# **Supporting Information**

### Thompson et al. 10.1073/pnas.1201398109

#### SI Methods

Generation of pET YtgR and YtgCR Expression Vectors. The sequence coding for the final 147 amino acids of CT069 was amplified from C. trachomatis serovar D genomic DNA using primers CT1003 and CT1004, which engineered an additional CACC onto the 5' end of the PCR product for directional insertion into the pET151-D/TOPO vector (Invitrogen) to make pET-YtgR. To generate pET-YtgCR, the full-length ct069 coding sequence was amplified from pBAD-YtgCR (see below) using primers CT1101 AND CT1102, which engineered an additional 3' NcoI restriction site. The NcoI restriction site was used to ligate the ct069 PCR product to a double-stranded oligonucleotide coding for the FLAG epitope, which was generated through the annealing of two custom-synthesized oligonucleotides (CT1033 and CT1034). The ligated sequence was amplified with primers CT1103 and CT1104, which added a 5' CACC for directional insertion into the pET151-D/TOPO vector.

To generate pBAD-YtgCR, the full-length *ct069* coding sequence was amplified from genomic DNA using primers CT1036 and CT1035, which added an additional 5' KpnI restriction site. This site was used to ligate a FLAG epitope coding sequence, which was generated through the annealing of two custom synthesized oligonucleotides (CT1033 and CT1034). PCR amplification of the ligated sequence with primers CT1037 and CT1035 generated a PCR product, to which 3' A-overhangs were added with the addition of Taq polymerase and ATP for 5 min at 72 °C. The PCR product was ligated into the pBAD-TOPO/TA expression vector.

Generation of the pCCT Reporter Plasmids. A nucleotide region of 2,100 bp containing a tetracycline resistance cassette (TetR) and a p15A origin of replication sequence was amplified from pACYC184 using primers CT1018 and CT1019, which incorporated a BgIII and XhoI restriction endonuclease sites on the 5' and 3' flanks, respectively. A nucleotide region of 4,878 bp was amplified from the pBAD-TOPO/LacZ/V5-His control expression vector using primers CT1020 and CT1021. The resultant PCR product contained an *araC* gene, an arabinose inducible promoter ( $P_{BAD}$ ), a sequence coding for  $\beta$ -galactosidase tagged C-terminally with V5 and polyhistidine epitopes, rrnB T1 and T2 transcriptional termination sites, and incorporated XhoI and BgIII restriction endonuclease sites at the 5' and 3' flanks, respectively. The two PCR products were digested with XhoI and BgIII simultaneously, before resolution on 0.7% Tris-acetate-EDTA (TAE) agarose gel and purification. Digested products were circularized using T4 ligase treatment overnight at 16 °C to create pCCT100. A KpnI restriction site was inserted into the flanking region of P<sub>BAD</sub> using inverse PCR with primers CT1022 and CT1023 within the framework of the Site-directed Mutagenesis kit (Invitrogen), to generate pCCT101. A sequence containing the YtgR operator sequence was PCR amplified from C. trachomatis serovar D genomic DNA with primers CT1024 and CT1025, which generated KpnI restriction sites on both flanks. Vector and the PCR product were digested with KpnI and ligated together to create pCCT102. Colony PCR verified insert direction, and plasmids were verified by sequencing (GATC).

**Expression and Purification of Recombinant YtgR.** Overnight cultures of BL21\* (DE3) *E. coli* harboring pET-YtgR were subcultured into 200 mL of LB plus carbenicillin, grown to an  $OD_{600} = 0.5$  and supplemented with Isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.75 mM for 5 h before

sedimentation by centrifugation (10,000  $\times g$  for 30 min) and storage at -80 °C.

After determining recombinant YtgR remained predominantly insoluble, we used a purification process previously described for the purification of other insoluble transcription factors (1, 2), with slight modifications. Pellets of E. coli harboring pET-YtgR were thawed on ice, and all subsequent steps were carried out at 4 °C or on ice. The thawed pellet was resuspended in 20 mL of Buffer A [50 mM Tris HCl, 50 mM NaCl, 0.1 mM DTT, 5% glycerol, 0.1 mM EDTA, 2% (wt/vol) deoxycholate (pH 7.0)] supplemented with 0.2 mg/mL lysozyme for 30 min. The bacterial suspension was then sonicated and centrifuged at  $12,000 \times g$  for 30 min. The insoluble pellet was washed twice with buffer A (without lysozyme), with each wash lasting 1 h with gentle agitation. After the second wash step, the insoluble fraction was sedimented by centrifugation at  $16,000 \times g$  for 30 min. The YtgR recombinant was liberated from the insoluble fraction by resuspending the pellet in buffer B [50 mM Tris HCl, 50 mM NaCl, 0.1 mM DTT, 5% glycerol, 0.25% (wt/vol) sarkosyl (pH 7.0)] for 1 h with gentle agitation. The soluble fraction was collected after centrifugation  $(16,000 \times g)$ , and another round of protein liberation was performed. Soluble fractions were pooled and recombinant YtgR was purified using the Ni-NTA kit (QIAGEN) as described by the instructions of the manufacturer for nondenaturing purification. Samples from each step in this process were analyzed by SDS/PAGE. Elutions containing a single product by Coomassie staining were pooled, and dialyzed into a buffer suitable for low-temperature storage (50 mM Tris HCl, 50 mM NaCl, 0.1 mM DTT, 5% glycerol, pH = 7.0). Half of the sample was incubated with Chelex-100 resin (Bio-Rad) for 2 h at 4 °C. Non-Chelex-treated (referred to as purified YtgR) and Chelex treated (referred to as apo-YtgR) were aliquoted, flash frozen, and stored at -80 °C. Protein concentrations were determined using the BCA Protein Assay Kit (Pierce).

**Generation of Biotin-Tagged DNA Oligonucleotides.** The region upstream of the *ytgA* coding sequence, containing the putative promoter elements and YtgR operator sequence was amplified from genomic DNA purified from *C. trachomatis* Serovar D EBs using primers CT1011 and CT1012 (Dataset S1). Primer CT1011 was engineered with a 5' Biotin-tag (Custom ordered from Invitrogen). A single amplified PCR product was ethanol precipitated, resuspended in nuclease free water and stored at -20 °C.

Dot-Blot Assay. Whatman Optitron BA-5 83 0.2 µM NC (Millipore) membrane was soaked in 0.4 M KOH for 5 min, before 3× 5-min washes with distilled deionized H<sub>2</sub>O. The membrane was equilibrated in wash buffer [20 mM Tris-HCl (pH 8.0), 50 mM KCl, 5% glycerol, 0.5 mM DTT, 2 mM MgCl<sub>2</sub>, 150 µM ascorbic acid] for at least 15 min at 4 °C. The apo-YtgR was diluted into 38-µL reaction buffer (wash buffer plus 150 µM FeSO4 or MnCl<sub>2</sub>) and allowed to equilibrate for 15 min at room temperature. A 2-µL volume of a 20 nM concentration of the biotinylated-DNA sequence was added to the reaction, which was then incubated at 30 °C for 30 min. The NC membrane was placed on top of filter paper within the dot-blot hybridization manifold (DHM-48; Fischer), and wells were flushed with wash buffer two times. Without application of the vacuum, samples were added to respective wells. The vacuum was then applied and each used well was washed two more times with wash buffer. The apparatus was disassembled, and the NC membrane was blocked in TBS plus 0.1% Tween-20 containing 3% BSA

(Sigma). The NC blot was probed with HRP-conjugated streptavidin in the same blocking buffer at a dilution of 1:20,000 for 1 h at room temperature. Biotinylated DNA retention to the NC blot was detected using the Immobilon HRP-substrate chemiluminescent detection kit (Millipore) and images were captured using the FUJI-3000 LAS imager at -30 °C. Dot-blot intensities were measured from a single image, using ImageJ software. Values were transformed by the average intensity of an unused well. Results displayed in Fig. 2 A and B are one representative experiment of three showing the same trend.

Biolayer Interferometry. Biolayer interferometry kinetic studies of DNA:protein interactions were performed using the Octet QKe (ForteBio). Target DNA was PCR amplified with a 5' biotinylated oligo, as described above. A 25 nM final concentration of purified recombinant YtgR was equilibrated in Kinetics Buffer (KB) (ForteBio) supplemented with the various metal solutions (FeSO<sub>4</sub>, MnCl<sub>2</sub>, ZnCl<sub>2</sub>, CuCl<sub>2</sub>, or CoCl<sub>2</sub>) at a final concentration of 200 µM for ~20 min. During this time, the biotinylated PCR product was loaded onto the streptavidin-coated sensors for 15 min, which was ample time to achieve tip saturation. After washing in KB) which contains BSA to negate nonspecific protein binding, sensors were incubated in the recombinant  $YtgR^{+/-}$  various metal supplements. The change in internally reflected light attributable to DNA:protein interactions was collected in real time for 10 min, at which point the tips were washed once in KB and incubated for an additional 20 min in KB to collect dissociation data. Binding kinetics were calculated based on the change in the reflected light over time (measured as a change in distance between a reference reflection point and the bound material on the tip) using the software provided with the Octet QKe.

**Western Blots.** *C. trachomatis* D/UW-3/CX was obtained originally from Ted Hacksadt (Rocky Mountain Laboratory, National Institute of Allergy and Infectious Diseases, Hamilton, MT), and propagated/purified in HeLa cells as described (3). Infected HeLa cell monolayers (multiplicity of infection of 2) were grown for 1, 6, or 24 h postinvasion before being lysed into RIPA buffer. Samples were mixed with 5× SDS sample buffer and resolved on a 12% TGS/polyacrylamide gel. Protein transfer and immunoblotting were performed with standard methods, using rabbit polyclonal antisera, raised against the full-length CT069, which was a kind gift of Guangming Zhong (University of Texas Health Sciences Center, San Antonio, TX).

Top10 *E. coli* harboring the pBAD-YtgCR expression vector, or the pBAD-empty vector, were grown overnight in 5 mL of LB broth containing 0.2% (wt/vol) D-glucose and 50 µg/mL carbeni-

1. Nguyen LH, Jensen DB, Burgess RR (1993) Overproduction and purification of sigma 32, the Escherichia coli heat shock transcription factor. *Protein Expr Purif* 4:425–433.

 Posey JE, Hardham JM, Norris SJ, Gherardini FC (1999) Characterization of a manganese-dependent regulatory protein, TroR, from Treponema pallidum. Proc Natl Acad Sci USA 96:10887–10892. cillin. The next day, 1 mL of the cultures were pelleted and rinsed with LB broth twice, to remove traces of D-glucose. Each bacterial culture was then subcultured to an  $OD_{600}$  of 0.1 in LB supplemented with carbenicillin, in triplicate. After 2 h, cultures were either induced for expression of the YtgCR [addition of 0.002% (wt/vol) L-arabinose], repressed for expression [addition of 0.2%(wt/vol) D-glucose], or were left unchanged (uninduced). After 4 h of additional growth, 0.1 OD units were pelleted and resuspended in 100  $\mu$ L of 1× SDS sample buffer. Samples were boiled for 10 min at 100 °C and resolved by SDS/PAGE. Proteins were transferred, immunoblotted, and detected using standard techniques, in this case using a 1:2,000 dilution of an anti-V5 epitope monoclonal antibody that was conjugated to HRP (AbCam). To determine the affinity of the CT069 antisera, E. coli harboring pBAD-YtgCR were subcultured as before and supplemented with 0.02% (wt/vol) L-arabinose (induced) or 0.2% (wt/vol) D-glucose (repressed) for 4 h.

Reporter Gene Expression Assay. Chemically competent BL21\* E. coli were initially transformed with pCCT101 or pCCT102 and plated onto LB agar containing tetracycline (15 µg/mL). Selected colonies were verified for the presence of the reporter plasmid, grown in selective LB broth culture, made electrocompetent, transformed with pET151-EV, pET-YtgR, or pET-YtgCR by electroporation and were plated onto LB agar containing tetracycline (15 µg/mL), carbenicillin (50 µg/mL), and X-gal (40 µg/mL). Single transformants of either set of plasmids did not grow on double selective media. Colonies of cotransformants were grown overnight in double selective LB broth containing D-glucose (0.2% wt/vol) at 37 °C and 200 rpm. The next day, cultures were pelleted and resuspended in LB broth (to remove D-glucose) and subcultured to an OD<sub>600</sub> of 0.1 in 5 mL of double-selective LB broth supplemented with 5  $\mu L$  of a 50 mM FeSO4 solution. Samples were grown at 37 °C at 200 rpm. After 1 h, cultures were supplemented with 200 mM IPTG, to induce expression of the pET vectors. Following an additional hour of induction, Larabinose was supplemented to a final concentration of 0.2% (wt/vol) to abrogate AraC repression of the P<sub>BAD</sub> promoter. Samples were collected 2 h after L-arabinose supplementation, and LacZ expression was measured via the Miller assay, as described (4). The experiment was performed three times independently, with each trial including the full complement of cotransformant samples. Interexperimental error was corrected by transforming the values of each trial, such that the mean of all samples within each batch were identical. The mean MU of each sample from the three batch-corrected experiments is shown. Error bars represent 1 SD from the mean.

 Caldwell HD, Kromhout J, Schachter J (1981) Purification and partial characterization of the major outer membrane protein of Chlamydia trachomatis. *Infect Immun* 31: 1161–1176.

4. Miller JH (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

#### Cor

#### Ide

Consensus	xxx	FLTX	xgxx	XAXX	XXRXHRLWE	XYL	xxxxx:	xxxxxvHx
Identity		h			-			
	70		378		388		398	40
1. 084072 Y069_CHLTR extraction	70   QWS	-LT Ir	G	A epend	RHRLWE	SYL	protei	FNKNE <b>VH</b> Å
	47		355		365		374	38 TPENEVH
2. C0QMH4 C0QMH4_DESAH extraction 3. B5ECZ6 B5ECZ6_GEOBB extraction	52	LT	G 360 G	e <b>a</b> rr	370 R HRLWE	TYL SVI	379	28 38 VH
4. C6E6W6 C6E6W6_GEOSM extraction	52 1	LT	360 G	O <b>R</b> AN	370 R HRLWE	SYL	379	38 TPEEAVH
5. E8WRP7 E8WRP7_GEOS8 extraction	52 I GYL	LT		BARO	370 VER HRLWE	SYL	379 - ASIG	38 TPOBEVHP
6. D4H2B6 D4H2B6_DENA2 extraction	51 TIS	LT	G 359 G	EALR	369 LKKAHRVWE	TYL	378 	38 VPSDQLHB
7. A3ZXJ0 A3ZXJ0_9PLAN extraction	53 GFI- 83	LT	361 G 391	ARR	371 NR HR WE 401	TYL	380 - ORVGI 411	39 MPEDSLH 0 42
8. D5SYP0 D5SYP0_PLAL2 extraction	HYV- 76	LT	1 <b>G</b> 384		IVRAHRLWE 394	TFL	AORLGI 404	LRPEQWHD 41
9. E8R461 E8R461_ISOPI extraction	ALH 89	LT	G 397		VR HRLWE	SYL	DRHFDI 417	LPLDHLHD 42
10. D2R7Y8 D2R7Y8_PIRSD extraction	0 WK	LT	G 368	SAQR	R HRLWE	AYL	DTHFD1 388	LPRDHLHË 39
11. F0SLH1 F0SLH1_PLABD extraction 12. F2AP05 F2AP05_RHOBT extraction	03	LTP	G 411 G	LGER	VVR HRLWE 421 VVR HRLWE	SPL	431 1	LPODHLHĖ 44 LEGSRIHO
13. Q7UIM9 Q7UIM9_RHOBA extraction	03	-LT	411 G	ASS	421	OXP.	431	44 LEGSRIHO
14. A6C9P2 A6C9P2_9PLAN extraction	68 1	LT	376	ARO	386 URSHRLWE	TYL	396 I VEHAGI	40 INSTRUCT
15. E6SJ31 E6SJ31_THEM7 extraction	18   GVR	LT	G 426 G	ARR	436 R HRLWE	RYL	446 TDVAGI	45 MAWED <b>VH</b> O
16. E4M786 E4M786_9FIRM extraction	09 GAR	LT	417 	EARR	427 R HRLWE 452	RYL	437 TDVAGI	44 MAWED <b>VH</b> 47
17. F3B501 F3B501_9FIRM extraction	34 SIC- 34	LT	G 442	ARA	452 452	LFL	462 VEYLG 462	Y SWKNVH 5 47
18. E6LLW2 E6LLW2_9FIRM extraction	SIC 34	LT	G 442	RARA	LLTKHRLWE 452	LFL	VEYLG 462	Y SWKNVH 47
19. G5GEW3 G5GEW3_9FIRM extraction	DIY- 47	LT	G 355	ART	LLTKHRLWE 365	LFL	VEHLG 375	Y SWQEVHA 38
20. D6YSH0 D6YSH0_WADCW extraction	RYR- 70	LT	G 378	RAAH	IVRUHRLWE 388	VYL	ADYLG 398	VGSERVHR 40
21. F8LF66 F8LF66_9CHLA extraction 22. F8KVM5 F8KVM5_PARAV extraction	RYR 70	LT	G 378	RAAH	R HRLWE	VYL	398 1	40 40 MOSERVH
23. D1R644 D1R644_9CHLA extraction	70 1	LT	G 378	8 <b>A</b> 80	388 R HRLWE	UYL	398	40 MGSERVH
24. Q6MEL7 Q6MEL7_PARUW extraction	70	-LT	G 378 G	AAK	388	VYL	398	40 ARTER <b>VH</b> H
25. F8L6P3 F8L6P3_SIMNZ extraction	62 1	LT	370 G		380 R HRLWE	YL	390 TSELK	40 VCEDRVHF
26. Q9Z8J6 Y347_CHLPN extraction	70 1 YYR-	LT	378 G	EALR	388 VR HRLWE	YL	398 VNSLDI	40 FSKESVH
27. D1A0Z6 D1A0Z6_CHLPP extraction	70 1 9 68	LT	378 G 376	EALR	388 VRAHRLWE 386	YL	398 VNSLD 396	40 FSKESVH 40
28. F6FAP8 F6FAP8_CHLPS extraction	RWS- 68	LSS	G 376 G	RAKK	NR HRLWE	YL	VRSLEI 396	FKEEEVHG 40
29. E5AHT0 E5AHT0_CHLP1 extraction	RWS 68	LSS	G 376	BAKK	UVRAHRLWE 386	_AF	VRSLEI 396	FKEEEVHG 40
30. F3NV98 F3NV98_CHLPS extraction	RWS 68	-LSS	G 376	BAKK	NRAHRLWE 386	YL	VRSLE 396	FREEEVHG 40
31. F6F5J6 F6F5J6_CHLPS extraction 32. F0T3X1 F0T3X1_CHLP6 extraction	RWS 68	-LSS	G 376	RAKK	R HRLWE 386	CAT.	396 1	40 40
33. F6FD68 F6FD68_CHLPS extraction	68 1	-LSS	G 376 G	BAKK.	R HRLWE 386 R HRLWE		396	40 FREEVHG
34. F6F882 F6F882_CHLPS extraction	68 1 RWS	LSS	376 G	A	386 VR HRLWE		396 VRSLE	40 FREEVHG
35. Q5L653 Q5L653_CHLAB extraction	68   RWS	LSE	376 G	BAKK	386 VRAHRLWE	YL	396 VRSLEI	40 FREE <b>VH</b> G
36. Q254E8 Q254E8_CHLFF extraction	68 LWS 68	LSE	376 G 376	RAKK	386 VRAHRLWE 386	YL	396 VRSLE 396	40 FKEEDVHS 40
37. Q823H1 Q823H1_CHLCV extraction	RWS- 70	LSE	G 378	EARR	NR HRLWE	YL	VHSLE 398	FKEENVH 40
38. B0B9H7 B0B9H7_CHLT2 extraction	0WS- 70	LT	G 378	RAAK	R HRLWE	YL	VSOLGI 398	PNKNEVH 40
39. F9YDB8 F9YDB8_CHLTC extraction	0WS- 70	LT	G 378	RAAK	R HRLWE	SYL	VSOLGI 398	PNKNE <b>VH</b> H 40
40. B0BB56 B0BB56_CHLTB extraction 41. D3UTN1 D3UTN1_CHLTS extraction	0₩S- 70	LT	G 378	BAAK	388	YL	VSÓLGI 398	PNKNEVH 40
41. D3UTN1 D3UTN1_CHLTS extraction 42. D6YP98 D6YP98_CHLT1 extraction	70	LT	G ' 378 G	RAAK	R HRLWE 388 R HRLWE		398	VH 40 VH
43. D6YCH7ID6YCH7 CHLT5 extraction	70 J	LT	378 G	A	388 R HRLWE		398	40 FNKNEVH
44. G4NM41 G4NM41_CHLTR extraction	70 0WS	LT	378 G	RAAK	388 UVRAHRLWE	SYL	398 VSQLDI	40 FNKNEVH
45. D6YEM3 D6YEM3_CHLT7 extraction	70   QWS-	LT	378	RAAK	388 VR HRLWE	YL	398 VSQLDI	40 FNKNEVH
46. D6YXA0 D6YXA0_CHLT9 extraction	70 0115 70	LT	378 G	RAAK	388 NR HRLWE	SYL	398 VSOLDI 398	40 FNKNEVH 40
47. D6YJW4 D6YJW4_CHLTG extraction	0 0WS- 70	LT	378 G 378	RAAK	UVRAHRLWE 388	YL	398 VSQLDI 398	40 FNKNEVH 40
48. D7DFW0 D7DFW0_CHLTL extraction	0115 70	LT	G 378	BAAK	R HRLWE	SYL	VSOLDI 398	PNKNEVH 40
49. D6YH87 D6YH87_CHLT0 extraction	0₩S- 70	LT	G 378	RAAK	NRAHRLWE	SYL	VSOLDI 398	PNKNE <b>VH</b> H 40
50. D7DCP1 D7DCP1_CHLTD extraction	0WS- 70	LT	378 G 378 G 378	BAAK	388		VSOLDI 398	FNKNEVH 40
51. Q3KMV2 Q3KMV2_CHLTA extraction 52. C4PLS2 C4PLS2_CHLTZ extraction	70	LT	G 378 G	A	388 JVR HRLWE		398	VH 40 VH
53. Q9PKX0 Y341_CHLMU extraction	70   x	LT	378 G	A	388 R HRLWE		398 VSELD	40 FNKSKVH
54. F4DIR0 F4DIR0_CHLPE extraction	70 1	LT	G	TAAK	388 VR HRLWE	YL	398 VHSLDI	40 FNETOVH
55. Q7UIM8 Q7UIM8_RHOBA extraction	62 1 EFA	LT	370 G	EARR	380 R HRLWE 380	YL	390 IHHAD	40 I APORVD R 40
56. F2AP06 F2AP06_RHOBT extraction	62 EFA 08	LT	370 G 416	EARR	380 TRHRLWE 426	YL	390 IHHAD 436	40 I APORVD R 44
57. A6C9P1 A6C9P1_9PLAN extraction	01R- 69	LT	G 377	E <b>A</b> AR	420 IVHEHRLWE 387	YL	430 ITYAD 397	VASSK <b>V</b> DR 40
58. D5EQF0 D5EQF0_CORAD extraction	AIV- 55	LT	G 363	AAAK	R HRLWE		18HAE 383	VAASR <b>V</b> DR 39
59. B3ET27 B3ET27_AMOA5 extraction	KWR- 55	LT	G 363		IAORHRLWE		383	ткраті <b>н</b> е 39
60. G8R720 G8R720_9FLAO extraction 61. Q11TH1 Q11TH1_CYTH3 extraction	GFR- 55	LSE	G 363	EAKR	R HRLWE		TORMR 383	LKADHIHP 39
62. E4TRG1/E4TRG1_MARTH extraction	56 GYP	-LSD	G 364 G	RGKR	IVR HRLWE 374 IVK HRLWE		384	LPSDEVHD 39 LAPDEVHD
63. Q31DN8 Q31DN8_THICR extraction	79   GLS	LT	387 G	ANT	397		407	41 MDADEVH
64 PEIMVOIREIMVO ORACT avtraction	13		422		432		442	45

438 DPH 428 ization domain 404 IDYLDKKLGSPK 409 VDYLDOKLGOPE 409 A EH 399 TAHRLEH 399 434 AREH TLOCKLGOPE 409 399 434 ARLEH хτ. 408 408 398 428 ЕН S P 400 435 410 DRLGHPT 441 434 434 ABLGSGS 447 -DPHOGEIPED 451 457 -DPOGROIPE A EH VDYL 431 447 RAEH ELRHEL 424 461 EH L 437 472 / PAEYMEHS PLQQE: AELAGRS 418 EOLGSPA 461 408 AHOMEH SQSAL 1D C 451 EH Q**L**QDQ**L** SP. 451 461 481 QLQDQL AHOLEH SP. 416 426 445 AFRIEH **LREKL** 466 1 501 ЕН LARE P**IP**TPAGEV 457 492 HLAEEN .P**IP**TPAGI A 513 482 DAKALEY GLKDRL EIYENHF 482 513 ALES LKDRL 181 482 513 LHRI 405 TKLLEDPK-428 TKLLEDT 428 'LJ VI.EH NLENEL A 395 425 -SAEEMEH ELEKEL 418 448 EMER ELEKEL 447 418 VLLKDPK 428 SABBMEHI ELEKEL 418 447 LTPELEKELI 418 428 VLLK DPK 428 420 RVLK NPT 428 ABBIEH 449 A ELEKEL 410 439 EH IEERL A 8 447 01IPNKKKE 1 447 )1IPNKKKE 418 418 ABBIEH 428 L P ARRIEH LOHTL 416 448 ACCH ELDYAI IIPEKPO 416 448 ELDYA АЕН IPERP 448 A EH ELDY 416 448 BMEH ELDYA: 416 448 420 TELLONPH-426 TELLONPH-426 TELLONPH-426 A EH ELDYA IIPEK 448 ABBEH ELDYA IPER 448 416 ELDYA EMER IP 416 448 426 TOMLDNPH-426 TEMLDNPD-426 TEMLDNPH-428 AEH ELDYA IPER 416 448 A ELDHA IPET 448 416 A EH ELDYAI IP 418 450 928 LTDELDSTLSOMLODPD 418 428 а ен EIPER LTDELDSTLS 450 928 428 MLO P 428 428 ABER EIPER 450 ABBEH ELDSTL IP 418 450 428 418 428 418 428 -Y--DPHOR 434 438 A EH IP DG 450 428 428 0 LO P 428 0 LO P 428 438 -DPH 438 -DPH 838 -DPH 838 o A E H ELDSTLS ETPERS 418 434 DG 450 A EH ELDSTL IP 418 428 110ELDSTLSOMLOPD 418 428 418 434 450 AEH 450 428 428 0/L0 PD 428 428 434 438 DPH 434 438 - DPH 434 438 - DPH 434 438 - DPH 434 438 - DPH A ELDSTL EIPERS JSTL JSTL JETDELDSTLS 418 LTDP<sup>\*</sup> DG 450 A EH EIPERTR 450 AEH ELDSTLS 0 DP EIPERT LTDELDSTLS 418 450 428 01L0 PD-428 01L0 PD-428 438 438 - DPH 438 - DPH 438 AEH EIPERTH 434 DG 450 ABER ELDSTL EIPERTRI 418 434 450 +13 428 TTPELOSTLSONL 418 428 -DPHOR 438 A EH DP1 EIPERTI 434 450 428 428 0 LO PO-428 428 A ELDSTL EIPKRTR 418 450 A ELDSTL IP 418 JLOF JLOF 428 .LTERLROPS-420 VAELSSLENARLO 420 TVAELSSLENARLO 466 SELET.7 L EMER LOTTL IP 418 011PEFKR 443 449 A ELDSELT 410 430 430 430 476 1 2 476 437 437 437 437 ADAIEH 443 410 AIBH 456 DADATEH 417 449 1 . DAC AVER OLVAQLE PHP L 419 403 1 ELVKELNKLL 413 LIEH 423 --DPH 427 --DPH 427 427 424 449 --PH 424 449 --PH 5 460 --SPH LEATS 403 419 A IEEL LESRLEKKL 414 PELEKKLERLEVP. 7 437 DEETVULKOLLOOG--472 VRRLERLEVPS 445 KILLRIF IEEELL ALGYPD 403 419 A T EH T H 4 404 A T EH T H 7 427 X C EH T H 2 462 A V EH T H A V E H H A 420 443 464 422 432 APLNOG ACAVR HRLWE YLT 395 405 R-MTEIG AAAVVII HRLE YLI 478 497 13 SF 87 452 VHDAEVIEH 425 GORTADLEH 64. B5JMV0|B5JMV0 9BACT extraction 415 470 455 65. D5SYP1|D5SYP1\_PLAL2 extraction

•• EHXXX XXLXXXLXXLX XPXXXX XXPMPX XXDPHX XXIPXX XXXXXX XXX

Fig. S1. Permease-repressor fusion proteins are highly conserved at metal-coordinating residues. All of the fusion proteins with predicted DtxR iron-dependent repressor domains in the C terminus were subjected to multiple sequence alignment. The region aligning to the dimerization domain was extracted, and a >75% sequence identity was used to generate a consensus sequence. The six metal-coordinating residues, as determined by the DtxR crystal structure, are marked with a closed circle above the consensus sequence.

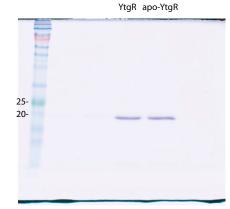


Fig. S2. Recombinant YtgR is purified to a single product by Coomassie staining. Aliquots of purified, recombinant YtgR, or apo-YtgR (Chelix-100-treated) were resolved on a 12% TGS/polyacrylamide gel and stained by Coomassie blue. After destaining, only one product was visible.

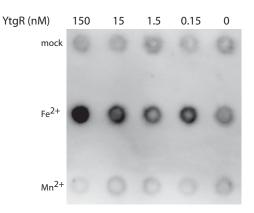
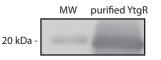


Fig. S3. Manganese does not activate apo-YtgR DNA-binding. Various concentrations of apo-YtgR were mixed with FeSO<sub>4</sub> or MnCl<sub>2</sub> (150  $\mu$ M) before incubation with a biotin-labeled dsDNA molecule that corresponded to the region upstream of *ytgA*. Reactions were filtered through a NC membrane and washed once, before detection of biotinylated DNA retained on the NC. Iron-supplemented protein samples retained the DNA oligonucleotide, whereas no appreciable retention over the no protein control (NPC) in the manganese-supplemented samples was observed.



**Fig. S4.** CT069 antisera recognize purified recombinant YtgR. An aliquot of recombinant YtgR was resolved by TGS/PAGE and transferred to NC membrane. The membrane was blotted using the CT069 antisera and chemiluminescence detected with the Millipore HRP detection kit. Chemiluminescence was observable without use of the Western blot imager. Visible light picture is shown.

#### Table S1. Plasmids used in this study

lasmid Relevant details		Source	
pACYC184	Subcloning vector	New England Biolabs	
pBAD-TOPO/TA	Expression vector	Invitrogen	
pBAD-LacZ-V5-pHis	Control Expression vector	Invitrogen	
pET151-D/TOPO	Expression vector	Invitrogen	
pET151-EV	circularized empty vector	This paper	
pET-YtgR	Expression of the YtgR domain with an N-terminal pHis tag	This paper	
pET-YtgC-YtgR	Expression of recombinant full-length YtgC-YtgR with an N-terminal pHis tag	This paper	
pBAD-YtgC-YtgR	Expression of YtgC-YtgR with a C-terminal V5 and pHis tag	This paper	
pCCT100	LacZ reporter plasmid with arabinose inducible promoter	This paper	
pCCT101	pCCT100 with KpnI RE site next to promoter	This paper	
pCCT102	pCCT101 with YtgR operator containing sequence inserted into the KpnI site	This paper	

Table S2. Oligonucleotide primers used in this study				
Primer ID	Sequence (5' to 3')			
CT1003	CACCATGCATCTATTGAAAATCTTTGGTATTTAC			
CT1004	CTAGCAACCATCCGACTTCCTTG			
CT1018	CCTCCTCTCGAGGCGCATTCACAGTTCTCCGC			
CT1019	CCTCCTAGATCTCGGAGATTTCCTGGAAGATGCCA			
CT1020	TATAAGATCTCCGGCGGATTTGTCCTACTCAGGAG			
CT1021	ATATCTCGAGGCGAAGGCGAAGCGGCATG			
CT1022	ACGGGTATGGAGAAACAGTAGAGAGTTGCG			
CT1023	TACTGTTTCTCCATACCCGTGGTACCTTTTTGGGCTAG			
CT1024	TAATGGTACCAGATAATCGATACGGGGACATAC			
CT1025	ATATGGTACCGAATAGCTCCTTAGTGGGTAG			
CT1033	TAATCCATGGACTACAAGGACGATGACAAGGGTACCTAAT			
CT1034	ATTAGGTACCCTTGTCATCGTCCTTGTAGTCCATGGATTA			
CT1035	GCAACCATCCGACTTCCTTGTTCG			
CT1036	ATATGGTACCCTGAGTTGTATATTTCAGGACACTATCTTTCTATCCAG			
CT1037	CCATGGACTACAAGGACGATGACAAGGG			
CT1101	ATGCTGAGTTGTATATTTCAGGACACTATC			
CT1102	ATACCATGGCGCAACCATCCGACTTCCTTGTT			
CT1103	CACCATGCTGAGTTGTATATTTCAGGACACTATC			
CT1104	CTAGGTACCCTTGTCATCGTCCT			

## **Other Supporting Information Files**

Dataset S1 (XLSX)

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