Supporting Information

The *czcD* (NiCo) riboswitch responds to iron(II)

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EXPERIMENTAL SECTION

General considerations. Chemical reagents were obtained from Fisher-Thermo Scientific (Waltham, MA), Millipore Sigma (St. Louis, MO), Roche (Basel, Switzerland), Amresco (Solon, OH), or VWR (Randnor, PA), unless noted otherwise, at the highest purity available. Primers and gBlocks were ordered from Integrated DNA Technologies (IDT) (Coralville, IA), and fragmentGenes were ordered from Genewiz (South Plainfield, NJ). *E. coli* strains [5alpha, and BL21(DE3)] for cloning and recombinant protein expression, as well as cloning reagents (restriction enzymes, Q5 DNA polymerase, OneTaq DNA polymerase, T4 DNA ligase, and NEBuilder HiFi DNA Assembly Master Mix) were obtained from New England Biolabs (Ipswich, MA). The *E. coli* BL21 Star (DE3) strain for recombinant RNA expression was purchased from Invitrogen (Carlsbad, CA). DFHBI-1T was either purchased from Tocris or synthesized as described.[1] PCR cleanup and miniprep kits were from Omega Bio-tek (Norcross, GA). Gel extractions used the Zymoclean gel DNA recovery kit from Zymo Research (Irvine, CA). Ni-NTA agarose resin was purchased from Thermo Scientific. Protein gel electrophoresis was carried out using Life Tech 16% Tris-glycine gels and a mini gel apparatus. The Sequagel reagents used for urea polyacrylamide gel electrophoresis were purchased from National Diagnostics (Atlanta, GA). T7 RNA polymerase was purified from a cell stock [BL21(DCAT4) transformed with pDL19][2] that was a gift from Dr. Philip Bevilacqua (Penn State). *Bacillus subtilis* 168 1A1 was obtained from the Bacillus Genetic Stock Center (Columbus, OH). pET31b-T7-Spinach2 was a gift from Ming Hammond (Addgene #79783)[3] and pHT01-ads was a gift from Heng-Phon Too (Addgene #47382)[4] (**Table S4** for all plasmids used in this study). Sanger sequencing of plasmids using primers shown in **Table S5** was performed by Penn State Huck Institute of the Life Science Genomics Core Facility and Genewiz.

UV-visible absorption spectra were obtained on an Agilent Cary 60 UV-visible spectrophotometer using a quartz cuvette (Starna Cells). Nucleic acid UV-visible absorption spectra and cell culture OD_{600nm} measurements were obtained on Mettler Toledo UV5Nano. Well plate analyses were carried out using a Tecan infinite m1000pro microplate reader or BioTek Synergy H1 microplate reader. Flow cytometry assays were carried out using a BD LSRFortessa Flow Cytometer. Experiments utilizing Fe^{II} were conducted within a vinyl anaerobic chamber (Coy Lab Products) or an MBraun Unilab anaerobic box. All glassware was acid-washed with TraceMetal grade nitric acid (Fisher) followed by extensive rinsing with filtered ddH_2O prior to usage.

Expression and purification of T7 RNA polymerase. *E. coli* BL21(DCAT4) cells transformed with pDL19 for T7 RNA polymerase expression[2] were streaked from a glycerol stock onto LB-agar plates containing ampicillin (Amp, 100 µg/mL in all growth media). A single colony was used to inoculate 50 mL of LB-Amp, which was incubated overnight (16 h) at 37 °C with 180 rpm shaking. Of this culture, 25 mL was used to inoculate 2 L of LB-Amp in a 6-L flask, which was grown at 37 °C with 180 rpm shaking. At OD_{600nm} = 0.6-0.8, the culture was induced with 125 µM isopropyl β-D-1-thiogalactopyranoside (IPTG), and growth was continued for 4 h. The remaining operations were performed at 4 °C. The cells were pelleted by centrifugation (7000×*g*, 15 min), resuspended in 50 mL of **Buffer A** (20 mM Tris, 20 mM NaCl, 2 mM EDTA, pH 7.5), and centrifuged again. The resulting cell pellet (5.87 g) was resuspended in **Buffer B** [50 mM Tris, 100 mM NaCl, 1 mM imidazole, 10 mM β-mercaptoethanol (β-ME), 0.1% Triton X-100, 5% glycerol, pH 8.4] at 5 mL/g cell paste with 1 U/mL DNase (NEB) and a Complete Mini protease

inhibitor cocktail tablet (Roche). Cells were lysed by sonication with a 1/4" (6 mm) microtip at 50% amplitude, with cycles of 20 s pulse on / 40 s pulse off for 20 min (QSonica). After sonication, the suspension was centrifuged at 30,000 ×*g* for 45 min. The supernatant was loaded onto a 2 mL (0.8 × 2 cm) Ni-NTA agarose column (Qiagen). The column was washed with 10 column volumes (CV) of Buffer B, followed by 40 CV of **Buffer C** (50 mM Tris, 100 mM NaCl, 10 mM imidazole, 10 mM β-ME, 0.1% Triton X-100, 5% glycerol, pH 8.4). Protein was eluted using 10 CV of **Buffer D** (50 mM Tris, 100 mM NaCl, 250 mM imidazole, 10 mM β-ME, 20% glycerol, pH 8.0). The eluted protein was concentrated to 1.5 mL using an Amicon Ultra 30-kDa MWCO centrifugal filtration unit (Millipore Sigma) and exchanged into **Buffer E** [50 mM Tris, 100 mM NaCl, 10 mM dithiothreitol (DTT), 1 mM EDTA, 0.1% Triton X-100, 50% glycerol, pH 8.0] using a Sephadex G25 column (15 mL, 1.5 × 9 cm). The resulting protein was quantified using ε_{280nm} = 141 mM^{-1} cm^{-1}.[5] Activity was tested using a control reaction transcribing Spinach2 (1 µL - 8µL enzyme) before flash-freezing in liquid N_2 and storing at -80 °C. The purification yielded 44 mg/L culture.

RNA transcription, purification, and folding. RNAs were transcribed following the NEB standard RNA synthesis protocol. DNA template (10-20 µg) was added to transcription buffer mix (0.5 mM NTP mix, 40 mM Tris, 6 mM $MgCl_2$, 1 mM DTT, 2 mM spermidine, 0.4 µM T7 RNA polymerase, 0.1 U inorganic pyrophosphatase, pH 7.9) and incubated at 37 °C for 3-4 h. Transcription reactions were quenched with the addition of 2× urea loading buffer [18 M urea, 20% sucrose, 0.1% sodium dodecyl sulfate (SDS), 90 mM Tris (pH 8.0), 90 mM boric acid, 1 mM ethylenediaminetetraacetic acid (EDTA)] followed by purification by gel electrophoresis (6-8% polyacrylamide, 7.5 M urea, National Diagnostics kit). The product band was visualized with a handheld UV light and excised and diced with razor blade to be soaked in crush-soak buffer (10 mM Tris, 200 mM NaCl, 1 mM EDTA, pH 8.0) overnight at 4 °C. The purified RNAs were isolated as a solid white pellet by ethanol precipitation at -20 °C followed by centrifugation at 18000 ×*g*. The RNA pellets were dissolved in 50 µL of autoclaved RNase-free water or 0.5× TE buffer (5 mM Tris, 0.5 mM EDTA, pH 8.0). RNA concentrations were calculated using the approximated sequence-specific extinction coefficients at 260 nm following hydrolysis digestion by incubating the RNA in a PCR machine at 95 °C for 1 h. All RNAs were refolded by incubating in a PCR machine at 75 °C for 2 min followed by gradual cool-down to room temperature prior to use.

RNA sensor template library generation and screening. Full-length Spinach2 and cp-Spinach2 sequences were generated by PCR of two self-priming primers (**Table S6** for all relevant primer sequences for library generation). The sensor template library was generated by PCR primer extension of parent Spinach2 or cp-Spinach2 templates, replacing the loop region in each riboswitch stem. For P1 stem templates, the Spinach2 sequence was added onto the *Eba* riboswitch sequence using each set of fwd/rev primers for P1 primers. P2 stem templates were generated by addition of the riboswitch sequence onto the cpSpinach2 sequence by using each set of fwd/rev primers labeled with P2, followed by the addition of the riboswitch sequence numbered 52-111. For the P3 stem templates, the riboswitch sequence was first added to the cpSpinach2 sequence using sets of fwd/rev primers labeled as P3, followed by the addition of the 5' 1-28 and 3' 73-111 sequences from the riboswitch sequence using the appropriate primers. For P4 stems, the riboswitch sequence was added onto the cp-Spinach2 sequence using sets of fwd/rev primers labeled as P4, followed by addition of the 1-42 sequence from the riboswitch. All templates were amplified using the T7amp_fwd and amp_rev primers to add the T7 promoter in order to enable

in vitro transcription by T7 RNA polymerase. All PCR reactions used Q5 DNA polymerase and followed the manufacturer's protocol. PCR reactions were confirmed via 1% or 2% agarose gel and purified with E.Z.N.A. Cycle Pure Kit (Omega Bio-tek) or QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's protocol. The purified DNA templates were transcribed, purified, and refolded before use as described in the previous section.

RNA sensor characterization in vitro. For each construct, fluorescence response was evaluated at different magnesium concentrations (0.5, 1, 5, and 10 mM) and either 0 or 100 μM $CoCl_2$, using 100 nM sensor and 10 μM DFHBI-1T, in 30 mM MOPS, 100 mM KCl, pH 7.2, at 20 °C. Constructs were incubated for 1 h prior to acquisition of fluorescence readings to allow for full DFHBI-1T binding. Fluorescence readings were acquired in 96-well plates (Greiner μClear half-area fluorescence plates) on a Tecan Infinite M1000Pro plate reader (λ_{ex}=475 nm, λ_{em}=505 nm). The three best-performing constructs (P1-1, P1-2, and P1-3) were evaluated further in the same buffer with 3 mM $MgCl_2$ added (**Buffer F**) and containing various concentrations of $MnCl_2$, $CoCl_2$, $NiCl_2$, $ZnSO_4$, and ferrous ammonium sulfate hexahydrate. Fe^{II} experiments were carried out in an anaerobic chamber. The purified, ethanol-precipitated RNA was brought into the anaerobic chamber and resuspended in water. An aliquot (1 μL diluted with 9 μL water) was removed from the chamber for RNA concentration determination. Titration samples were prepared in 96-well plates, which were sealed using 96-well sealing tape (Thermo Fisher) to prevent Fe^{II} oxidation, and after 1 h, the plates were removed from the chamber and immediately fluorescence readings were acquired. Data were analyzed using GraphPad Prism 6 software using the following non-linear regression equation (1):

$$Y = Bottom + \frac{(Top - Bottom)}{(1+10^{((logK_d - X)\times Hillslope)})} \quad (1)$$

where X is log([M^{II}]), Y is the fluorescence value, Hillslope is the Hill coefficient (n), and K_d is the apparent dissociation constant. Top and Bottom is determined for each data set.

Citrate-buffered metal titrations.

Preparation of low- and high-metal citrate buffers. For buffered metal ion titrations of the **czcD** sensors, citrate was used as the metal ion buffer.[6] The citrate buffer was prepared by dissolving MOPS, KCl, and citric acid (to give final concentrations of 30 mM, 100 mM, and 1 mM, respectively, in 50 mL) in water to give a final volume of 40 mL. The buffer was incubated with 2 g Chelex-100 for 12 h and the pH was adjusted to pH 7.2 using 6 M KOH. After decanting to remove Chelex, high-metal citrate buffers were made by addition of 0.1 M metal stocks [$MnCl_2$, $(NH_4)_2Fe(SO_4)_2$, $CoCl_2$, $NiCl_2$, and $ZnSO_4$] into the buffer to the following final metal concentrations, followed by filling up to a final volume of 50 mL: for **czcD1**, 300 μM for Fe, Co, and Ni and 800 μM for Mn and Zn; for **czcD-2** and **czcD-3**, 100 μM for Fe, Co, Ni, and Zn and 500 μM for Mn. The low-metal buffer was made by filling up to final volume of 50 mL, without metal addition.

Citrate buffered metal titrations. High and low metal citrate buffer stocks were first prepared by the addition of 100 nM RNA and 10 μM DFHBI-1T. The desired free metal concentrations were achieved by mixing high and low metal citrate buffers. Following incubation for 1 h, the resulting samples were measured in 96-well plates (Greiner μClear half-area fluorescence plates) using a Biotek Synergy fluorescent plate reader (λ_{ex}=475 nm, λ_{em}=505 nm).

Fe^{II} experiments were carried out in an MBraun anaerobic chamber as described above. Data were analyzed using GraphPad Prism 6 software using equation 1, with the exception that X is the $\log_{10}$ of the free metal concentration, which was calculated for each titration point as described below.

Calculation of free metal concentrations for K_d determination. The free metal ion concentrations $[M_{free}]$ for each sample mixture were calculated as described[7, 8] using the following equation (2):

$$[M_{free}] = \frac{K_{d,M}[ML]}{[L]} \quad (2)$$

where [L] is the concentration of citrate that is not bound to metal ions, and [ML] is the concentration of metal-bound citrate. $K_{d,M}$ is the effective K_d for each metal adjusted for pH using the following equation (3):

$$K_{d,M} = \frac{1+10^{pK1-pH}+10^{pK2-pK1-2pH}}{K_M} \quad (3)$$

where pK_1 and pK_2 are the published first and second pK_a values of citrate at an ionic strength of 0.1,[9] and the pH is the pH of the buffer. The K_M is defined as K_M = [ML] / ([M][L]), given for each metal ion in ref. [9]. The $K_{d,M}$ values determined for the metal solutions used in our experiments are shown in **Table S3**.

Because the concentration of ligand (1 mM) was much greater than the RNA concentration (100 nM), eq. 2 was approximated by eq. 4 for each sample, where V_{high} and V_{low} are the volumes of the high and low metal solutions mixed together.

$$[M_{free}] = K_{d,M} \bullet V_{high} / V_{low} \quad (4)$$

The potential contribution of Mg^{II} binding to citrate – which could compete with the transition metal ions for citrate binding, increase the free transition metal ion concentration, and therefore lead to an underestimate of the actual K_d values – was likely to be relatively minor based on the relative $K_{d,M}$ values of the transition metal ions and Mg^{II}. As a result, this potential contribution was ignored in these calculations. Still, in order to estimate the magnitude of the effect, assays of **czcD-2** with Co^{II} in the presence of 1 mM, 3 mM, and 10 mM $MgCl_2$ were carried out. The results (**Figure S8**) indicate that the overall effect is at most 2-3 fold, which does not change the basic conclusions of this manuscript. We also note that knowledge of how much of the added Mg^{II} is citrate-bound is also complicated by the high concentration of Mg^{II}-binding sites on the RNA itself, and interpretation of the effect of Mg concentrations on sensor response is complicated by potential effects on RNA structure and outcompetition of other metal ions for their cognate binding sites on the riboswitch. The effects of Mg^{II} on riboswitch function is an understudied area in general[10] but especially for metal-binding riboswitches.[11, 12] A more detailed analysis and accounting of these effects on function of the present riboswitch is in progress.

Plasmid construction for sensor expression in *E. coli.* pET31b-T7-Spinach2 (**Table S4**, Addgene #79783) contains Spinach2, flanked by EagI sites, inserted into the human $tRNA^{Lys}$ scaffold.[3, 13] The **czcD-2** sensor was inserted into this construct for expression in *E. coli.* pET31b-T7-Spinach2 (1.5 µg) was linearized and Spinach2 removed by digestion with EagI-HF (20 U) at 37 °C for 1 h followed by purification by gel electrophoresis (1% agarose). EagI sites were added

onto **czcD-2** by PCR using primers Spinach 2-EagI-F and Spinach 2-EagI-R (**Table S5**) and the PCR product was digested with EagI-HF at 37 °C for 1 h followed by purification by agarose gel electrophoresis. The insert was ligated into the vector at a 3:1 insert:vector ratio using T4 DNA ligase (NEB) for 1 h at room temperature, and the product was transformed into NEB 5-alpha cells, plated on LB-agar-Amp, and incubated at 37 °C overnight. Colonies were screened for insert using colony PCR (OneTaq DNA polymerase, primers T7P and T7T), and the DNA sequences of the plasmids were confirmed by Sanger sequencing.

Analysis of cellular iron by czcD-2 using flow cytometry. *E. coli* BL21Star(DE3) cells were transformed with pET31b-T7-czcD-2 or pET31b-T7-Spinach2 (as a control) and plated on M9-agar-carbenicillin (Cb, 100 µg/mL in all media for this experiment) plates, which were incubated at 37 °C overnight. The M9 media consisted of 42 mM Na_2HPO_4, 22 mM KH_2PO_4, 8.5 mM NaCl, 19 mM NH_4Cl, 2 mM Mg_2SO_4, 0.1 mM $CaCl_2$, 0.2% glucose, and 0.1% casamino acids. A single colony was inoculated into a 5 mL M9-Cb culture and grown overnight for 16 hrs at 37 °C with 180 rpm shaking. This culture was used to inoculate at 40× dilution 5 mL M9-Cb cultures in 14-mL polypropylene culture tubes, which were grown at 37 °C with 180 rpm shaking. At OD_{600nm} = 0.1, each culture was induced with 1 mM IPTG, and growth was continued for 2 h. Metal ions were added to the desired concentrations (0-100 µM ferric ammonium citrate, 20 µM $MnCl_2$, $CoCl_2$, $NiCl_2$, or $ZnSO_4$) from 100 mM or 1 mM stock solutions, and the cultures were grown for an additional 1 h. Cells were harvested by centrifugation (3200 ×*g*, 10 min, 4 °C). Experiments were carried out with 2 biological replicates in each of 3 independent experiments.

Cell pellets were resuspended in 0.5 mL 1× PBS (1.9 mM NaH_2PO_4, 8.4 mM Na_2HPO_4, 175 mM NaCl, pH 7.0) containing 50 µM DFHBI-1T (added from a 50 mM stock in DMSO). Cellular fluorescence was measured with the 488-nm excitation laser line and 530-nm green fluorescence channel using the Special Order 16-color BD LSRFortessa flow cytometer at the Penn State Huck Institutes of the Life Sciences Microscopy and Cytometry Facility. For each sample, ≥30,000 cells were measured. The data of each flow cytometry sample were analyzed using Flowjo v10, gating for *E. coli* cells based on FSC/SSC, plotting the histogram then histogram (e.g., **Figure S9A**), and determining the mean fluorescence value for the population. Fluorescence response of **czcD-2**-expressing cells was normalized to the Spinach2-expressing cells grown under the same conditions, in order to account for differences in cell size (see **Figure S9B**).

czcD-2 competition against the *Lmo* riboswitch. The metal-binding ability of the *Lmo* riboswitch (vs. an mSpinach2 control) was assessed via competitive titration. The RNAs (0.05-3.2 µM) were titrated into RNA sensor mix (Buffer F containing 100 nM **czcD-2**, 10 µM DFHBI-1T, and either 2 µM Co^{II} or 2 µM Fe^{II}) and fluorescence measured in 96-well plates on a Tecan Infinite M1000Pro plate reader (λ_{ex}=475 nm, λ_{em}=505 nm). Fe^{II} experiments were carried out in an anaerobic chamber. The ethanol-precipitated RNA was brought into the anaerobic chamber and resuspended in water. An aliquot (1 µL diluted with 9 µL water) was removed from the chamber for RNA concentration determination. Titration samples were prepared in 96-well plates, which were sealed using 96-well sealing tape (Thermo Fisher) to prevent Fe^{II} oxidation, and after 1 h, the plates were removed from the chamber and immediately fluorescence readings were acquired.

Cloning of putative metal exporters into *B. subtilis* expression vectors. The genes encoding PfeT, Eba3544, LMO3448, and sfGFP (**Figures S16-S19**) were each inserted into pHT01-ads (MoBiTec, Addgene #47382), which contains an IPTG-inducible promoter, for protein expression

in *Bacillus subtilis* 1A1 (BGSC). pHT01-ads (1.5 µg) was linearized and *ads* was removed by digestion with BamHI-HF (20 U) and XbaI (20 U) at 37 °C for 1 h, followed by purification by gel electrophoresis (1% agarose). The inserts were obtained as codon-optimized gBlocks. *pfeT* was synthesized with the Gibson ends included. Due to synthesis requirements, Gibson ends had to be added by PCR to the gBlocks for the Eba3544 and LMO3448 genes, using primers eba-Gib-F, eba-Gib-R, lmo-Gib-F and lmo-Gib-R. The sfGFP gene was amplified from pET21b-RL015A[14] and BamHI and XbaI sites were added using sfGFP-BamHI-F and sfGFP-XbaI-R, followed by digestion with BamHI-HF (20 U) and XbaI (20 U) for 1 h at 37 °C and PCR clean-up kit purification. The *pfeT*, Eba3544, and LMO3448 inserts were ligated into the linearized pHT01 vector at a 3:1 insert:vector ratio using the NEBuilder HiFi DNA Assembly Master Mix for 1 h at 50 °C. The sfGFP insert was ligated into the linearized pHT01 vector at a 3:1 insert:vector ratio using T4 DNA ligase (NEB) for 1 h at room temperature. The products were transformed into NEB 5-alpha cells, plated on LB-agar-Amp, and incubated at 37 °C overnight. The colonies were screened for insert using colony PCR (OneTaq DNA polymerase, primers LacI-R and pHT01-R) and the DNA sequences of plasmids were confirmed by Sanger sequencing.

Complementation assay in *B. subtilis*. Transformation of *B. subtilis* was done according to MoBiTec's protocol. *B. subtilis* 1A1 cells were streaked onto an LB-agar plate from a glycerol stock. A single colony was used to inoculate 5 mL of LB, which was grown overnight (16 h) at 37 °C with 180 rpm shaking. This culture was inoculated at 100× dilution into 10 mL GM1 (1× Spizizen salts, 0.5% glucose, 0.02% casamino acids, 0.1% yeast extract, 1.5 mM $MgSO_4$) and grown at 37 °C with 180 rpm shaking until growth reached stationary phase (OD_{600nm} = 3.0). This culture was diluted 20× into 10 mL GM2 (1× Spizizen salts, 0.5% glucose, 0.02% casamino acids, 0.1% yeast extract, 2.5 mM $MgCl_2$, 5 mM $CaCl_2$) and grown for 2 h at 30 °C with 180 rpm shaking. The cells were used for transformations immediately after GM2 growth. EGTA was added to a final concentration of 1 mM to 1 mL of GM2 cell culture and incubated at room temperature for 5 min. Plasmid (1 µg) was added and incubated at 37 °C for 2 h with 180 rpm shaking. The cells (200 µL) were plated on LB-agar-Cm (5 µg/ml) and incubated at 37 °C overnight.

The complementation assay to determine the ability of the metal exporters (PfeT, Eba3544, LMO3448, and sfGFP as a control) to rescue metal toxicity was performed as described[15] with minor modifications. A single colony was used to inoculate 5 mL of LB (5 µg/mL Cm in all growth media), which was grown overnight (16 h) at 37 °C with 180 rpm shaking. This culture was inoculated at 100× dilution into 5 mL of LB-Cm and grown at 37 °C with 180 rpm shaking. At OD_{600nm} = 0.4, this culture was induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG), and growth was continued for 30 min. This culture was then inoculated at 100× dilution into a 500-mL baffled flask containing 100 mL LBC medium (LB medium containing 1 g/L sodium citrate dihydrate) or 100 mL LBC medium supplemented with 2.5 mM ferrous ammonium sulfate, 1 mM $MnCl_2$, 0.4 mM $CoCl_2$, 1.25 mM $NiCl_2$, or 3 mM $CuCl_2$ 0.6 mM $ZnSO_4$. This culture was grown at 37 °C with 180 rpm shaking and cell growth was monitored at 600 nm every hour for 8 h.

Verification of expression and membrane insertion of putative metal transporters. *B. subtilis* 1A1 cells transformed with pHT01-sfGFP, pHT01-lmo-tev-sfGFP, or pHT01-eba-tev-sfGFP were streaked onto an LB-agar plate from a glycerol stock (5 µg/mL Cm in all growth media). A single colony was used to inoculate 5 mL of LB-Cm, which was grown overnight (16 h) at 37 °C with 180 rpm shaking. This culture was inoculated at 100× dilution into 200 mL of LB-Cm and grown at 37 °C with 180 rpm shaking. At OD_{600nm} = 0.4, this culture was induced with 1 mM IPTG, and

growth was continued for 30 min. The cells were pelleted by centrifugation (7000 ×*g*, 15 min), and frozen at -80 °C overnight. The membrane fractions were collected as described with minor modifications.[16] The resulting cell pellets (0.55 g) were resuspended with 15 mL of **Buffer G** (25 mM Tris, 100 mM sucrose, pH 7.5) and lysed by sonication with a ¼" (6 mm) microtip at 50% amplitude, with cycles of 20s pulse on/ 40s pulse off for 10 min (QSonica). After sonication, the lysates were first clarified by centrifugation at 8000 ×*g* at 4 ºC for 40 min followed by isolation of the cell membrane fraction by centrifugation at maximum speed (75600 ×*g*) for 1 h. The supernatants were decanted completely from the cell membrane pellets. The cell membrane pellets were resuspended in 1 mL of Buffer G. Fluorescence readings of each supernatant and resuspended membrane were determined in 96-well plates using a fluorescence plate reader (λ_{ex}=485 nm, λ_{em}=513 nm), and scaled according to volume in order to calculate total fluorescence values reported in **Figure S14**.

SUPPLEMENTARY TABLES

Table S1. Sequences of the DNA templates used for the riboswitches and riboswitch-based sensors. The DNA template sequences for the *Eba* and *Lmo czcD* riboswitches are derived from Furukawa et al.[17] Spinach2[18] or cpSpinach2[19] sequences are shown in red. In the sensor constructs, components of the appropriate truncated stem are shown in green. Px-y denotes insertion of Spinach2 or cpSpinach2 into stem x retaining y base pairs. Sensors **czcD-1**, **czcD-2**, and **czcD-3** are the P1-1, P1-2, and P1-3 constructs, respectively. Spinach2 was used for the P1 constructs and cpSpinach2 was used for the P2, P3, and P4 constructs.

Name	Sequence
Eba riboswitch	5'-GTACAAACTGAGCAGGCAAATGACCAGAGCGGTCATGCAGCCGGGCTGCGAAAGCGGCAACAGATGATTACACGCACATCTGTGGGACAGTTGTATATTCCACAGATGTTTTTT -3'
Lmo riboswitch	5'-GTCATTGTGATCTGAACAGGCGGTGAACGTAACACGAGGTTCATGCAGCTGGGCTGCAATTATTTGCGGCAGCAGACTATGTATTCTAAGGGCATATCTGTGGGACAGTTAATTCTGTTCTACGGGTATGCCCTTTTTT -3'
Spinach2	5'-GATGTAACTGAATGAAATGGTGAAGGACGGGTCCAGTAGGCTGCTTCGGCAGCCTACTTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATC-3'
cp-Spinach2	5'-TTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATCGCAAGATGTAACTGAATGAAATGGTGAAGGACGGGTCCA-3'
mSpinach2	5'-GATGTAACTGAATGAAATGGTGAATTACTTGTCCAGTAGGCTGCTTCGGCAGCCTACTTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATC-3'
czcD-1	5'-GATGTAACTGAATGAAATGGTGAAGGACGGGTCCAGAGCAGGCAAATGACCAGAGCGGTCATGCAGCCGGGCTGCGAAAGCGGCAACAGATGATTACACGCACATCTCTGTGGGACTTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATC -3'
czcD-2	5'-GATGTAACTGAATGAAATGGTGAAGGACGGGTCCATGAGCAGGCAAATGACCAGAGCGGTCATGCAGCCGGGCTGCGAAAGCGGCAACAGATGATTACACGCACATCTCTGTGGGACATTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATC -3'
czcD-3	5'-GATGTAACTGAATGAAATGGTGAAGGACGGGTCCACTGAGCAGGCAAATGACCAGAGCGGTCATGCAGCCGGGCTGCGAAAGCGGCAACAGATGATTACACGCACATCTCTGTGGGACAGTTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATC -3'
P1-5	5'-GATGTAACTGAATGAAATGGTGAAGGACGGGTCCAAACTGAGCAGGCAAATGACCAGAGCGGTCATGCAGCCGGGCTGCGAAAGCGGCAACAGATGATTACACGCACATCTCTGTGGGACAGTTTTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATC -3'
P1-4	5'-GATGTAACTGAATGAAATGGTGAAGGACGGGTCCAACTGAGCAGGCAAATGACCAGAGCGGTCATGCAGCCGGGCTGCGAAAGCGGCAACAGATGATTACACGCACATCTCTGTGGGACAGTTTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATC -3'
P1-0	5'-GATGTAACTGAATGAAATGGTGAAGGACGGGTCCAAGCAGGCAAATGACCAGAGCGGTCATGCAGCCGGGCTGCGAAAGCGGCAACAGATGATTACACGCACATCTCTGTGGGATTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATC -3'
P2-6	5'-GTACAAACTGAGCAGGCAAATGACCTTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATCGCAAGATGTAACTGAATGAAATGGTGAAGGACGGGTCCAGGTCATGCAGCCGGGCTGCGAAAGCGGCAACAGATGATTACACGCACATCTCTGTGGGACAGTTGTATATTCCACAGATGT-3'

P2-5	5'-GTACAAACTGAGCAGGCAAATGACTTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATCGCAAGATGTAACTGAATGAAATGGTGAAGGACGGGTCCAGTCATGCAGCCGGGCTGCGAAAGCGGCAACAGATGATTACACGCACATCTCTGTGGGACAGTTGTATATTCCACAGATGT-3'
P2-4	5'-GTACAAACTGAGCAGGCAAATGATTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATCGCAAGATGTAACTGAATGAAATGGTGAAGGACGGGTCCATCATGCAGCCGGGCTGCGAAAGCGGCAACAGATGATTACACGCACATCTCTGTGGGACAGTTGTATATTCCACAGATGT-3'
P2-3	5'-GTACAAACTGAGCAGGCAAATGTTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATCGCAAGATGTAACTGAATGAAATGGTGAAGGACGGGTCCACATGCAGCCGGGCTGCGAAAGCGGCAACAGATGATTACACGCACATCTCTGTGGGACAGTTGTATATTCCACAGATGT-3'
P2-2	5'-GTACAAACTGAGCAGGCAAATTTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATCGCAAGATGTAACTGAATGAAATGGTGAAGGACGGGTCCAATGCAGCCGGGCTGCGAAAGCGGCAACAGATGATTACACGCACATCTCTGTGGGACAGTTGTATATTCCACAGATGT-3'
P3-5	5'-GTACAAACTGAGCAGGCAAATGACCAGAGCGGTCATGCAGCCGGGCTGCTTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATCGCAAGATGTAACTGAATGAAATGGTGAAGGACGGGTCCAGCGGCAACAGATGATTACACGCACATCTCTGTGGGACAGTTGTATATTCCACAGATGT-3'
P3-4	5'-GTACAAACTGAGCAGGCAAATGACCAGAGCGGTCATGCAGCCGGGCTGTTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATCGCAAGATGTAACTGAATGAAATGGTGAAGGACGGGTCCACGGCAACAGATGATTACACGCACATCTCTGTGGGACAGTTGTATATTCCACAGATGT-3'
P3-3	5'-GTACAAACTGAGCAGGCAAATGACCAGAGCGGTCATGCAGCCGGGCTTTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATCGCAAGATGTAACTGAATGAAATGGTGAAGGACGGGTCCAGGCAACAGATGATTACACGCACATCTCTGTGGGACAGTTGTATATTCCACAGATGT-3'
P3-2	5'-GTACAAACTGAGCAGGCAAATGACCAGAGCGGTCATGCAGCCGGGCTTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATCGCAAGATGTAACTGAATGAAATGGTGAAGGACGGGTCCAGCAACAGATGATTACACGCACATCTCTGTGGGACAGTTGTATATTCCACAGATGT-3'
P4-5	5'-GTACAAACTGAGCAGGCAAATGACCAGAGCGGTCATGCAGCCGGGCTGCGAAAGCGGCAACAGATTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATCGCAAGATGTAACTGAATGAAATGGTGAAGGACGGGTCCATCTGTGGGACAGTTGTATATTCCACAGATGT-3'
P4-4	5'-GTACAAACTGAGCAGGCAAATGACCAGAGCGGTCATGCAGCCGGGCTGCGAAAGCGGCAACAGTTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATCGCAAGATGTAACTGAATGAAATGGTGAAGGACGGGTCCACTGTGGGACAGTTGTATATTCCACAGATGT -3'
P4-3	5'-GTACAAACTGAGCAGGCAAATGACCAGAGCGGTCATGCAGCCGGGCTGCGAAAGCGGCAACATTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATCGCAAGATGTAACTGAATGAAATGGTGAAGGACGGGTCCATGTGGGACAGTTGTATATTCCACAGATGT-3'
P4-2	5'-GTACAAACTGAGCAGGCAAATGACCAGAGCGGTCATGCAGCCGGGCTGCGAAAGCGGCAACTTGTTGAGTAGAGTGTGAGCTCCGTAACTAGTTACATCGCAAGATGTAACTGAATGAAATGGTGAAGGACGGGTCCAGTGGGACAGTTGTATATTCCACAGATGT-3'

Table S2. Response of **czcD-1**, **czcD-2**, and **czcD-3** – apparent K_d ($K_{d,app}$), Hill coefficient (n), and fluorescence turn-on (F_{max}/F_{min}) – to divalent first-row transition metal ions in unbuffered titrations. For sensor assays, experiments were carried out in 30 mM MOPS, 100 mM KCl, 3 mM $MgCl_2$, pH 7.2, at 20 °C, using 100 nM sensor and 10 µM DFHBI-1T. Experiments were performed aerobically except for those involving Fe^{II}, which were performed in an anaerobic chamber. For **czcD-1**, for all metals except for Fe^{II}, no saturation of response was observed, so fitting parameters could not be reliably obtained. Lower limits of K_ds for Mn^{II}, Co^{II}, Ni^{II}, and Zn^{II} are reported as the highest metal concentration experimentally tested, because the sensor did not reach 50% of the total response to Fe^{II} (assuming that the sensor would hypothetically saturate at the same value for all metal ions). The $K_{d,app}$ values we determined for **czcD-2** and **czcD-3** are slightly smaller than those determined for the native riboswitch by Furukawa et al. This discrepancy may result from the 20 mM Mg^{II} present in the buffer used in the original report (see **Figure S4**). Values are reported as the mean ± S.D. for 3 independent titrations. N.D.: cannot be determined from data in **Figure S2** due to poor fluorescence response.

		Mn^{II}	**Fe^{II}**	**Co^{II}**	**Ni^{II}**	**Zn^{II}**
czcD-1	$K_{d,app}$ (µM)	N.D.	23 ± 4	>50	>25	>100
	F_{max}/F_{min}	N.D.	8.0	N.D.	N.D.	N.D.
	n	N.D.	2.2 ± 0.3	N.D.	N.D.	N.D.
czcD-2	$K_{d,app}$ (µM)	66 ± 3	2.2 ± 0.8	3.1 ± 0.1	2.2 ± 0.5	12 ± 1
	F_{max}/F_{min}	5.1	3.7	6.2	6.6	7.6
	n	2.5 ± 0.2	1.6 ± 0.1	2.9 ± 0.2	1.7 ± 0.1	2.8 ± 0.5
czcD-3	$K_{d,app}$ (µM)	25 ± 12	1.3 ± 0.5	2.2 ± 0.1	0.9 ± 0.1	5.1 ± 0.5
	F_{max}/F_{min}	2.0	1.9	2.9	2.2	2.7
	n	2.5 ± 0.8	1.2 ± 0.2	3.9 ± 2.3	3 ± 1.1	1.8 ± 0.7
Eba riboswitch (in-line)[a]	$K_{d,app}$ (µM)	220	no response[b]	5.6	12	no response[b]
	n	1.2	N/A	2	1.6	N/A

[a] Values from Furukawa et al.[17] Determined by in-line probing in 50 mM Tris, 100 mM KCl, 20 mM $MgCl_2$, pH 8.3, at 23 °C. (For Mn^{II} determinations, pH 7.5 and 30 °C were used.)
[b] Our data suggest that the lack of response was likely a result of precipitation of hydroxides of Fe^{III} and Zn^{II} under the experimental conditions (see main text).

Table S3. Calculated $K_{d,M}$ values used for determination of apparent K_ds of **czcD** sensors

Chelator	Metal	log K_M	Adjusted $K_{d,M}$
Citric acid	Mn^{II}	4.15	7.30×10^{-5}
	Fe^{II}	4.40	4.10×10^{-5}
	Co^{II}	5.00	1.05×10^{-5}
	Ni^{II}	5.40	4.10×10^{-6}
	Zn^{II}	4.98	1.08×10^{-6}
	Mg^{II}	3.37	4.60×10^{-4}

Table S4. Plasmids used in this study

Name	Notes	Source
pDL19	Amp^R; *E. coli* expression of T7 RNA polymerase	Ref. 2, Philip Bevilacqua
pET31b-T7-Spinach2	Amp^R	Ref. 3, Addgene #79783
pET31b-T7-czcD-2	Amp^R; expression of **czcD-2** in *E. coli*	This work
pET21b-RL015A	Amp^R	Ref. 14, Addgene #42134
pHT01-ads	Cm^R, Amp^R	Ref. 4, Addgene #47382
pHT01-sfGFP	Cm^R, Amp^R; *B. subtilis* expression of sfGFP	This work
pHT01-pfeT	Cm^R, Amp^R; *B. subtilis* expression of PfeT	This work
pHT01-eba	Cm^R, Amp^R; *B. subtilis* expression of Eba3544	This work
pHT01-eba-tev-sfGFP	Cm^R, Amp^R; *B. subtilis* expression of Eba3544-sfGFP, for verification of expression and localization	This work
pHT01-lmo-tev	Cm^R, Amp^R; *B. subtilis* expression of LMO3448 with TEV-cleavable C-terminal His_6 tag	This work
pHT01-lmo-tev-sfGFP	Cm^R, Amp^R; *B. subtilis* expression of LMO3448-sfGFP, for verification of expression and localization	This work

Table S5. Primers used for cloning and sequencing

Name	Sequence[a,b]
Spinach 2-EagI-F	5'-CCAAGCGGCCGGATGTAACTGAATGAAATGGTGAAGG-3'
Spinach 2-EagI-R	5'-CTTGGCGGCCGGATGTAACTAGTTACGGAGCTCA -3'
sfGFP-BamHI-F	5'-ATAAGGATCCATGCGTAAAGGCGAAGAACTG-3'
sfGFP-XbaI-R	5'-TTATTCTAGATTACTTATACAGCTCGTCCATACCG-3'
eba-Gib-F	5'-CCAATTAAAGGAGGAAGGATCCATGACAACTGAAAAAAAACCATATG-3'
eba-Gib-R	5'-GGACGTCGACTCTAGATTACTCGTTCGCCGG-3'
lmo-Gib-F	5'-CCAATTAAAGGAGGAAGGATCCATGATTAGTTACCTTATTAAATCTCGCC-3'
lmo-Gib-R	5'-GGACGTCGACTCTAGATCAGTGGTGGTGGTGGT-3'
eba-tev-sfGFP-gib-F	5'-CCCGGCGAACGAGGAAAACCTGTATTTTCAGGGC-3'
lmo-tev-sfGFP-gib-F	5'-CATTAAAGAGTGTTAAGGTTGAAAACCTGTATTTTCAGGGC-3'
pHT01-gib-R	5'-GGACGTCGACTCTAGATCA-3'
Sequencing primers	
pHT01-R	5'- TCCGAGCTTCGTCCAAAATA-3'
LacI-R	5'- GGCATACTCTGCGACATCGT-3'
lmo-mid	5'-TATCGTTAACGAGAGCG-3'
pfeT-mid	5'-GATTGTATGGCCAGATTG-3'
T7P	5'-TAATACGACTCACTATAGGG-3'
T7T	5'-GCTAGTTATTGCTCAGCGG-3'

[a] Restriction sites are underlined, [b] Gibson ends are in red.

Table S6. Primers used for sensor library construction (continued on the next page).

Name	Sequence
Spinach2-top	5'-aagTAATACGACTCACTATAGATGTAACTGAATGAAATGGTGAAGGACGGGTCCAGTAGGCTGC-3'
Spinach2-bottom	5'-GATGTAACTAGTTACGGAGCTCACACTCTACTCAACAAGTAGGCTGCCGAAGCAGCCTACTGGACCCGTC-3'
cpSpinach2-top	5'-aagTAATACGACTCACTATAGTGGTGGTGGTGGTGCTCGAGTTACTTGTATAGCTCGTCCATGCCG-3'
cpSpinach2-bottom	5'-TGGTCCTGCTGGAGTTCGTGTAACTCGAGCACCACCACCACCA-3'
NiCo_T7amp_fwd	5'-ccaagTAATACGACTCACTATAGGTACAAACTGAGCAGGCAA-3'
NiCo_amp_rev	5'-ACATCTGTGGAATATACAACTGTCC-3'
NiCo_P1-5_fwd	5'-GATGTAACTGAATGAAATGGTGAAGGACGGGTCCAAACTGAGCAGGCAAATGACC-3'
NiCo_P1-5_rev	5'-GATGTAACTAGTTACGGAGCTCACACTCTACTCAACAAAACTGTCCCACAGAGATGTG-3'
NiCo_P1-4_fwd	5'-GATGTAACTGAATGAAATGGTGAAGGACGGGTCCAACTGAGCAGGCAAATGACCA-3'
NiCo_P1-4_rev	5'-GATGTAACTAGTTACGGAGCTCACACTCTACTCAACAAACTGTCCCACAGAGATGTG-3'
NiCo_P1-3_fwd	5'-GATGTAACTGAATGAAATGGTGAAGGACGGGTCCACTGAGCAGGCAAATGACCA-3'
NiCo_P1-3_rev	5'-GATGTAACTAGTTACGGAGCTCACACTCTACTCAACAACTGTCCCACAGAGATGTG-3'
NiCo_P1-2_fwd	5'-GATGTAACTGAATGAAATGGTGAAGGACGGGTCCATGAGCAGGCAAATGACCAGA-3'
NiCo_P1-2_rev	5'-GATGTAACTAGTTACGGAGCTCACACTCTACTCAACAATGTCCCACAGAGATGTGC-3'
NiCo_P1-1_fwd	5'-GATGTAACTGAATGAAATGGTGAAGGACGGGTCCAGAGCAGGCAAATGACCAGA-3'
NiCo_P1-1_rev	5'-GATGTAACTAGTTACGGAGCTCACACTCTACTCAACAAGTCCCACAGAGATGTGC-3'
NiCo_P1-0_fwd	5'-GATGTAACTGAATGAAATGGTGAAGGACGGGTCCAAGCAGGCAAATGACCAGAG-3'
NiCo_P1-0_rev	5'-GATGTAACTAGTTACGGAGCTCACACTCTACTCAACAATCCCACAGAGATGTGCG-3'
NiCo_P2-5_fwd	5'-GTACAAACTGAGCAGGCAAATGACTTGTTGAGTAGAGTGTGAGCTC-3'
NiCo_P2-5_rev	5'-GTAATCATCTGTTGCCGCTTTCGCAGCCCGGCTGCATGACTGGACCCGTCCTTCAC-3'
NiCo_P2-4_fwd	5'-GTACAAACTGAGCAGGCAAATGATTGTTGAGTAGAGTGTGAGCTC-3'
NiCo_P2-4_rev	5'-GTAATCATCTGTTGCCGCTTTCGCAGCCCGGCTGCATGATGGACCCGTCCTTCAC-3'
NiCo_P2-3_fwd	5'-GTACAAACTGAGCAGGCAAATGTTGTTGAGTAGAGTGTGAGCTC-3'
NiCo_P2-3_rev	5'-GTAATCATCTGTTGCCGCTTTCGCAGCCCGGCTGCATGTGGACCCGTCCTTCAC-3'
NiCo_P2-2_fwd	5'-GTACAAACTGAGCAGGCAAATTTGTTGAGTAGAGTGTGAGCTC-3'
NiCo_P2-2_rev	5'-GTAATCATCTGTTGCCGCTTTCGCAGCCCGGCTGCATTGGACCCGTCCTTCAC-3'
NiCo_P3-5_fwd	5'-GCAGGCAAATGACCAGAGCGGTCATGCAGCCGGGCTGCTTGTTGAGTAGAGTGTGAGCTC-3'
NiCo_P3-5_rev	5'-TCCCACAGAGATGTGCGTGTAATCATCTGTTGCCGCTGGACCCGTCCTTCAC-3'
NiCo_P3-4_fwd	5'-GCAGGCAAATGACCAGAGCGGTCATGCAGCCGGGCTGTTGTTGAGTAGAGTGTGAGCTC-3'
NiCo_P3-4_rev	5'-TCCCACAGAGATGTGCGTGTAATCATCTGTTGCCGTGGACCCGTCCTTCAC-3'
NiCo_P3-3_fwd	5'-GCAGGCAAATGACCAGAGCGGTCATGCAGCCGGGCTTTGTTGAGTAGAGTGTGAGCTC-3'
NiCo_P3-3_rev	5'-TCCCACAGAGATGTGCGTGTAATCATCTGTTGCCTGGACCCGTCCTTCAC-3'
NiCo_P3-2_fwd	5'-GCAGGCAAATGACCAGAGCGGTCATGCAGCCGGGCTTGTTGAGTAGAGTGTGAGCTC-3'
NiCo_P3-2_rev	5'-TCCCACAGAGATGTGCGTGTAATCATCTGTTGCTGGACCCGTCCTTCAC-3'
NiCo_P4-5_fwd	5'-AGCGGTCATGCAGCCGGGCTGCGAAAGCGGCAACAGATTGTTGAGTAGAGTGTGAGCTC-3'
NiCo_P4-5_rev	5'-ACATCTGTGGAATATACAACTGTCCCACAGATGGACCCGTCCTTCAC-3'

NiCo_P4-4_fwd	5'-AGCGGTCATGCAGCCGGGCTGCGAAAGCGGCAACAGTTGTTGAGTAGAGTGTGAGCTC-3'
NiCo_P4-4_rev	5'-ACATCTGTGGAATATACAACTGTCCCACAGTGGACCCGTCCTTCAC-3'
NiCo_P4-3_fwd	5'-AGCGGTCATGCAGCCGGGCTGCGAAAGCGGCAACATTGTTGAGTAGAGTGTGAGCTC-3'
NiCo_P4-3_rev	5'-ACATCTGTGGAATATACAACTGTCCCACATGGACCCGTCCTTCAC-3'
NiCo_P4-2_fwd	5'-AGCGGTCATGCAGCCGGGCTGCGAAAGCGGCAACTTGTTGAGTAGAGTGTGAGCTC-3'
NiCo_P4-2_rev	5'-ACATCTGTGGAATATACAACTGTCCCACTGGACCCGTCCTTCAC-3'
NiCo_1-28_fwd	5'-ccaagTAATACGACTCACTATAgGTACAAACTGAGCAGGCAAATGACCAGA-3'
NiCo_1-42_fwd	5'-GGCTGCATGACCGCTCTGGTCATTTGCCTGCTCAGTTTGTAC-3'
NiCo_52-111_rev	5'-ACATCTGTGGAATATACAACTGTCCCACAGAGATGTGCGTGTAATCATCTGTTGCCGCTT-3'
NiCo_73-111_rev	5'-ACATCTGTGGAATATACAACTGTCCCACAGAGATGTGCG-3'

SUPPLEMENTARY FIGURES

Figure S1. Screening of the library of *Eba czcD* riboswitch-based sensors. For each construct, fluorescence response was evaluated at different magnesium concentrations (0.5, 1, 5, and 10 mM) and either 0 or 100 µM $CoCl_2$, using 100 nM sensor and 10 µM DFHBI-1T, in 30 mM MOPS, 100 mM KCl, pH 7.2, at 20 °C. The three best-performing constructs (boxed) were evaluated further.

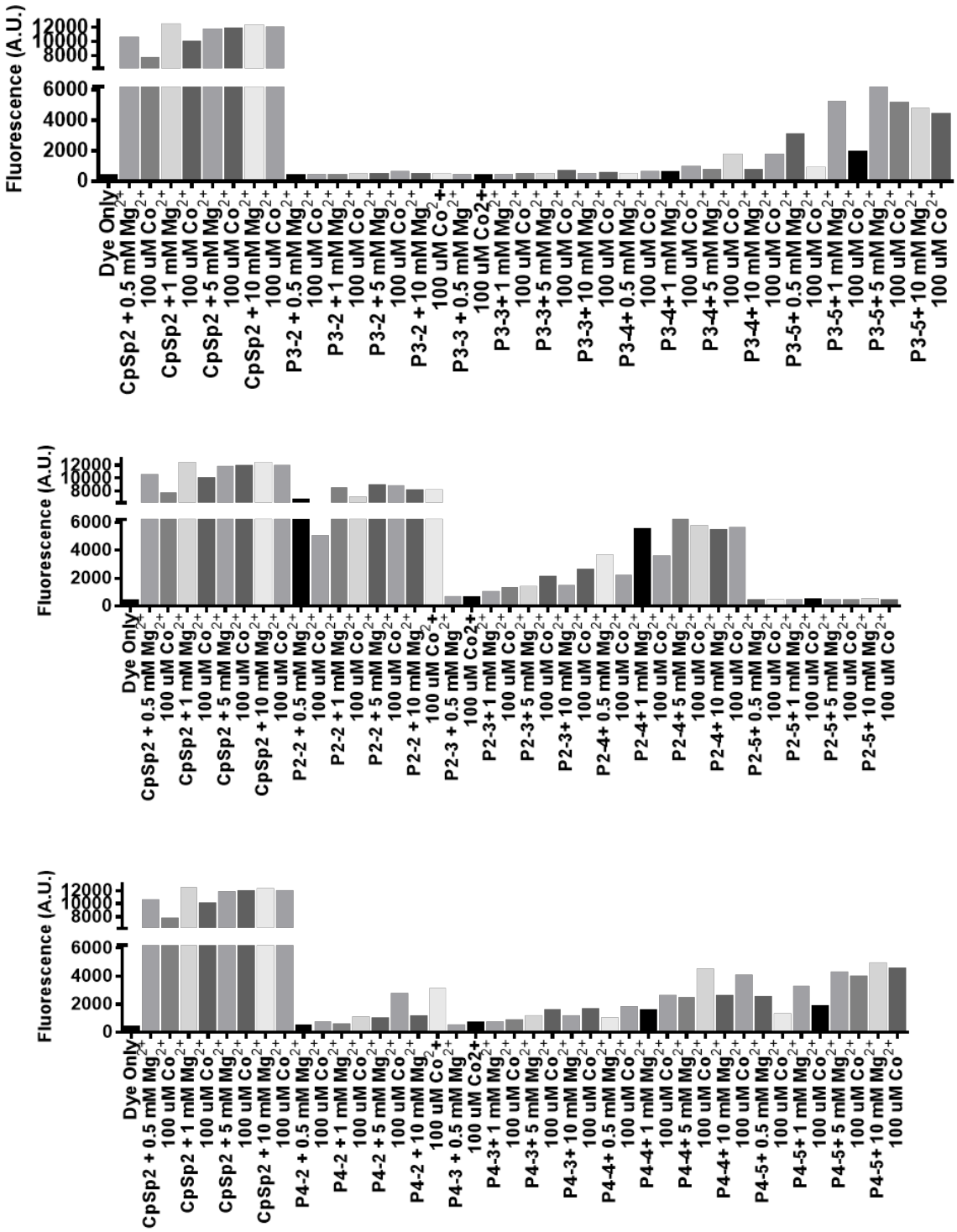


Figure S1, continued

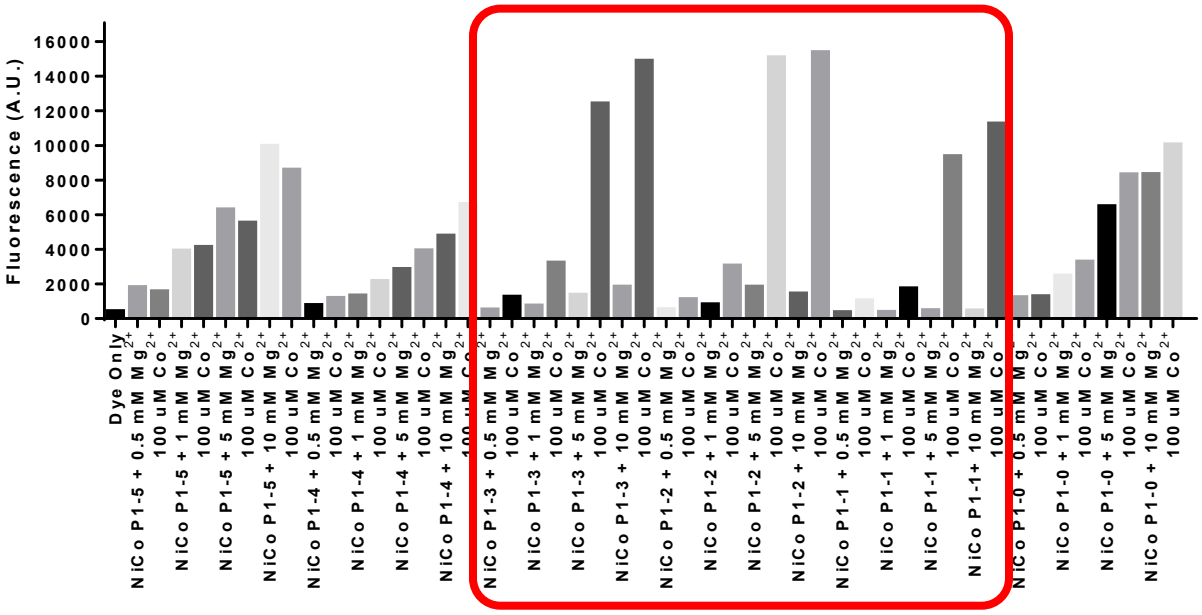


Figure S2. Unbuffered fluorescence titrations of **czcD-1**, **czcD-2**, and **czcD-3** with Mn^{II}, Fe^{II}, Co^{II}, Ni^{II}, and Zn^{II}. Experimental conditions and results are summarized in **Table S2**.

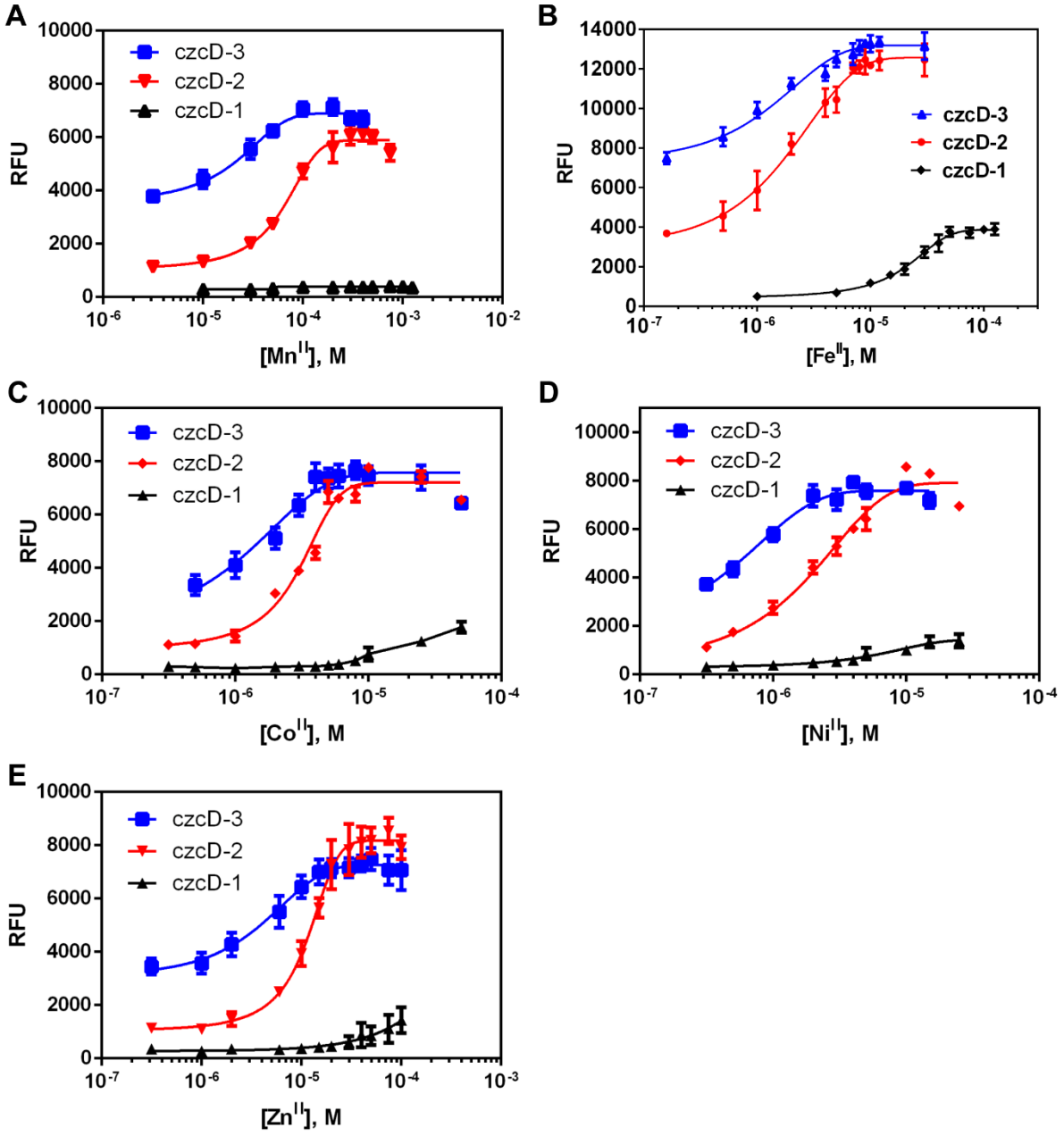


Figure S3. Detail of the metal-binding sites from the x-ray crystal structure of the Co^{II}-bound *Eba* riboswitch (PDB code: 4RUM), showing a possible origin of cooperative response, as discussed previously.[17] Co1 (C1) is coordinated by N7 of G47, N7 of G87, the 2'-OH of G46, and three solvent molecules. Co2 (C2) is coordinated by N7 of G46 and N7 of G88, the 2'-OH of G87, and three solvent molecules. One of those molecules is hydrogen bonded to the 2'-OH of G45, which in turn is ligated to Co3 (C3) via N7. N7 of A14 also coordinates Co3 (C3), along with four solvent molecules. The connectivity between the C1 and C2 sites, in particular, but possibly also C3, could ensure cooperativity. We speculate that Co4, interacting only with G18 (via O6 and N7), may be an artefact of the 2 mM Co^{II} present in the crystallization condition. It is unknown at present whether differences in Hill coefficient for various metal ions reflect different binding stoichiometries.

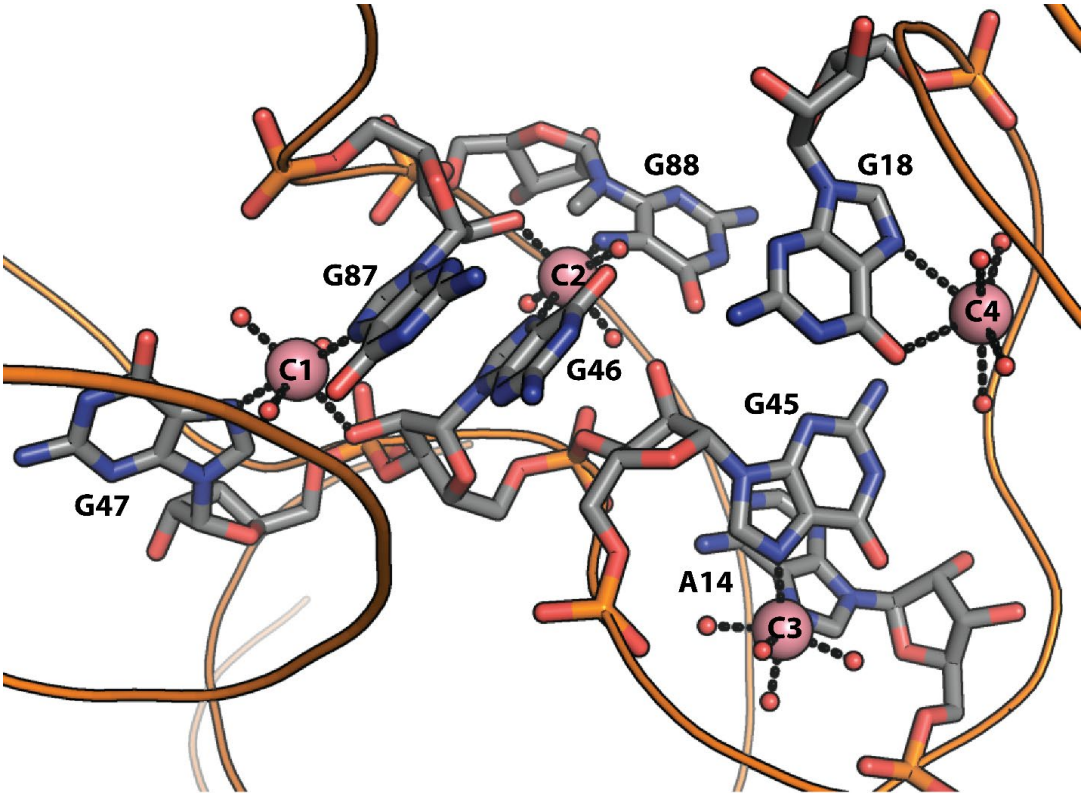


Figure S4. Assays of **czcD-2** response under conditions used by Furukawa et al.[17] **czcD-2** was titrated with unbuffered Co^{II} stocks using the following buffer conditions: Buffer 1 – 50 mM Tris, 100 mM KCl, 20 mM $MgCl_2$, pH 8.3 (in-line probing buffer, **Table S2**), and Buffer 2 – 40 mM Tris, 150 mM KCl, 10 mM $MgCl_2$, 1% glycerol, pH 7.5. Fits of these titration curves give: $K_{d,app}$ = 8.7 ± 2.1 μM, n = 1.3 ± 0.1 (Buffer 1) and $K_{d,app}$ = 3.1 ± 0.2 μM, n = 1.9 ± 0.4 (Buffer 2). These results are similar to those obtained from in-line probing assays of the riboswitch, suggesting that differences in buffer composition (Tris vs. MOPS) and/or Mg concentration (3 mM in our studies vs. 10 mM or 20 mM) likely account for the minor differences between these data.

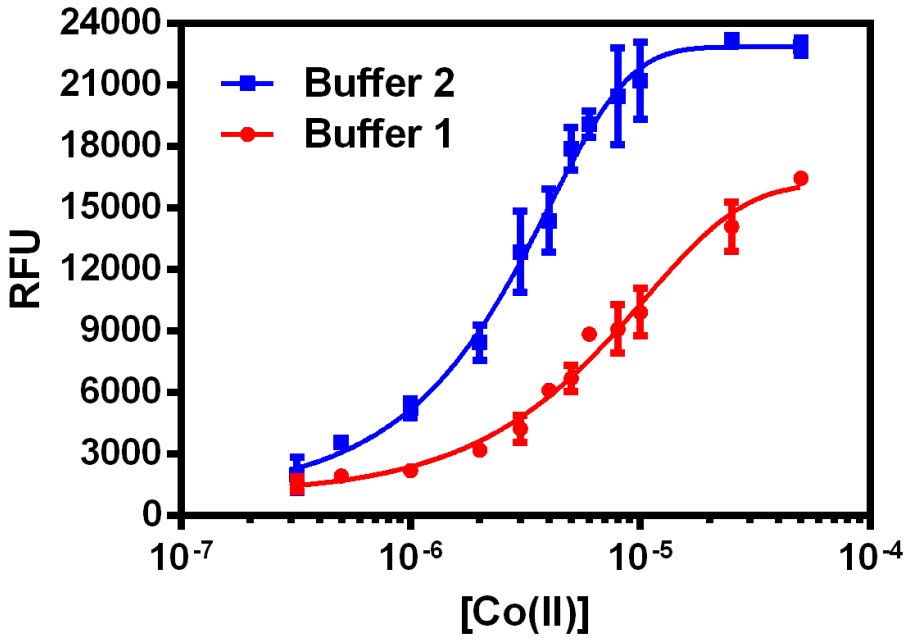


Figure S5. czcD-2 cleavage assay. **czcD-2** sensor was incubated with the highest metal concentrations used in this study, demonstrating that the RNA sensor is stable under the conditions used for RNA sensor characterization and citrate-buffered metal titrations. Buffering and incubation conditions (1 h, 20 ºC) were carried out as described in the Experimental Section. The samples were analyzed using a 6% urea PAGE gel and the gel was stained for 20 min with 1× SYBR™ Gold in 50 mL of 1× TBE. The stained gel was imaged using a Bio-Rad Gel Doc™ EZ imager. The Lewis acidities of Co^{II} and Ni^{II} are similar to those of the other divalent metal ions we have studied here, suggesting that RNA hydrolysis is likely negligible under these conditions.

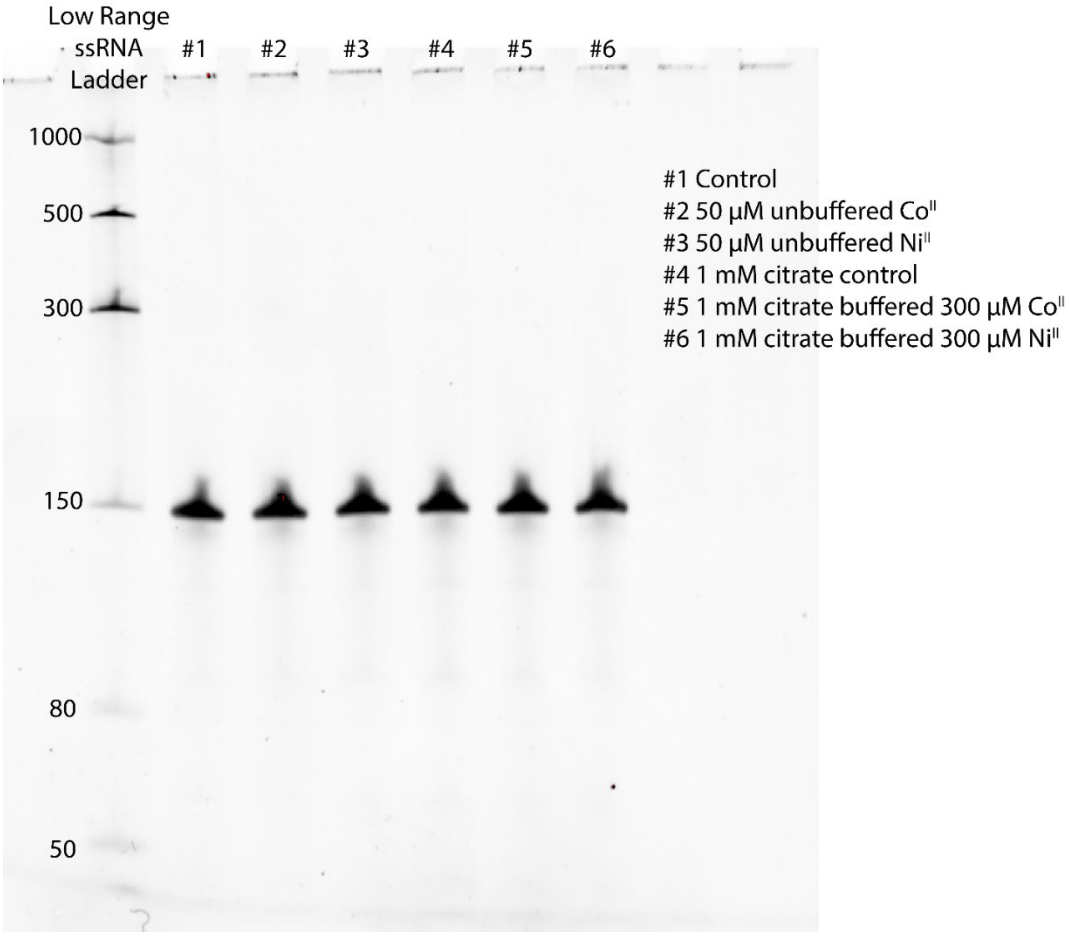


Figure S6. czcD-2 does not respond to iron under aerobic conditions. Iron was added from a freshly made ferrous ammonium sulfate stock solution to the sensor mix, followed by aerobic incubation for 1 h at room temperature. After incubation, fluorescence was measured using λ_{ex}=475 nm and λ_{em}=505 nm. These data, distinct from our characterization of **czcD-2** with Fe^{II} under anaerobic conditions (**Figure 1, Table 1, Table 2**) suggest that Fe^{II} oxidation to insoluble Fe^{III} likely accounts for the failure of the *czcD* riboswitch to respond to iron in the initial studies by Furukawa et al.[17]

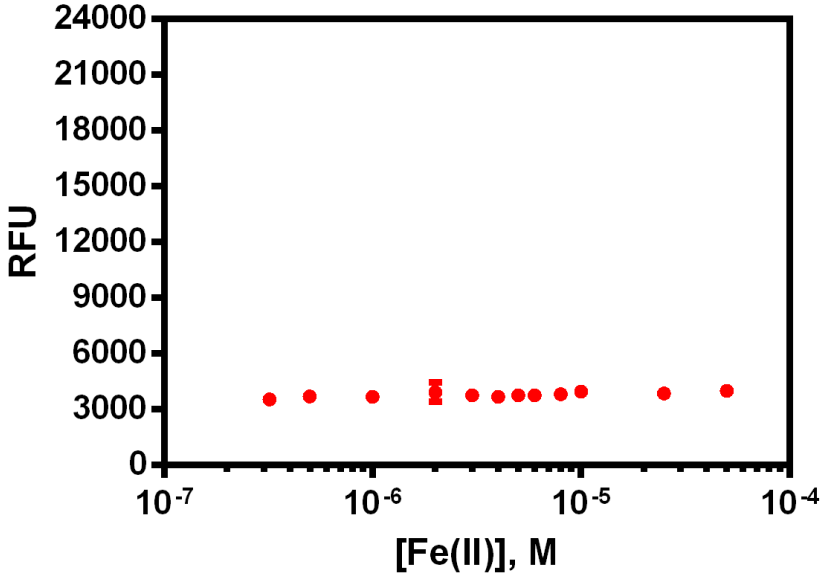


Figure S7. Citrate-buffered fluorescence titrations of **czcD-1** and **czcD-3** with Mn^{II}, Fe^{II}, Co^{II}, Ni^{II}, and Zn^{II}. Experimental conditions and results are summarized in **Table 2**.

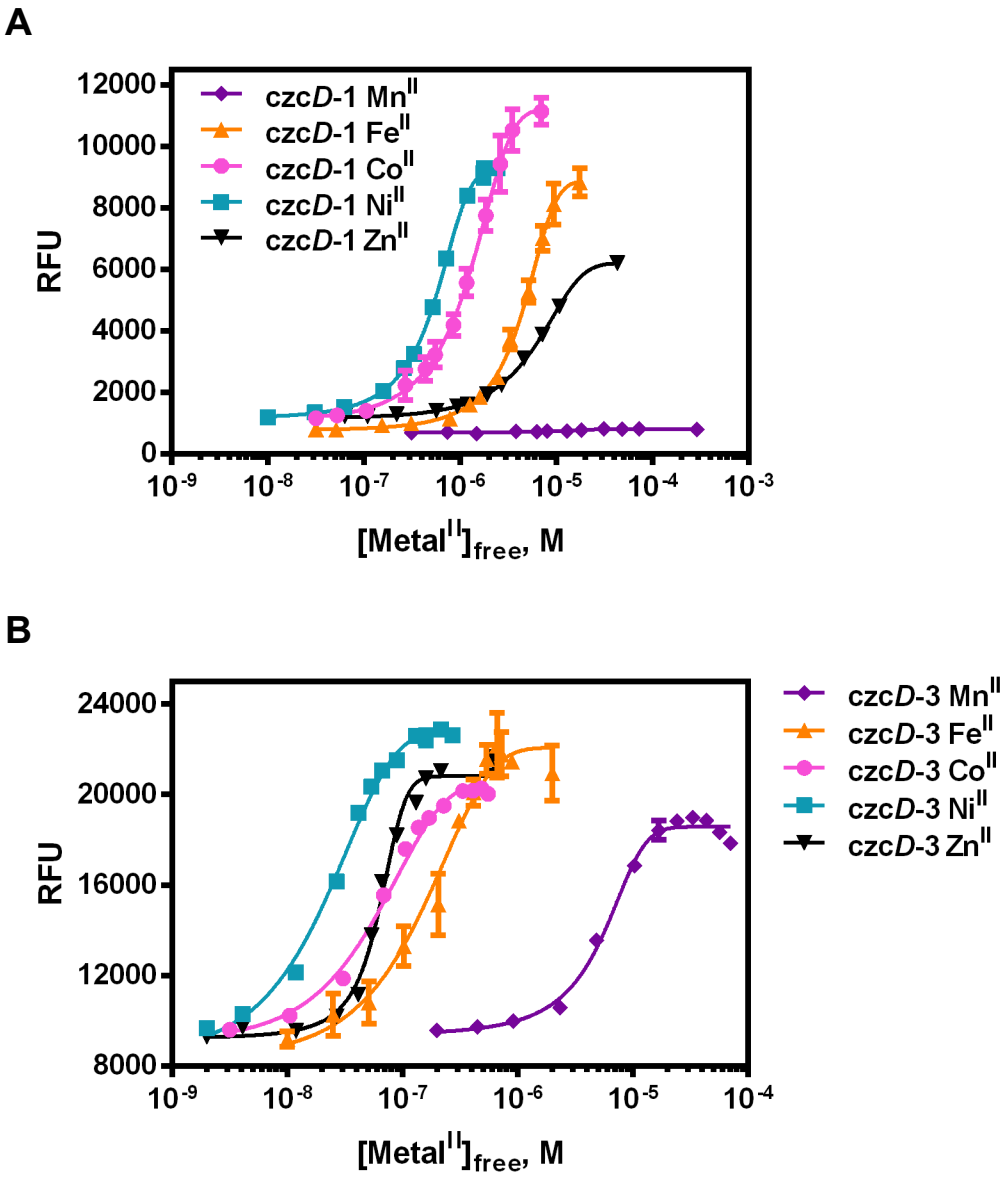


Figure S8. Magnesium dependence of the fluorescence response of **czcD-2** (100 nM sensor, 10 μM DFHBI-1T) to citrate-buffered Co^{II}. Titrations were carried out as described in the Experimental Procedures, with either 1, 3, or 10 mM $MgCl_2$ (3 mM $MgCl_2$ data shown in **Figure 2**). The data are plotted as mean ± S.E.M. for 3 experiments, and the calculated fitting parameters are expressed as mean ± S.D. The similarity in the calculated $K_{d,app}$ values (110 nM and 210 nM for 10 mM and 1 mM Mg^{II}, respectively) to each other and to the value for 3 mM Mg^{II} (62 nM) suggests that the effects of Mg^{II} concentration on metal responsiveness are relatively minor.

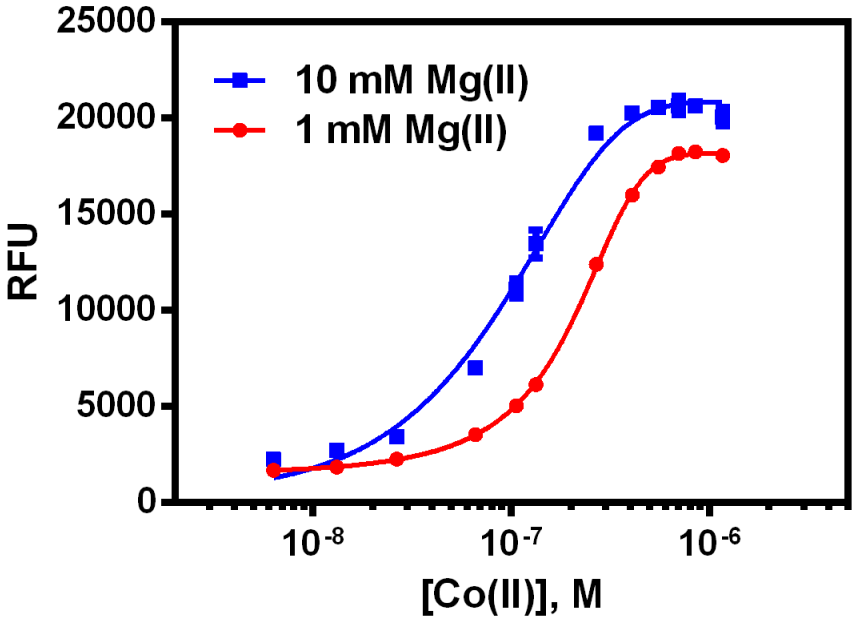


Figure S9. Analysis of flow cytometry data to determine iron responsiveness of **czcD-2** in *E. coli* cells. A) Flow cytometry histogram ($n \geq 30000$ cells) of **czcD-2**-expressing cells, revealing an iron-dependent increase in mean cellular fluorescence. B) (*Left*) Cell sizes of Spinach2- and **czcD-2** expressing cells, as represented by FSC (forward scatter), showing similar, iron-dependent increases in cell size, consistent with previous reports.[20, 21] (*Right*) Mean cellular fluorescence of Spinach2- and **czcD-2** expressing cells. While fluorescence of Spinach2-expressing cells increases with iron concentration, the trend mirrors that of increasing cell size. Moreover, the fluorescence of **czcD-2**-expressing cells increases to a greater extent with iron concentration. The response of **czcD-2** was normalized to the fluorescence increase of Spinach2 for each iron concentration to yield the data in **Figure 3**. Mean ± S.E.M. for 3 independent experiments (2 biological replicates each).

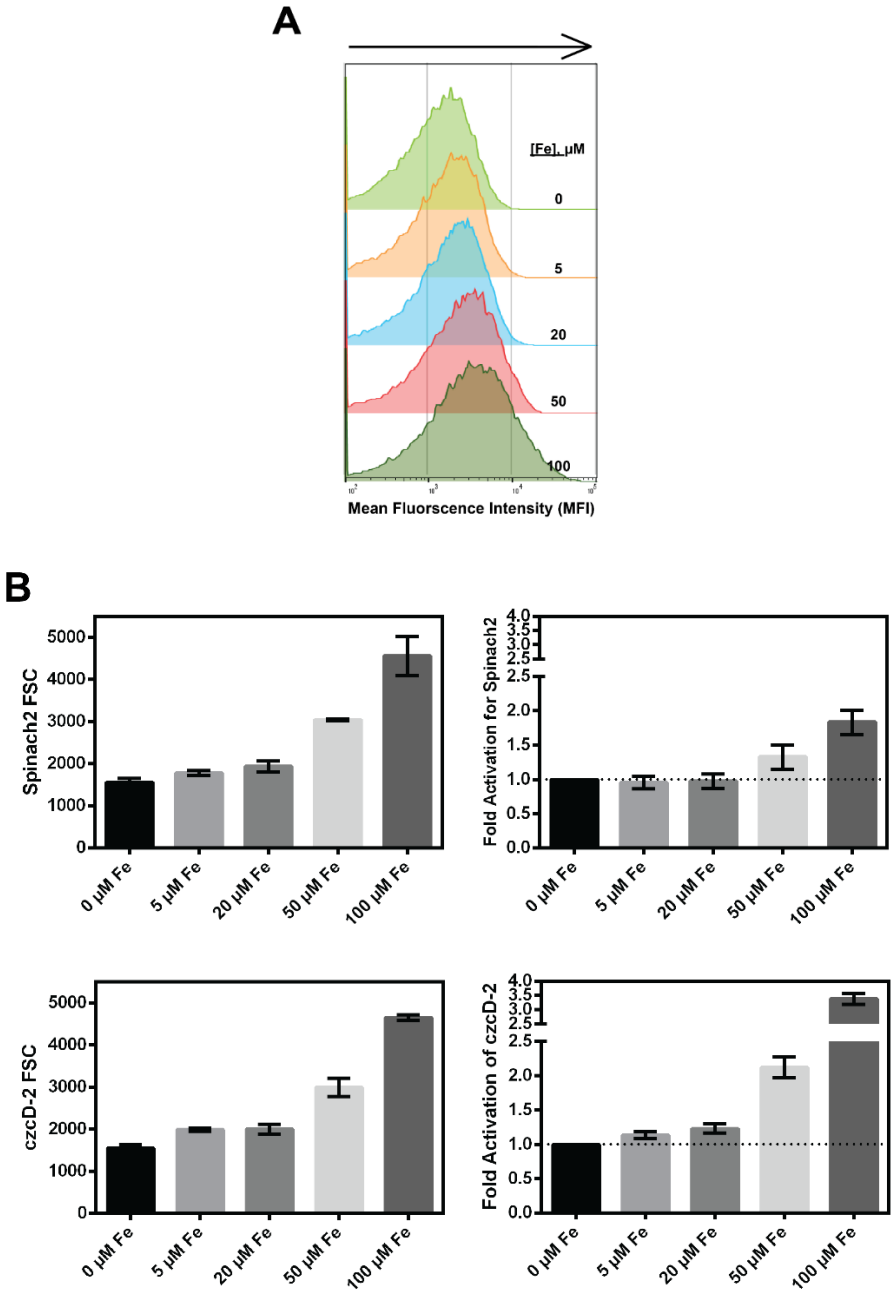


Figure S10. Assessment of response of **czcD-2** to divalent first-row transition metals in *E. coli* cells by flow cytometry. Cells were exposed to 20 μM Fe^{II}, Co^{II}, Ni^{II}, Zn^{II}, or Mn^{II} or no added metal for 1 h. Fluorescence response of **czcD-2**-expressing cells was normalized to that of Spinach2-expressing cells under the same conditions; that ratio was set to 1 for the no added metal experiment, to which the other experiments were compared. Mean ± S.E.M. for 3 independent experiments (2 biological replicates each). * $p < 0.05$ vs. no metal added.

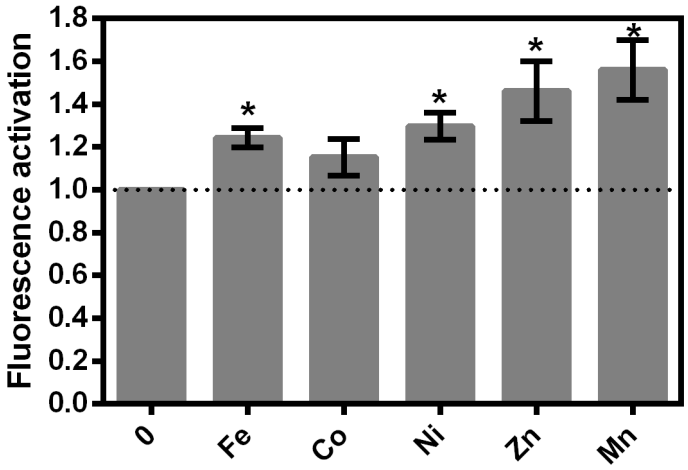


Figure S11. Sequence alignment of P_{1B4}-type ATPases: *Bacillus subtilis* 168 PfeT (UniprotKB O31688),[22] *Listeria monocytogenes* EGD-e FrvA (lmo0641, UniprotKB Q8Y992),[15, 23] *Mycobacterium tuberculosis* H37Rv CtpD (Rv1469, UniprotKB P9WPT3),[24] and *Listeria monocytogenes* FSL_J1-194 LMO3448 (UniprotKB T1YRD8). The SPC motif characteristic of P_{1B4}-type ATPases is shown bolded in red.[25]

```
PfeT      MN---EQVIVQRDPHE------PLKTDKREKNWAQH---AELIAALVSGALILAGWLLSG      48
FrvA      ----------------------------MKDWMKQ--NWQFITTGISGILIVIGCLVGS      29
CtpD      MTLTACEVTAAEAPFDRVSKTIPHPLSWGAALWSVVSVRWATVA----LLLFLAGLVAQL      56
LMO       ----------------------------MISYLIKSRQGQFLA---IGILFAAAGFIFG      28
                                            :         ::      *:  . .

PfeT      --YQVLSIIL-FLLAFVIGGFAKAKEGIEETLESKTLNVELLMIFAAIGSALIGYWAEGA      105
FrvA      DVGDFWTAVI-FLSAFVIGGFEQAKEGIQATIKTKKLNVELLMILAATGASIIGYWFEGA      88
CtpD      NGAPEAMWWTLYLACYLAGGWGSAWAG-AQALRNKALDVDLLMIAAAVGAVAIGQIFDGA      115
LMO       TMNSEYSRWL-FYAAIFFLGFYASKNAIVETVRYKSPNVDLLMILAALGAVIFDFESEGA      87
                    :  . .  *:  :  .   ::. *  :*:**** ** *:  :.   :**

PfeT      ILIFIFSLSGALETYTMNKSSRDLTSLMQLEPEEAT-LMVNGETKRVPVSDLQAGDMIVI      164
FrvA      ILIFIFSVSGALETYTTNKSKREITKLMAFQPERAFRLLSNGDLEEVAAKELQLDDMVFV      148
CtpD      LLIVIFATSGALDDIATRHTAESVKGLLDLAPDQAVVVQGDGSERVVAASELVVGDRVVV      175
LMO       ALLLIFAAAEVLEDYANNKSTSAISELMAQVPETAQVLKENGEVVTVPTEDLNVGERVVV      147
           *:.**: : .*:  : .::   :. *:   *: *  :  :*.   * ..:*  .: :.:

PfeT      KPGERVAADGIIESGSTSLDESALTGESMPVEKNTGDTVFTGTVNRNGSLTVRVTKANED      224
FrvA      RPGESVPIDGVIVRGSTTLNEAAINGESVPATKTVGADVFGGTVNVSSAITVKVTQTFEN      208
CtpD      RPGDRIPADGAVLSGASDVDQRSITGESMPVAKARGDEVFAGTVNGSGVLHLVVTRDPSQ      235
LMO       SKGEQIPIDGIIDRKS-IVNESALTGESVPVVKEAEDEVFAGTINEGDVFYIDVTKSSDE      206
            *: :  ** :   :  ::: ::.***:*. *     ** **:* .. : : **:  .:

PfeT      SLFRKIIKLVESAQNSVSPAQAFIERFENAYVKGVLIAVALLLFVPHFALGWSWSETFYR      284
FrvA      TIFSKIIRLVETAQSEPSKTARFIERFEDVYVKAVLLFVLVMMFLPHFALGWSWNETFYR      268
CtpD      TVVARIVELVADASATKAKTQLFIEKIEQRYSLGMVAATLALIVIP-LMFGADLRPVLLR      294
LMO       TVFSNIIRMVEEAQSRPSRISKFIDRIESKYVISVLVIVPIFIVVMYALMDLPFEEAFYR      266
          ::. .*:.:*  *.   :    **:::*. *  .::  .  ::.:    :.     .: *

PfeT      AMVFMVVASPCALVASIMPAALSLISNGARNGMLVKGSVFLEQLGSVQMIAFDKTGTVTK      344
FrvA      AMVLLTVASPCALVASVTPATLAAISNGARHGILFKGGVHLENLRGVKAIAFDKTGTLTN      328
CtpD      AMTFMIVASPCAVVLATMPPLLSAIANAGRHGVLVKSAVVVERLADTSIVALDKTGTLTR      354
LMO       GMVFLTVASPCALVASATPATLSAISNGAKNGILFKGGAAMEALSTMDILYTDKTGTLTY      326
          .*.:: ******:* :  *  *: *:*..::*:*.*... :* *   . :  *****:*

PfeT      GQPAVETIRIA--EGFSEAEVLEAVYAIETQSSHPLAQAITAYAESRGV-NQSGYISIEE      401
FrvA      GTPALTDRLFA--ENVDKQLVINVVGAMERQSLHPLAAAITQDLEPEIT-EKLTEIEVTD      385
CtpD      GIPRLASVAPLDPNVVDARRLLQLAAAAEQSSEHPLGRAIVAEARRRGI-AIPPAKDFRA      413
LMO       GEFKVDEYS-------APDDVLKEVIYMEQQSSHPIARAIVTAFKETDLSSVDHNEPVSE      379
          *   :               ::: .   * .* **:. **.   .            .

PfeT      TSGFGVMAEVSGAKWKVGKAGFIGEEMAAQFMKQTASDVIQSGHTIVFVKKDDQIAGCIA      461
FrvA      VPGWGVQAIYREGNWQVGKAGFVGKEAAAAFSNGAFERLASEGKTIVYVAKDGVIQAMFA      445
CtpD      VPGCGVHALVGNDFVEIASPQSYRGA-----PLAELAPLLSAGATAAIVLLDGVAIGVLG      468
LMO       IAGSGIK----KGTVRVGKPSAFST----FKNYDRFKQYFQKGNTIILAAKEEEVVGYFS      431
            * *:         .:..                      . * *   .  :    . :.

PfeT      LKDQIRPEAKEVMEELNRLG-IKTAMLTGDHEDTAQAIAKEAGMTTVVAECLPDQKVNEI      520
FrvA      LKDTCRPEAIRTIKALQAKG-IKTIMVTGDNEQTGAAIQAELGMDYVVSGCLPEKKVDVL      504
CtpD      LTDQLRPDAVESVAAMAALTAAPPVLLTGDNGRAAWRVARNAGITDVRAALLPEQKVEVV      528
LMO       LSDQIRRQSADAVANFQKEG-IKVTLLTGDNEEVTETVAEVVGVDDYKASMLPEDKIAYV      490
          *.*  * ::   :  :         ::***:  .   :    *:    :  **:.*:  :
```

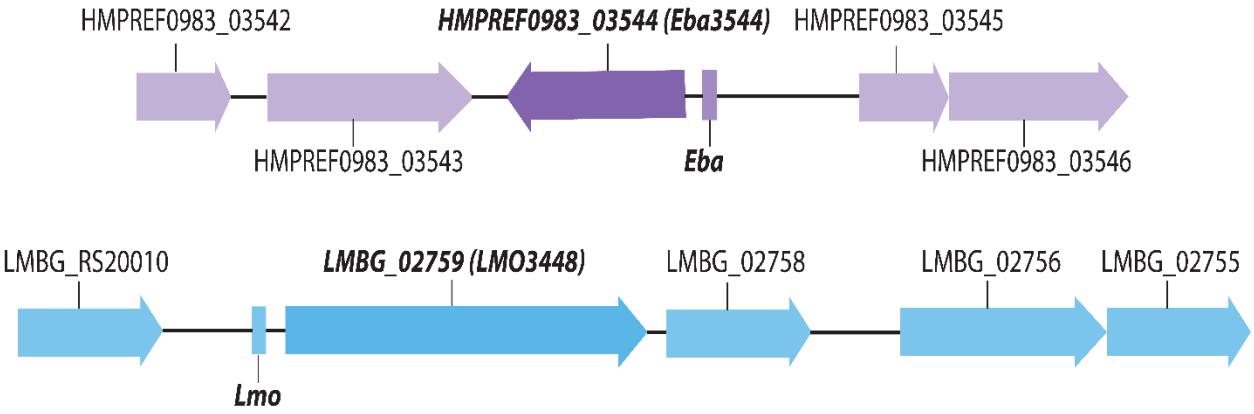
Figure S11, continued

```
PfeT      KRLKEEFGTIAMVGDGINDAPALKAADVGIAMGGGTDVALETADMVLMKNDLKKLVNMCR      580
FrvA      RELSVTYGSVAMVGDGINDAPALAHAAVGIAMGEGTDIAMETADVVLMKNDLEKIPYAYT      564
CtpD      RNLQAGGHQVLLVGDGVNDAPAMAAARAAVAMGAGADLTLQTADGVTIRDELHTIPTIIG      588
LMO       RESQDKEEVVGMIGDGINDAPALANADIGIAMGSGSSVAMESSDVVVVKNDLSKLFYSYK      550
          :. .     : ::***:*****:  *  .:*** *:.:::::::* * ::::* .:

PfeT      LSRKMNRIIKQNIVFSLAVICLLICANFLQAMELPFGVIGHEGSTILVILNGLRLLK---      637
FrvA      LSERLHWITWQNICFAIAVILVLITANVFQLINLPFGVVGHEGSTILVILNGLRLLRSNR      624
CtpD      LARQARRVVTVNLAIAATFIAVLVLWDLFGQLPLPLGVVGHEGSTVLVALNGMRLLTNRS      648
LMO       LSKKLNKIILQNVIFSISVIVTLIVLNLFGVLGLPLAVLFHEGSTILVILNGLRLLGSKG      610
          *:.: . :   *: :: :.*  *:  :.:  : **:.*: *****:** ***:***

PfeT      -----------------       637
FrvA      KK---------------       626
CtpD      WRAAASAAR--------       657
LMO       PKQEERVSDPSLKSVKV       627
```

Figure S12. Genomic neighborhoods of the *Eba* and *Lmo* riboswitches. In *Erysipelotrichaceae bacterium* 3_1_53 (NCBI Reference Sequence: NZ_GL520149.1), the *Eba* riboswitch is upstream of and putatively controlling HMPREF0983_03544 (Eba3544), encoding a putative metal efflux protein. In *L. monocytogenes* FSL J1-194 (NCBI Reference Sequence: NZ_AARJ02000030.1), the *Lmo* riboswitch is upstream of and putatively controlling LMBG_02759 (LMO3448), encoding a predicted P_{1B4}-type ATPase. The riboswitch and ATPase gene are flanked by two predicted transposases, suggesting that the riboswitch-ATPase unit are a mobile genetic element.[26]



Eba genes (predicted functions):
3542: MerR family transcriptional regulator
3543: DUF2185 domain-containing protein
3545: GntR family transcription regulator
3546: ABC transporter, ATP-binding protein

Lmo genes (predicted functions):
20010: transposase
02758: IS6 family transposase
02756: ATP-binding protein
02755: hypothetical protein

Figure S13. Control experiments showing expression of PfeT, LMO3448, and Eba3544 does not affect *B. subtilis* growth rate in absence of added metal ions. Growths and induction of protein expression were carried out as described in **Figure 5** and the Experimental Section.

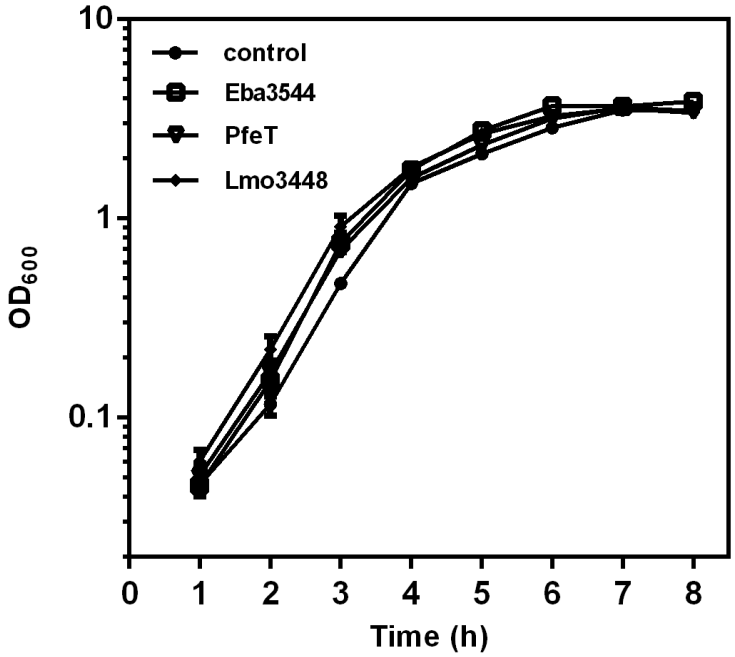


Figure S14. LMO3448 and Eba3544 are expressed and inserted into the membrane. *B. subtilis* cultures were grown under the same conditions as the complementation assay (30 min induction with IPTG) and membrane fractions were harvested to confirm sfGFP-tagged protein insertion into the cell membrane. The y-axis represents total sfGFP fluorescence of the supernatant and membrane fractions, normalized by volume of each fraction. Approximately one-third of the sfGFP-tagged Eba3544 and LMO3448 proteins, but not an sfGFP control, are inserted into the cell membrane. At this time, we are unable to assess whether the lesser ability of Eba3544 to rescue metal toxicity (**Figure 5**) is a result of inherent properties of the protein, its lower expression, or a combination of these factors.

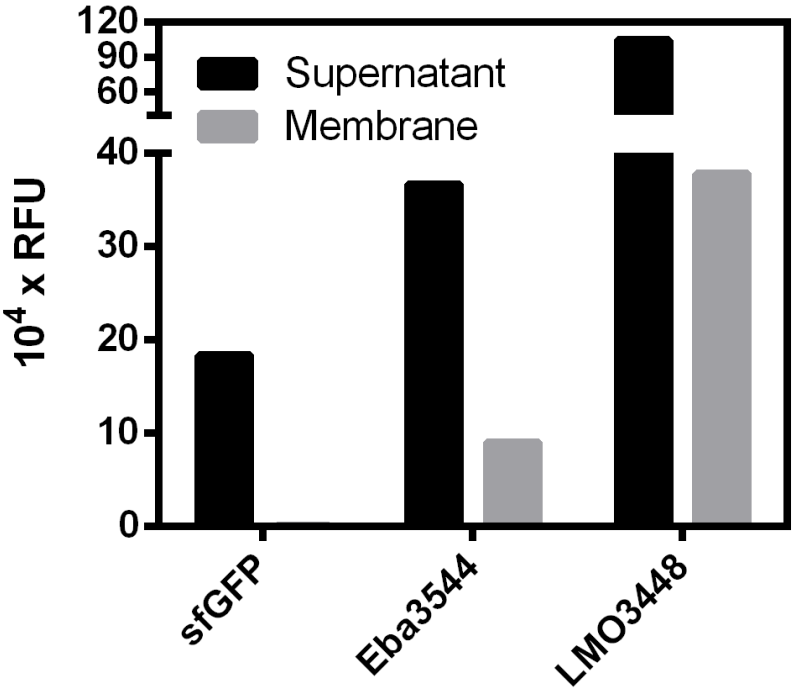


Figure S15. Predicted domain and secondary structure of PfeT, LMO3448, and Eba3544. (A) Protein sequences of PfeT and LMO3448 were aligned with the sequence of *Legionella pneumophila* CopA, a P_{1B1}-type ATPase that transports Cu^{I}. Based on the x-ray crystal structure of CopA (PDB: 3RFU),[27] the 8 transmembrane helices (red; A, B, and 1-6), actuator domain (gray), N-domain (teal), and P-domain (magenta) in CopA are shown. The characteristic three-residue motifs in TM4 that are thought to be involved in metal recognition are highlighted in yellow in all three sequences; this motif is CPC in P_{1B1} ATPases and SPC in P_{1B4}s.[25] The sequence alignment shows that, although PfeT and LMO3448 feature short ~15-amino acid extensions at the N-terminus and C-terminus, respectively, with potential metal ligands, both proteins lack a metal-binding domain (e.g. the N-terminal ~80 aa of CopA). (B) Eba3544's secondary and transmembrane structures were predicted using Jpred4[28] and TMHMM2.0.[29] Eba3544 is predicted to feature a small domain at its C-terminus.

A

```
CopA        MKHDHHQGHTHSGKGHACHHEHNSPKTQQASSKMEGPIVYTCPMHPEIRQSAPGHCPLCG   60
LMO3448     ------------------------------------------------------------   0
PfeT        ------------------------------------------------------------   0

CopA        MALEPETVTVSEVVSPEYLDMRRRFWIALMLTIPVVILEMGGH-GLKHFISGNGSSWIQL   119
LMO3448     --MIS-----------YLIKSRQGQF---------LA--IGILFAAAGFIFGTMNSEYSR   36
PfeT        --MNEQVIVQRDPHEPLKTDKREKNWAQHAELIAALV--SGAL-ILAGWLLSGY-QVLSI   54
              :                . *.  :         :    *       :: .   .  .

CopA        LLATPVVLWGGWPFFKRGWQSLKTGQLNMFTLIAMGIGVAWIYSMVAVLWPGVFPHAFRS   179
LMO3448     WLFYAAIFFLG-------FYASKNAIVETVRYKSPNVDLLMILA---------------A   74
PfeT        ILFLLAFVIGG-------FAKAKEGIEETLESKTLNVELLMIFA---------------A   92
             *   ...  *       :   * .  : .   : .: :  * :              :

CopA        QEGVVAVYFEAAAVITTLVLLGQVLELKAREQTGSAIRALLKLVPESAHRIKEDGSEEEV   239
LMO3448     LGAVIFDFESEGAALLLIFAAAEVLEDYANNKSTSAISELMAQVPETAQVLKENGEVVTV   134
PfeT        IGSALIGYWAEGAILIFIFSLSGALETYTMNKSSRDLTSLMQLEPEEAT-LMVNGETKRV   151
              ..:  :   .* :  :.  . .**  : :::   :  *:   ** *  :  :*.   *

CopA        SLDNVAVGDLLRVRPGEKIPVDGEVQEGRSFVDESMVTGEPIPVAKEASAKVIGATINQT   299
LMO3448     PTEDLNVGERVVVSKGEQIPIDGIIDRKS-IVNESALTGESVPVVKEAEDEVFAGTINEG   193
PfeT        PVSDLQAGDMIVIKPGERVAADGIIESGSTSLDESALTGESMPVEKNTGDTVFTGTVNRN   211
              .:: .*: : :  **::  ** ::     ::** :*** :** *::   *: .*:*.

CopA        GSFVMKALHVGSDTMLARIVQMVSDAQRSRAPIQRLADTVSGWFVPAVIL-VAVLSFIVW   358
LMO3448     DVFYIDVTKSSDETVFSNIIRMVEEAQSRPSRISKFIDRIESKYVISVLVIVPIFIVVMY   253
PfeT        GSLTVRVTKANEDSLFRKIIKLVESAQNSVSPAQAFIERFENAYVKGVLIAVALLLFVPH   271
            . : : . : ..:::: .*:::*..**   :  . : : ... :* .*:: * :: .:

CopA        ALLGPQPALSYGLIAAVSVLIIACPCALGLATPMSIMVGVGKGAQSGVLIKNAEALERME   418
LMO3448     ALMDLP--FEEAFYRGMVFLTVASPCALVASATPATLSAISNGAKNGILFKGGAAMEALS   311
PfeT        FALGWS--WSETFYRAMVFMVVASPCALVASIMPAALSLISNGARNGMLVKGSVFLEQLG   329
              :.     .  :  .: .: :*.****  :   : :  :.:**:.*:*.*..  :* :

CopA        KVNTLVVDKTGTLTEGHPKLTRIV-TDDFVEDNALALAAALEHQSEHPLANAIVHAAKEK   477
LMO3448     TMDILYTDKTGTLTYGEFKVDEYS-----APDDVLKEVIYMEQQSSHPIARAIVTAFKET   366
PfeT        SVQMIAFDKTGTVTKGQPAVETIRIAEGFSEAEVLEAVYAIETQSSHPLAQAITAYAESR   389
            .:: :  *****:* *.  :            :.*  .  :* **.**:*.**.   :.
```

Figure S15, continued

```
CopA        GLSL-GSVEAFEAPTGKGVVGQVDGHHVAIGNARLMQEHGGDNAPLFEKADELRGKGASV   536
LMO3448     DLSSVDHNEPVSEIAGSGIKK----GTVRVGKPSAFSTF-----KNYDRFKQYFQKGNTI   417
PfeT        GVNQ-SGYISIEETSGFGVMAEVSGAKWKVGKAGFIGEEMAA-QFMKQTASDVIQSGHTI   447
            .:.  .    ..  :* *:          :*:   :           :  .:   .* ::

CopA        MFMAVDGKTVALLVVEDPIKSSTPETILELQQSGIEIVMLTGDSKRTAEAVAGTLGIKKV   596
LMO3448     ILAAKEEEVVGYFSLSDQIRRQSADAVANFQKEGIKVTLLTGDNEEVTETVAEVVGVDDY   477
PfeT        VFVKKDDQIAGCIALKDQIRPEAKEVMEELNRLGIKTAMLTGDHEDTAQAIAKEAGMTTV   507
            ::   : : .. : :.* *: .: :.: :::: **: .:**** : .::::*   *:

CopA        VAEIMPEDKSRIVSELKDKGLIVAMAGDGVNDAPALAKADIGIAMGTGTDVAIESAGVTL   656
LMO3448     KASMLPEDKIAYVRESQDKEEVVGMIGDGINDAPALANADIGIAMGSGSSVAMESSDVVV   537
PfeT        VAECLPDQKVNEIKRLKEEFGTIAMVGDGINDAPALKAADVGIAMGGGTDVALETADMVL   567
             *. :*::*   : . :::   :.* ***:******  **:***** *:.**:*::.:.:

CopA        LHGDLRGIAKARRLSESTMSNIRQNLFFAFIYNVLGVPLAAGVLYPLTGLLLSPMIAAAA   716
LMO3448     VKNDLSKLFYSYKLSKKLNKIILQNVIFSISVIVTLIVL---NLFGVLGLPLA----VLF   590
PfeT        MKNDLKKLVNMCRLSRKMNRIIKQNIVFSLAVICLLICA---NFLQAMELPFG----VIG   620
            ::.**  :    :**..    * **:.*::      :      :     * :.    .

CopA        MALSSVSVIINALRLKRVTL----------------- 736
LMO3448     HEGSTILVILNGLRLLGSKGPKQEERVSDPSLKSVKV 627
PfeT        HEGSTILVILNGLRLLK-------------------- 637
               *:: **:*.***
```

B

```
>Eba3544 predicted secondary and transmembrane structure
MTTEKKPYEM AVGITDRREA LIIRKMSLIS LIGNTVFSGF KLFAGVIGNS GAMISDAIHS FSDVLTTLIA
IIIIIIIIII IIIIIIIIII IIIIITTTTT TTTTTTTTTT TTTTTTTOO OTTTTTTTTT TTTTTTTTTT

WIGVKVSKKA ADEAHPYGHE RMECVASLLL GLVLMATGLG VGRVGVDNII ANNYEALAIP KMIALAASVV
TTTTIIIIII IIIIIIIIII IIIITTTTTT TTTTTTTTTT TTTTTTTOOO OOOOOOOOOO OTTTTTTTTT

SILGKEAMFW YTRYYAKLIN SSAFMADAWH HRSDAISSIG SFIGIAGAML GFPVMDSVAS VVICLFILKV
TTTTTTTTTT TTTTIIIIII IIIIIIIIII IIIITTTTTT TTTTTTTTTT TTTTOOTTTT TTTTTTTTTT

AYDILRDALM KMLDTSCGEA YENQLTHYIA EKEDVRSVDL LHSRMFGNKV FIDLEISVDG DKSLRDAHAV
TTTTTIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII

AELVHEDVEL NFPEIKHIMI HVNPANE
IIIIIIIIII IIIIIIIIII IIIIIII
```

I-inside
O-outside
T-transmembrane
Alpha helix
Beta sheet

Figure S16. DNA and amino acid sequences of *Bacillus subtilis* 168 *pfeT*.

```
>B. subtilis 168 BSU_13850 (pfeT, UniProtKB-O31688)
ATGAATGAAC AAGTTATCGT TCAACGCGAC CCGCATGAGC CATTGAAAAC AGACAAGAGG GAAAAAAACT
GGGCACAGCA CGCGGAGCTT ATTGCAGCAC TTGTATCAGG CGCTCTTATT TTGGCAGGCT GGCTCTTATC
CGGATATCAG GTATTATCCA TAATACTTTT CTTGCTGGCT TTTGTGATCG GCGGATTTGC TAAAGCGAAG
GAAGGAATCG AAGAAACACT CGAATCCAAA ACGCTGAATG TAGAACTTTT AATGATTTTC GCTGCCATTG
GTTCCGCCTT GATCGGATAC TGGGCAGAGG GTGCTATTTT AATATTTATT TTTTCCTTAA GCGGAGCTTT
AGAAACATAC ACGATGAATA AAAGCAGCAG AGATTTAACG TCTTTAATGC AGCTTGAGCC TGAAGAAGCG
ACATTGATGG TCAATGGTGA AACCAAAAGA GTTCCGGTTT CAGACTTGCA GGCCGGTGAT ATGATTGTGA
TCAAACCTGG AGAACGCGTA GCGGCAGACG GTATCATTGA ATCGGGCTCG ACAAGTCTTG ACGAGTCGGC
TTTAACAGGT GAATCAATGC CTGTAGAAAA AAACACAGGC GACACTGTAT TCACAGGAAC GGTGAATCGC
AATGGCTCCT TGACAGTCCG TGTCACGAAA GCAAATGAAG ATTCGTTATT CAGAAAAATT ATCAAACTGG
TTGAATCAGC GCAAAATAGT GTTTCACCCG CGCAGGCTTT CATCGAACGA TTTGAAAATG CTTATGTGAA
AGGTGTGCTA ATTGCGGTGG CGCTCCTTTT ATTCGTCCCG CACTTTGCGC TGGGCTGGAG CTGGAGTGAA
ACCTTCTACC GCGCAATGGT GTTTATGGTT GTCGCGTCAC CTTGTGCGCT TGTCGCCTCT ATTATGCCGG
CGGCGCTGTC CCTGATTTCA AATGGCGCCC GCAACGGTAT GCTTGTGAAA GGAAGCGTCT TTCTTGAACA
GCTGGGCTCA GTGCAAATGA TCGCCTTTGA TAAAACCGGA ACTGTAACAA AAGGCCAGCC TGCCGTAGAG
ACGATCAGAA TAGCAGAAGG ATTCAGTGAA GCGGAAGTTC TTGAGGCTGT CTATGCCATC GAAACGCAAT
CAAGCCATCC GCTCGCCCAA GCCATAACGG CGTACGCTGA AAGCCGCGGC GTGAATCAGT CCGGCTACAT
ATCTATAGAA GAAACCTCAG GGTTTGGCGT TATGGCAGAA GTGTCGGGTG CGAAATGGAA GGTCGGTAAA
GCAGGTTTTA TCGGCGAGGA AATGGCAGCA CAATTTATGA AACAAACAGC ATCAGATGTC ATTCAAAGCG
GTCATACAAT TGTATTTGTG AAAAAGGATG ATCAAATAGC AGGCTGTATC GCACTGAAGG ACCAAATCAG
GCCTGAAGCA AAAGAGGTCA TGGAAGAACT GAACCGACTT GGGATTAAAA CGGCCATGCT GACAGGAGAT
CACGAAGACA CGGCTCAAGC GATTGCCAAG GAAGCCGGCA TGACAACTGT CGTGGCAGAA TGCCTGCCTG
ACCAAAAAGT GAATGAAATC AAACGGTTAA AAGAAGAATT CGGAACGATT GCAATGGTGG GTGACGGAAT
CAATGATGCG CCGGCACTCA AAGCAGCGGA TGTCGGCATT GCGATGGGCG GCGGAACAGA TGTAGCACTT
GAGACCGCTG ATATGGTCCT CATGAAAAAC GATTTGAAAA AGCTCGTAAA CATGTGCCGC TTGTCTCGGA
AAATGAACAG GATCATCAAA CAAAATATCG TGTTTTCTCT AGCTGTAATC TGCCTGCTGA TTTGTGCAAA
CTTTTTGCAG GCGATGGAAT TGCCATTTGG CGTGATTGGT CATGAGGGCA GCACGATTTT AGTCATACTA
AACGGTTTAA GACTCCTAAA ATAA

>PfeT amino acid sequence
MNEQVIVQRD PHEPLKTDKR EKNWAQHAEL IAALVSGALI LAGWLLSGYQ VLSIILFLLA FVIGGFAKAK
EGIEETLESK TLNVELLMIF AAIGSALIGY WAEGAILIFI FSLSGALETY TMNKSSRDLT SLMQLEPEEA
TLMVNGETKR VPVSDLQAGD MIVIKPGERV AADGIIESGS TSLDESALTG ESMPVEKNTG DTVFTGTVNR
NGSLTVRVTK ANEDSLFRKI IKLVESAQNS VSPAQAFIER FENAYVKGVL IAVALLLFVP HFALGWSWSE
TFYRAMVFMV VASPCALVAS IMPAALSLIS NGARNGMLVK GSVFLEQLGS VQMIAFDKTG TVTKGQPAVE
TIRIAEGFSE AEVLEAVYAI ETQSSHPLAQ AITAYAESRG VNQSGYISIE ETSGFGVMAE VSGAKWKVGK
AGFIGEEMAA QFMKQTASDV IQSGHTIVFV KKDDQIAGCI ALKDQIRPEA KEVMEELNRL GIKTAMLTGD
HEDTAQAIAK EAGMTTVVAE CLPDQKVNEI KRLKEEFGTI AMVGDGINDA PALKAADVGI AMGGGTDVAL
ETADMVLMKN DLKKLVNMCR LSRKMNRIIK QNIVFSLAVI CLLICANFLQ AMELPFGVIG HEGSTILVIL
NGLRLLK
```

Figure S17. DNA and amino acid sequences of *L. monocytogenes* FSL_J1-194 LMO3448.

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>Listeria_monocytogenes_FSL_J1-194_LMBG_02759 (Lmo3448)
ATGATCAGTT ATTTAATAAA GAGTAGGCAG GGCCAGTTTT TAGCTATCGG TATTTTGTTT GCCGCAGCTG
GCTTTATATT TGGAACGATG AATAGTGAGT ACAGTCGCTG GCTTTTCTAT GCTGCTATAT TTTTCTTAGG
CTTTTATGCG TCCAAAAATG CGATCGTAGA AACAGTGCGC TACAAGTCGC CCAACGTGGA TCTACTTATG
ATCCTCGCTG CACTTGGTGC TGTGATCTTT GATTTTGAAT CAGAAGGAGC AGCCTTACTA CTAATTTTTG
CCGCAGCTGA AGTACTAGAA GATTATGCGA ACAACAAATC GACCTCAGCG ATCTCGGAAT TGATGGCTCA
GGTACCTGAA ACAGCTCAAG TCTTGAAAGA AAATGGTGAA GTGGTAACCG TTCCTACTGA AGACCTGAAT
GTAGGGGAAA GGGTCGTCGT TTCTAAAGGG GAACAGATCC CGATCGACGG AATCATTGAC CGCAAATCGA
TCGTCAATGA ATCAGCTCTC ACTGGCGAAT CGGTTCCTGT CGTGAAAGAG GCCGAAGATG AAGTGTTCGC
AGGAACGATC AATGAAGGCG ACGTTTTTTA TATCGATGTA ACCAAATCCA GCGATGAAAC AGTCTTCTCC
AATATCATTC GGATGGTCGA AGAAGCGCAG AGCCGTCCTT CACGCATCTC TAAATTCATT GACCGGATAG
AGAGCAAGTA TGTTATTTCT GTACTAGTTA TCGTGCCGAT TTTCATCGTT GTGATGTATG CATTGATGGA
TCTGCCATTC GAAGAAGCCT TTTATCGCGG TATGGTCTTT CTAACAGTCG CCAGTCCCTG TGCCTTAGTC
GCTTCGGCCA CGCCAGCAAC GTTGAGTGCG ATCAGTAATG GAGCAAAAAA CGGGATTTTA TTCAAAGGGG
GGGCTGCAAT GGAAGCTTTG AGCACGATGG ATATCTTATA CACAGACAAG ACAGGCACGC TGACTTATGG
TGAATTCAAG GTCGATGAAT ACAGTGCTCC CGATGATGTA TTGAAAGAAG TGATCTATAT GGAACAGCAG
TCGAGTCATC CGATCGCTCG AGCTATCGTG ACGGCGTTTA AAGAGACGGA TCTGAGTTCA GTCGATCATA
ATGAACCTGT TAGTGAAATA GCCGGTTCCG GTATCAAGAA AGGCACAGTA AGGGTGGGAA AACCTTCAGC
CTTCAGTACA TTCAAAAACT ACGATCGCTT CAAGCAGTAC TTCCAAAAAG GCAACACGAT CATTCTCGCA
GCAAAAGAAG AGGAGGTCGT TGGTTACTTT TCACTCAGTG ACCAGATTCG CAGACAGTCG GCAGATGCCG
TGGCTAACTT CCAGAAAGAA GGCATTAAAG TCACGTTATT AACGGGTGAT AACGAAGAAG TGACAGAGAC
GGTAGCAGAG GTCGTCGGTG TCGATGACTA TAAAGCGTCC ATGTTGCCGG AAGACAAGAT CGCCTATGTC
AGGGAGAGTC AGGATAAGGA AGAAGTGGTC GGCATGATCG GTGACGGGAT CAATGATGCG CCAGCTCTGG
CAAATGCAGA TATCGGGATC GCGATGGGCA GCGGTTCCTC TGTGGCGATG GAATCATCCG ATGTCGTTGT
CGTTAAAAAT GACTTGTCGA AACTGTTCTA CAGTTACAAG TTGAGTAAGA AACTGAACAA AATCATTTTG
CAGAATGTGA TTTTCTCGAT CAGCGTGATC GTGACCTTGA TCGTCTTGAA CCTCTTTGGC GTATTAGGAC
TTCCACTAGC GGTATTATTC CATGAAGGGT CAACAATACT CGTTATTCTT AATGGTCTCC GTCTACTTGG
ATCTAAAGGT CCGAAGCAAG AAGAGAGAGT CTCGGATCCA TCTTTAAAGT CTGTAAAGGT TTAA

>LMO3448 amino_acid_sequence
MISYLIKSRQ GQFLAIGILF AAAGFIFGTM NSEYSRWLFY AAIFFLGFYA SKNAIVETVR YKSPNVDLLM
ILAALGAVIF DFESEGAALL LIFAAAEVLE DYANNKSTSA ISELMAQVPE TAQVLKENGE VVTVPTEDLN
VGERVVVSKG EQIPIDGIID RKSIVNESAL TGESVPVVKE AEDEVFAGTI NEGDVFYIDV TKSSDETVFS
NIIRMVEEAQ SRPSRISKFI DRIESKYVIS VLVIVPIFIV VMYALMDLPF EEAFYRGMVF LTVASPCALV
ASATPATLSA ISNGAKNGIL FKGGAAMEAL STMDILYTDK TGTLTYGEFK VDEYSAPDDV LKEVIYMEQQ
SSHPIARAIV TAFKETDLSS VDHNEPVSEI AGSGIKKGTV RVGKPSAFST FKNYDRFKQY FQKGNTIILA
AKEEEVVGYF SLSDQIRRQS ADAVANFQKE GIKVTLLTGD NEEVTETVAE VVGVDDYKAS MLPEDKIAYV
RESQDKEEVV GMIGDGINDA PALANADIGI AMGSGSSVAM ESSDVVVVKN DLSKLFYSYK LSKKLNKIIL
QNVIFSISVI VTLIVLNLFG VLGLPLAVLF HEGSTILVIL NGLRLLGSKG PKQEERVSDP SLKSVKV
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Figure S18. DNA and amino acid sequences of *Erysipelotrichaceae_bacterium*_3_1_53 Eba3544.

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>Erysipelotrichaceae_bacterium_3_1_53_HMPREF0983_03544 (UniProtKB E2SQM5)
ATGACAACGG AAAAGAAGCC ATATGAAATG GCAGTTGGTA TCACAGACAG AAGGGAAGCA TTGATTATAC
GAAAAATGTC GTTGATTAGC CTGATTGGTA ATACGGTATT CTCCGGCTTT AAGCTGTTTG CCGGTGTTAT
CGGTAATTCC GGTGCAATGA TTTCCGATGC TATCCATTCT TTTTCAGATG TATTAACTAC CTTGATTGCC
TGGATCGGTG TCAAGGTTTC CAAAAAAGCT GCGGATGAGG CACATCCTTA CGGACATGAA CGTATGGAAT
GCGTCGCTTC TCTGCTTTTA GGTCTTGTGC TTATGGCAAC AGGTCTGGGA GTTGGCAGAG TTGGAGTAGA
TAACATTATA GCAAATAACT ATGAGGCTCT TGCAATACCC AAAATGATTG CCCTTGCTGC GTCAGTTGTT
TCCATCCTTG GAAAGGAAGC TATGTTCTGG TATACAAGGT ATTATGCAAA GCTGATAAAT TCCTCTGCCT
TCATGGCGGA TGCGTGGCAC CATCGCTCCG ATGCGATTTC CTCCATCGGC TCTTTCATAG GCATCGCCGG
CGCCATGCTT GGCTTCCCGG TTATGGATTC TGTGGCAAGC GTAGTGATTT GTCTGTTTAT TTTAAAGGTT
GCCTATGATA TCTTAAGGGA CGCATTGATG AAAATGCTGG ATACCTCATG CGGTGAAGCT TATGAGAATC
AGTTAACTCA CTATATAGCT GAGAAAGAGG ATGTACGTTC TGTAGATTTA CTTCATTCCC GAATGTTTGG
CAACAAGGTC TTTATTGATT TGGAAATTTC GGTAGACGGC GATAAATCAT TGCGTGATGC ACATGCGGTT
GCTGAGCTTG TACATGAGGA TGTTGAACTT AATTTCCCAG AGATAAAGCA CATCATGATT CATGTTAATC
CGGCAAATGA ATAA

>Eba3544 amino acid sequence
MTTEKKPYEM AVGITDRREA LIIRKMSLIS LIGNTVFSGF KLFAGVIGNS GAMISDAIHS FSDVLTTLIA
WIGVKVSKKA ADEAHPYGHE RMECVASLLL GLVLMATGLG VGRVGVDNII ANNYEALAIP KMIALAASVV
SILGKEAMFW YTRYYAKLIN SSAFMADAWH HRSDAISSIG SFIGIAGAML GFPVMDSVAS VVICLFILKV
AYDILRDALM KMLDTSCGEA YENQLTHYIA EKEDVRSVDL LHSRMFGNKV FIDLEISVDG DKSLRDAHAV
AELVHEDVEL NFPEIKHIMI HVNPANE
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Figure S19. Codon-optimized gBlocks for cloning of *pfeT*, Eba3544, and LMO3448 into pHT01. The sfGFP sequence inserted into pHT01 is also shown. Restriction sites are underlined and Gibson ends are in red. The LMO3448 gBlock also contains a TEV-cleavable His_6 tag (ENLYFQGLEHHHHHH, bolded).

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>pfeT gBlock
CAATTAAAGGAGGAAGGATCCATGAACGAACAGGTTATCGTACAACGCGACCCACATGAACCACTGAAGACCGATAAACGCG
AGAAGAATTGGGCTCAGCACGCCGAACTTATTGCAGCCCTGGTTTCTGGGGCTTTGATTTTAGCGGGCTGGTTACTTTCTGG
GTATCAGGTTTTGAGCATTATCTTATTTCTTTTAGCTTTTGTTATTGGTGGGTTTGCGAAAGCGAAAGAGGGCATTGAAGAG
ACATTGGAATCCAAAACTTTGAACGTCGAATTATTGATGATCTTTGCCGCGATCGGAAGCGCTCTTATCGGATATTGGGCAG
AGGGGGCAATTTTAATTTTTATCTTCTCTCTTTCGGGTGCACTGGAGACATACACTATGAACAAATCCTCCCGTGACCTTAC
AAGCCTTATGCAATTGGAACCCGAAGAAGCGACCTTGATGGTGAACGGAGAAACGAAACGCGTCCCGGTAAGCGACTTGCAG
GCAGGGGACATGATCGTGATTAAGCCAGGGGAACGTGTGGCTGCTGACGGAATTATCGAATCAGGATCGACTTCACTGGACG
AATCCGCGCTTACAGGCGAATCGATGCCTGTTGAGAAGAACACGGGGGACACGGTCTTTACTGGGACTGTGAATCGTAACGG
ATCCTTGACCGTTCGCGTTACCAAGGCAAACGAAGACTCCTTGTTTCGCAAAATCATTAAGCTGGTAGAATCGGCACAAAAC
TCGGTTAGCCCGGCGCAAGCATTTATTGAACGCTTTGAAAACGCATATGTCAAAGGGGTCCTTATCGCAGTAGCATTACTTT
TGTTTGTGCCACACTTCGCCCTTGGTTGGTCCTGGAGCGAAACGTTTTACCGCGCAATGGTTTTTATGGTAGTGGCTTCGCC
GTGCGCTTTAGTCGCTTCTATCATGCCCGCAGCCTTGTCCTTAATTTCTAATGGCGCTCGCAATGGAATGTTGGTAAAAGGT
TCGGTTTTTTTGGAGCAGCTGGGTTCAGTGCAGATGATTGCCTTTGATAAGACTGGGACGGTAACAAAGGGGCAACCCGCTG
TCGAGACAATCCGTATCGCCGAGGGGTTTAGCGAGGCCGAGGTGCTTGAGGCAGTGTACGCTATTGAAACACAATCCTCTCA
CCCTTTAGCCCAAGCGATTACGGCATATGCCGAGAGTCGTGGAGTGAATCAAAGCGGTTACATCTCCATTGAAGAGACCAGC
GGCTTTGGCGTAATGGCTGAGGTTTCAGGCGCCAAGTGGAAAGTTGGAAAGGCGGGTTTCATTGGTGAGGAAATGGCAGCGC
AATTTATGAAACAAACAGCGTCGGATGTTATTCAATCTGGCCATACAATCGTTTTCGTTAAAAAAGATGATCAGATTGCCGG
GTGTATCGCTCTGAAAGACCAGATTCGTCCGGAGGCAAAGGAAGTTATGGAAGAACTTAATCGTTTAGGGATCAAAACCGCG
ATGCTTACAGGAGATCACGAAGATACGGCCCAGGCAATTGCTAAAGAGGCAGGAATGACGACCGTGGTTGCCGAATGTTTAC
CCGATCAAAAAGTCAACGAGATCAAACGCTTAAAGGAAGAGTTCGGCACTATCGCAATGGTAGGGGACGGCATTAATGACGC
TCCTGCATTGAAGGCAGCGGATGTGGGTATTGCAATGGGTGGTGGGACAGATGTGGCATTGGAGACCGCAGACATGGTATTG
ATGAAGAATGATCTGAAAAAGTTAGTCAACATGTGCCGCTTAAGTCGTAAAATGAATCGCATCATCAAGCAAAATATCGTGT
TTTCGCTGGCTGTAATCTGCCTGTTAATCTGCGCAAACTTCCTTCAGGCCATGGAATTGCCTTTTGGTGTTATCGGACACGA
AGGCAGCACAATTTTAGTGATTCTGAATGGTCTTCGCTTGCTGAAATGATCTAGAGTCGACGTCC

>LMO3448 gBlock
aactttaagaaggagatataCATATGATTAGTTACCTTATTAAATCTCGCCAGGGTCAATTCTTGGCAATCGGAATCCTGTT
TGCGGCGGCGGGTTTTATTTTTGGTACCATGAACTCGGAATACTCACGCTGGCTTTTCTACGCGGCAATTTTTTTCTTAGGT
TTTTATGCATCCAAGAACGCCATTGTAGAAACGGTTCGCTATAAGAGTCCTAATGTGGACCTTCTGATGATTCTTGCCGCAC
TGGGTGCAGTAATCTTCGACTTTGAAAGTGAAGGAGCGGCATTGTTACTGATTTTCGCGGCAGCGGAAGTGTTGGAAGACTA
CGCTAACAACAAATCGACTTCAGCAATTTCGGAATTAATGGCGCAAGTGCCGGAAACCGCTCAGGTCCTGAAGGAAAACGGG
GAGGTTGTTACGGTTCCCACCGAAGATCTGAATGTTGGAGAACGTGTGGTAGTAAGCAAAGGAGAGCAAATCCCTATTGACG
GAATTATCGACCGTAAAAGTATCGTTAACGAGAGCGCCTTGACAGGTGAGTCGGTACCTGTCGTGAAGGAAGCGGAAGACGA
AGTTTTTGCAGGGACCATTAACGAAGGTGACGTGTTTTATATCGACGTGACAAAGAGTTCCGACGAAACCGTCTTTTCCAAC
ATCATTCGTATGGTAGAAGAAGCCCAGTCACGCCCCTCACGCATCAGCAAATTTATCGATCGCATTGAGTCAAAATATGTAA
TCTCGGTTCTTGTCATTGTACCCATCTTCATCGTTGTGATGTATGCATTGATGGACTTACCCTTCGAGGAGGCATTCTACCG
TGGCATGGTGTTTTTAACGGTCGCGTCTCCATGTGCTTTAGTTGCGTCAGCCACACCAGCAACACTTAGCGCAATCTCGAAC
GGGGCCAAGAACGGAATTCTTTTCAAGGGAGGAGCGGCTATGGAAGCACTTTCGACCATGGACATCTTGTATACAGATAAAA
CCGGCACATTAACTTATGGCGAGTTCAAAGTTGACGAATATAGTGCCCCCGACGACGTATTAAAGGAAGTGATTTACATGGA
GCAGCAATCAAGTCATCCGATCGCCCGTGCGATCGTAACTGCCTTTAAGGAGACGGACTTGTCCAGCGTCGATCATAATGAG
CCTGTGAGTGAAATTGCTGGATCTGGCATCAAGAAAGGCACTGTTCGTGTCGGGAAGCCGAGCGCCTTCAGTACATTCAAGA
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CTCGTTAAGCGATCAGATTCGCCGTCAGTCCGCGGATGCAGTGGCTAACTTCCAAAAGGAAGGAATCAAGGTAACATTATTG
ACAGGTGACAACGAAGAGGTAACCGAAACGGTAGCCGAAGTCGTAGGCGTAGACGACTACAAGGCATCAATGCTTCCAGAGG
ATAAAATTGCGTATGTGCGCGAATCGCAGGACAAGGAGGAGGTGGTAGGGATGATTGGAGACGGGATCAACGATGCCCCTGC
TCTGGCTAATGCCGACATCGGAATCGCAATGGGATCAGGATCAAGTGTGGCGATGGAGAGCTCTGACGTCGTCGTTGTGAAG
AACGACCTGAGCAAGTTGTTCTACTCATACAAATTAAGCAAAAAATTAAATAAAATTATCTTACAGAATGTGATTTTCTCGA
TTTCAGTTATTGTGACTCTTATTGTTTTGAATTTGTTCGGCGTTCTGGGTTTGCCTTTGGCTGTCTTGTTCCACGAAGGCAG
TACCATCCTTGTCATTTTAAATGGACTTCGCCTGTTGGGTTCTAAGGGACCCAAACAGGAGGAGCGCGTTTCAGATCCCTCA
TTAAAGAGTGTTAAGGTTGAAAACCTGTATTTTCAGGGCctcgagcaccaccaccacca
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Figure S19, continued

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>Eba3544 gBlock
CCCATGACAACTGAAAAAAAACCATATGAAATGGCGGTGGGCATTACTGACCGCCGCGAAGCGTTAATTATCCGCAAGATGA
GCTTGATTTCCTTGATTGGTAATACGGTGTTCAGCGGGTTCAAGCTGTTTGCCGGAGTCATCGGGAACTCAGGAGCAATGAT
TAGTGATGCTATCCACAGTTTTTCCGATGTTTTGACTACATTGATCGCATGGATTGGTGTTAAAGTGAGCAAAAAGGCTGCT
GATGAAGCACATCCCTACGGGCATGAACGCATGGAATGCGTTGCGAGCCTTCTTCTGGGGCTGGTGTTGATGGCAACCGGTC
TGGGTGTTGGACGCGTGGGAGTTGATAACATCATCGCTAATAATTACGAGGCTCTTGCGATTCCTAAGATGATCGCCCTTGC
TGCTTCCGTTGTCTCTATTCTTGGAAAGGAAGCGATGTTCTGGTACACTCGCTATTATGCTAAGCTGATTAACAGTAGCGCC
TTCATGGCGGATGCATGGCACCATCGTAGCGATGCTATCAGCTCCATTGGCTCCTTTATCGGCATCGCGGGCGCCATGCTTG
GTTTCCCCGTAATGGACAGCGTAGCTAGTGTTGTTATCTGTTTATTTATCCTTAAAGTCGCTTACGACATTTTGCGTGACGC
GCTGATGAAAATGCTGGATACTAGTTGTGGTGAAGCCTATGAAAACCAATTGACGCATTACATCGCAGAGAAAGAAGACGTG
CGTTCCGTAGATTTACTGCATTCTCGTATGTTCGGCAACAAAGTATTTATTGACCTTGAAATCTCAGTTGATGGCGATAAGT
CATTACGTGACGCCCATGCGGTCGCAGAGCTTGTCCATGAAGATGTTGAATTGAACTTCCCAGAGATTAAACACATTATGAT
CCATGTCAACCCGGCGAACGAGTAA

>sfGFP from pET21b-RL015A
ATGCGTAAAGGCGAAGAACTGTTCACGGGCGTAGTTCCGATTCTGGTCGAGCTGGACGGCGATGTGAACGGTCATAAGTTTA
GCGTTCGCGGTGAAGGTGAGGGCGACGCGACCAACGGCAAACTGACCCTGAAGTTCATCTGCACCACCGGTAAACTGCCGGT
GCCTTGGCCGACCTTGGTGACGACGTTGACGTATGGCGTGCAGTGTTTTGCGCGTTATCCGGACCACATGAAACAACACGAT
TTCTTCAAATCTGCGATGCCGGAGGGTTACGTCCAGGAGCGTACCATTTCCTTCAAGGATGATGGCTTCTACAAAACTCGCG
CAGAGGTTAAGTTTGAAGGTGACACGCTGGTCAATCGTATCGAATTGAAGGGTATCGACTTTAAAGAGGATGGTAACATTCT
GGGCCATAAACTGGAGTATAACTTCAACAGCCATAATGTTTACATTACGGCAGACAAGCAAAAGAACGGCATCAAGGCCAAT
TTCAAGATTCGCCACAATGTTGAGGACGGTAGCGTCCAACTGGCCGACCATTACCAGCAGAACACCCCAATTGGTGACGGTC
CGGTTTTGCTGCCGGATAATCACTATCTGAGCACCCAAAGCGTGCTGAGCAAAGATCCGAACGAAAAACGTGATCACATGGT
CCTGCTGGAATTTGTGACCGCTGCGGGCATCACCCACGGTATGGACGAGCTGTATAAGTAA
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