM]GCTACCGGGTACAGTTAAACC	This study
cpx-F1	CCTCCGAGGCAGAAATTACG	This study
cpx-R-FAM	[6FAM]AAATCTTTCGTGTCGCGATT	This study
SDM1-F	TATAACTTAAATGTTATTCCTGTTTTCTGGGAGGGG	This study
SDM1-R	AATAACATTTAAGTTATATAAGACAGACTCCTGTAAATAAAAC	This study
SDM2-F	TATCCGGTTTTATTTATAGGAGTCTGTCTTATGTAAC	This study
SDM2-R	TATAAATAAAACCGGATATTAAGTCACCGTTTTTTAATG	This study
SDM2-F2	TATCCGGTTTTATTTATAGGAGTCTGTCTTATATAAC	This study

Supplementary Table S3. Sequences of the wild-type and mutant *dmpM* and *cpx promoters* inserted in pUCIDT-AMP and pIDTSMART-AMP, respectively, used as templates to generate probes for EMSAs. Primer binding sites are indicated in italics. The *dmpM* fragment is flanked outside EcoRI sites by GC adapters (lower case), which were necessary to improve the synthesis reaction due to low GC content of the target sequence. Predicted TyrR binding sites in the *dmpM* and *cpx* promoters are underlined, and substituted nucleotides in mutant promoters are in bold lowercase.

Insert	Characteristics	Sequence 5'-3'
dmpM-wt	Wild type promoter	ctcacttgtagaacggtgatcagcctgtgctctagagcctgatagttgagcgatacac acGAATTC*CAACCGTTAATTTCGCATTC*AGATATATATAACAATATTTACAGGCCACA CCCGGAAATGAATTGAAAAACGCATGATCTTTAACGGTATTAGTTACCAGCATTAAAA AACGGTGACTTAATGTCCGGTTTTATTTACAGGAGTCTGTCTTATGTAACTTAAATGT TACTCCTGTTTTCTGGGAGGGGGCGTTACATATGAAGTAATAAA*GGTTTAACTGTACC* *CGGTAGC*TGTAAATAAGAAATTCACATTTTTTAATCCTTCAGGAGCTAATGAATTCtg atcgttgaagtcgacctacatcgagtgcgcactatcaagagtgttccagtcacgcgat
dmpM-mut1	Predicted TyrR box 1 mutated	ctcacttgtagaacggtgatcagcctgtgctctagagcctgatagttgagcgatacac acGAATTC*CAACCGTTAATTTCGCATTC*AGATATATATAACAATATTTACAGGCCACA CCCGGAAATGAATTGAAAAACGCATGATCTTTAACGGTATTAGTTACCAGCATTAAAA AACGGTGACTTAATGTCCGGTTTTATTTACAGGAGTCTGTCTTAT**a**TAACTTAAATGT TA**t**TCCTGTTTTCTGGGAGGGGGCGTTACATATGAAGTAATAAA*GGTTTAACTGTACC* *CGGTAGC*TGTAAATAAGAAATTCACATTTTTTAATCCTTCAGGAGCTAATGAATTCtg atcgttgaagtcgacctacatcgagtgcgcactatcaagagtgttccagtcacgcgat
dmpM-mut2	Predicted TyrR box 2 mutated	ctcacttgtagaacggtgatcagcctgtgctctagagcctgatagttgagcgatacac acGAATTC*CAACCGTTAATTTCGCATTC*AGATATATATAACAATATTTACAGGCCACA CCCGGAAATGAATTGAAAAACGCATGATCTTTAACGGTATTAGTTACCAGCATTAAAA AACGGTGACTTAAT**a**TCCGGTTTTATTTA**t**AGGAGTCTGTCTTATGTAACTTAAATGT TACTCCTGTTTTCTGGGAGGGGGCGTTACATATGAAGTAATAAA*GGTTTAACTGTACC* *CGGTAGC*TGTAAATAAGAAATTCACATTTTTTAATCCTTCAGGAGCTAATGAATTCtg atcgttgaagtcgacctacatcgagtgcgcactatcaagagtgttccagtcacgcgat

Insert	Characteristics	Sequence 5'-3'
dmpM-mut3	Predicted TyrR boxes 1 and 2 mutated	ctcacttgtagaacggtgatcagcctgtgctctagagcctgatagttgagcgatacac acGAATTC*CAACCGTTAATTTCGCATTC*AGATATATATAACAATATTTACAGGCCACA CCCGGAAATGAATTGAAAAACGCATGATCTTTAACGGTATTAGTTACCAGCATTAAAA AACGGTGACTTAAT**a**TCCGGTTTTATTTA**t**AGGAGTCTGTCTTAT**a**TAACTTAAATGT TA**t**TCCTGTTTTCTGGGAGGGGGCGTTACATATGAAGTAATAAA*GGTTTAACTGTACC CGGTAGC*TGTAAATAAGAAATTCACATTTTTTAATCCTTCAGGAGCTAATGAATTCtg atcgttgaagtcgacctacatcgagtgcgcactatcaagagtgttccagtcacgcgat
cpx-wt	Wild type promoter	GAATTCTTGTTTAAATA*CCTCCGAGGCAGAAATTACG*ACATCAAGGCTATCTAATCCA TGACTTTACGTTGTTTTACACCCCCTGACGCATGTTTGCAGCCTGAATCGTAGACTGT CTCTCGTTG*AATCGCGACACGAAAGATTTT*GGGAGCAAGTGGAATTC
cpx-mut1	CpxR site 1 mutated	GAATTCTTGTTTAAATA*CCTCCGAGGCAGAAATTACG*ACATCAAGGCTATCTAATCCA T**a**A**a**TTTACGTTGTTTTA**t**ACCCCCTGACGCATGTTTGCAGCCTGAATCGTAGACTGT CTCTCGTTG*AATCGCGACACGAAAGATTTT*GGGAGCAAGTGGAATTC
cpx-mut2	CpxR site 2 mutated	GAATTCTTGTTTAAATA*CCTCCGAGGCAGAAATTACG*ACATCAAGGCTATCTAATCCA TGACTTTACGTTGTTTTACACC**aaa**TGACGCATGTTTG**t**AGCCTGAATCGTAGACTGT CTCTCGTTG*AATCGCGACACGAAAGATTTT*GGGAGCAAGTGGAATTC
cpx-mut3	CpxR site 3 mutated	GAATTCTTGTTTAAATA*CCTCCGAGGCAGAAATTACG*ACATCAAGGCTATCTAATCCA TGACTTTACGTTGTTTTACACCCCCTGACGCATGTTTGCAGCCTGAATC**a**TAGACTGT CTCT**tt**TTG*AATCGCGACACGAAAGATTTT*GGGAGCAAGTGGAATTC

Supplementary Tables S4-S6 are available separately as excel files.

Supplementary Table S7. Proteins in the Protein Data Bank with a high probability of matching DmpM as determined by HHPred. The percent probability of the database match to the DmpM amino acid sequence and the percent amino acid identity are indicated. The E-value is defined as for BLAST.

PDB	Product	Species	Probability	E-value	Identity %
3GWZ	Mitomycin 7-O-methyltransferase	*Streptomyces lavendulae*	100.0	5.20E-30	27
3LST	Orsellinic acid methyltransferase	*Micromonospora echinospora*	100.0	1.50E-30	36
5EEG	Carminomycin 4-O-methyltransferase	*Streptomyces peucetius*	99.95	1.10E-27	23
1FP2	Isoflavone O-methyltransferase	*Medicago sativa*	100.0	4.50E-32	25
5XOH	Bergaptol O-methyltransferase	*Peucedanum praeruptorum*	100.0	1.80E-31	25
3P9C	Caffeic acid O-methyltransferase	*Lolium perenne*	99.95	9.30E-28	23

Supplementary Table S8. Quantification of band intensity in EMSAs (Figure 10) with probes containing wild-type or mutant CpxR boxes in the *cpxR*-*cpxP* intergenic region and various concentrations of TyrR. Band intensities (Int) were quantified using Image Lab™ software v. 6.0.1 (Bio-Rad).

DNA Probe	TyrR (μM)	Band No.	Relative Front	Background Adjusted Band Volume (Int)	Total Band Volume (Int)	Band %	Lane %
Wild-type	0	1	0.96	743392	877666504	100.0	87.2
Wild-type	0.08	1	0.97	712296	1374035832	100.0	86.8
Wild-type	0.87	1	0.93	721448	1496219920	100.0	89.9
Wild-type	2.19	1	0.17	454584	2107926808	53.6	51.6
Wild-type	2.19	2	0.94	393744	1434701112	46.4	44.7
Wild-type	4.39	1	0.13	418600	1489237256	48.5	45.7
Wild-type	4.39	2	0.94	444496	1217276944	51.5	48.6
Box 1 mutant	0	1	0.94	562848	1217279024	100.0	90.4
Box 1 mutant	0.08	1	0.92	439296	1332842368	100.0	86.3
Box 1 mutant	4.39	1	0.17	76692	2269970604	19.0	16.5
Box 1 mutant	4.39	2	0.93	327624	1208227812	81.0	70.4
Box 2 mutant	0	1	0.93	775320	1305836792	100.0	90.7
Box 2 mutant	0.08	1	0.93	658736	1516514792	100.0	89.8
Box 2 mutant	4.39	1	0.11	548392	1523632864	78.9	73.9
Box 2 mutant	4.39	2	0.94	147056	666276728	21.1	19.8
Box 3 mutant	0	1	0.94	616096	891051304	100.0	89.4
Box 3 mutant	0.08	1	0.91	583752	1672779368	100.0	87.0
Box 3 mutant	4.39	1	0.12	557994	1677489859	87.4	82.3
Box 3 mutant	4.39	2	0.94	80230	790273071	12.6	11.8

REFERENCES

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