

SI Appendix

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Strain and Plasmid Construction. All strains are derivatives of a laboratory stock of *E. coli* K-12 MG1655 and are listed in SI Dataset S1. Deletion and tagged strains were generated by λ -Red-mediated recombineering (1-3) using NM400 and the oligonucleotides listed in SI Dataset S3. The chromosomal P_{lac} -5'UTR_{*mgtA*}-*lacZ* fusion carrying the first 126 nt of the *mgtA* mRNA fused to the seventh codon of the *lacZ* coding sequence in PM1205 (4), was created using a P_{lac} -*mntP* 5'UTR-*lacZ* (5) strain and MG1655 as templates for overlapping PCR. The PCR product was integrated into the chromosome of PM1205 strain replacing the P_{BAD} promoter. Mutations and tags were moved between strains linked to antibiotic markers by P1 transduction. When necessary, kanamycin resistance cassettes were excised from the chromosome by FLP-mediated recombination (6). All mutations and fusions were confirmed by sequencing.

Growth Conditions. Cells were standardly grown overnight in LB medium (10 g of tryptone, 5 g of yeast extract, 10 g of NaCl per liter). All cultures were maintained at 37°C. The effects of high and low Mg²⁺ levels were assayed in N-minimal medium (pH 7.4) (7) containing 0.1% casamino acids and 0.4% glycerol with indicated concentrations of MgSO₄. Antibiotics were used at the following concentrations: kanamycin, 30 μ g/mL; chloramphenicol, 25 μ g/mL; ampicillin, 100 μ g/mL; tetracycline, 12.5 μ g/mL.

Northern Analysis. Cells (5 mL) were collected by centrifugation and flash frozen in liquid nitrogen. Subsequently, cell pellets were rapidly thawed at 37°C, resuspended in 100 μ L of 10

mM TE, pH 8.0 containing 30 mg/mL lysozyme and placed at 37°C for 10 min. After two more cycles of flash freezing in liquid nitrogen and thawing at 37°C, 1 mL of Trizol reagent (Ambion) was added to each sample, and cells were further lysed by repetitive pipetting and then incubated at room temperature for 5 min. After the addition of 0.2 mL of chloroform and further mixing, the top 0.6 mL of the aqueous phase was transferred to a new tube, and extracted once more with Trizol-chloroform. Total RNA was precipitated with 0.5 mL of isopropyl alcohol and washed with 75% ethanol before resuspension in 25 μ L of deionized water. For Northern blot analysis, 20 μ g of total RNA was separated on an 8% denaturing urea PAGE gel at 300 V for 1.5 h. The RNA was then transferred to Zeta-probe nylon membranes (BioRad) by electro-blotting for 18 h at 20 V at 4°C. Membranes were UV cross-linked and then incubated in hybridization buffer for 5 h at 45°C prior to the addition of a P³²-labeled oligonucleotide probe. Blots were incubated overnight and then washed 5X in SSC buffer (Corning) and exposed to X-ray film.

Immunoblot Analysis. Cells were mixed with SDS loading buffer [50 mM Tris 6.8, 2% (w/v) sodium dodecyl sulfate (SDS), 0.1% (w/v) bromophenol blue, 10% (v/v) glycerol, 100 mM dithiothreitol (DTT)] and then subjected to SDS/PAGE on 10–20% Tris-glycine or Any kD Mini-PROTEAN TGX gels (BioRad) and electro-blotted to a 0.2 μ m pore size nitrocellulose membrane (Novex). Membranes were blocked by incubation in phosphate buffered saline with Tween 20 (PBS-T) containing 5% (wt/vol) milk. To detect SPA-tagged or FLAG-tagged MgtS, membranes were incubated with a 1:1,000 dilution of monoclonal α -FLAG M2-horseradish peroxidase (HRP) antibody (Sigma) for ~1 h. To detect His₆-tagged proteins, the membranes were incubated with a 1:2,000 dilution of Peta His HRP conjugate (Qiagen) for 2 h. To detect MgtA-HA, the membranes were incubated with 1:2000 Anti-HA tag antibody (CHIP grade,

Abcam) for 4 h, and then incubated with a 1:5,000 dilution of Amersham ECL Rabbit IgG, HRP-linked whole Ab from donkey (GE Health). SuperSignal West Pico Chemiluminescent Substrate (Pierce) was used for visualization of all the aforementioned HRP-conjugated antibodies.

β -galactosidase Assays. Overnight cultures were diluted 1:100 into N medium with 500 μ M Mg^{2+} and grown to $OD_{600} \sim 0.4-0.5$. Two aliquots (3 mL) of cells from each culture were collected and then washed 2X in either N medium with 500 μ M Mg^{2+} or without added Mg^{2+} . After the addition of IPTG (1 mM) and arabinose (0.2%) for pBAD24-containing strains, cells were grown 30 min, and then β -galactosidase activity was assayed for 100 μ L as described (8).

FLAG-tagged Protein Purification. Overnight cultures were diluted 1:100 into 1 L of N medium with 15 μ M Mg^{2+} and grown to $OD_{600} \sim 0.4-0.6$. After arabinose (0.2%) was added to induce MgtS expression, cells were grown for another 4 h. Cells were collected, washed in 20 mL of buffer containing 10 mM Tris (pH 7.5) and 20% glycerol, collected a second time ($3,700 \times g$, 20 min) and resuspended in Basal buffer [10 mM Tris (pH 7.5), 100 mM NaCl, 10% glycerol] containing Protease inhibitor cocktail (Roche) and 500 μ M $MgSO_4$. The volume was adjusted with Basal buffer so that the OD_{600} of the sample was 50. The cells were lysed using a microfluidizer processor (Microfluidics) at 30,000 psi. The lysed cells were incubated with 50 mM dodecyl β -D-maltoside (DDM) in 4°C for 2.5 hours, and then the cell debris was removed by centrifugation ($20,000 \times g$, 20 min). The cleared lysate was incubated with 300 μ L of α -FLAG M2-agarose beads from mouse (Sigma) for 6 h at 4°C. The lysate and beads were applied to a Bio-Spin disposable chromatography column (Bio-Rad Laboratories) and allowed to drain by gravity. The columns were washed 3X with 10 mL of Basal buffer supplemented with 2 mM

DDM. The proteins were eluted with 1 mL Acidic Elution buffer (100 mM Glycine pH 2.3, 100 mM NaCl, 0.1% Triton-X). Eluate (1 mL) was mixed with 250 μ L trichloroacetic acid (TCA) and incubated on ice for 1 h. The protein was pelleted by centrifugation at $20,000 \times g$ for 30 min at 4°C . The pellet was washed 3X with 300 μ L of cold acetone ($20,080 \times g$, 4°C , 10 min) and dried in a vacuum evaporator or heat block at 95°C for less than 5 min. Subsequently, 50 μ L of SDS loading buffer was added to each pellet. The samples were heated at 95°C for 15 min, and aliquots were subjected to SDS/PAGE on Any kD Mini-PROTEAN TGX gel. Protein were visualized with Coomassie blue staining. Bands of interest were excised from the gel and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

His₆-tagged Protein Purification. Overnight cultures of strains overexpressing either MgtS-GG-FLAG or MgtS-D30A-GG-FLAG were diluted 1:100 into LB medium. At $\text{OD}_{600} \sim 0.4\text{--}0.6$, arabinose (0.2%) was added and cells were grown for another 1 h. Overnight cultures of strains overexpressing either MgtA-His₆ or AcrB-His₆ were diluted 1:100 into 1 L of N medium supplemented with 500 μ M MgSO₄. At $\text{OD}_{600} \sim 0.4\text{--}0.6$, cells were collected, washed 2X in N medium without added Mg²⁺, resuspended in N medium without added Mg²⁺ with arabinose (0.2%) and grown for another 2 h. Cells from each culture collected by centrifugation ($4,650 \times g$, 20 min) were washed in 20 mL of buffer containing 10 mM Tris (pH 7.5) and 20% glycerol, collected a second time ($3,700 \times g$, 20 min) and resuspended in Basal buffer containing Protease Inhibitor Cocktail (Roche) such that the OD_{600} of the sample was 50. Cells from the FLAG-tagged protein cultures were mixed with the His₆-tagged protein cultures at a 3:1 ratio. To ensure thorough mixing, cells were shaken gently at 4°C for 15 min. The cells were then homogenized as for the FLAG-tagged protein purification and then incubated with 50 mM DDM in 4°C for 3

h. The insoluble cellular debris was removed by centrifugation ($20,000 \times g$, 20 min), and the supernatant was applied to 600 μL Ni^{2+} -NTA agarose resin (Qiagen) in a Bio-Spin disposable chromatography column (Bio-Rad Laboratories) and allowed to drain by gravity. The columns were washed with 25 mL of Basal buffer supplemented with 2 mM DDM and 20 mM imidazole, and washed beads (50 μL) were mixed with 2X SDS loading buffer (50 μL) and heated at 95°C for 10 min. Eluted samples (15 μL) were analyzed on immunoblots using anti-His or M2 anti-FLAG antibodies.

HA-tagged Protein Purification. Overnight cultures were diluted 1:100 into 1 L of N medium supplemented with 500 μM MgSO_4 . At $\text{OD}_{600} \sim 0.4\text{--}0.6$, cells were collected, washed 2X in N medium without added Mg^{2+} , resuspended in N medium without added Mg^{2+} and grown for another 2.5 h. A small aliquot (100 μL) of cells was taken before lysis for immunoblot analysis (7.5 μL loaded). Cells from each culture were collected by centrifugation as for the His-tagged protein purification. The cells were homogenized as for the FLAG-tagged protein purification and then incubated with 50 mM DDM in 4°C for 3 h. The insoluble cellular debris was removed by centrifugation ($20,000 \times g$, 20 min), and the supernatant was applied to 300 μL Pierce Anti-HA agarose (Thermo Scientific) in a Bio-Spin disposable chromatography column and allowed to drain by gravity. The columns were washed with 25 mL of Basal buffer supplemented with 2 mM DDM. Washed beads (50 μL) were mixed with 50 μL of 2X SDS loading buffer and heated at 95°C for 10 min. Samples (15 μL) eluted from beads were analyzed on immunoblots using anti-His or M2 anti-FLAG antibodies.

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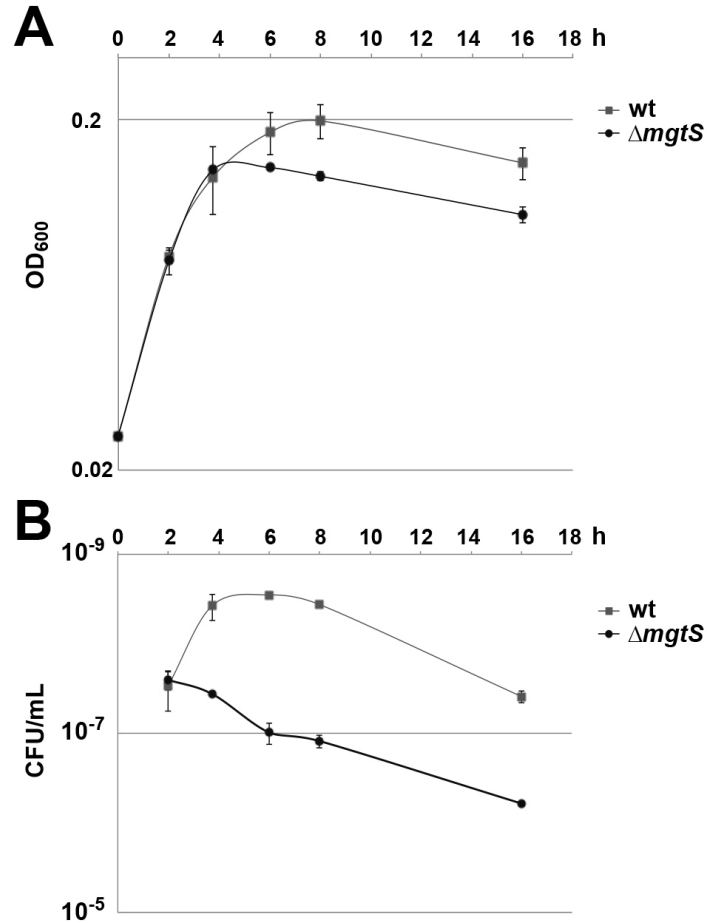


Fig. S1. Decrease in OD₆₀₀ correlates with decrease in number of colony forming units. Cells from overnight cultures of wild type (MG1655) and $\Delta mgtS$ (GSO775) strains were washed 1X with Mg²⁺ free N-minimal medium and diluted to an OD₆₀₀ = 0.025 in N-minimal medium containing 7.5 μ M Mg²⁺. (A) OD₆₀₀ of the cultures was measured and (B) aliquots were removed for CFU/mL enumeration at the indicated times. The average OD₆₀₀ and CFU/mL for three cultures are shown, and the error bars represent 1 SD.

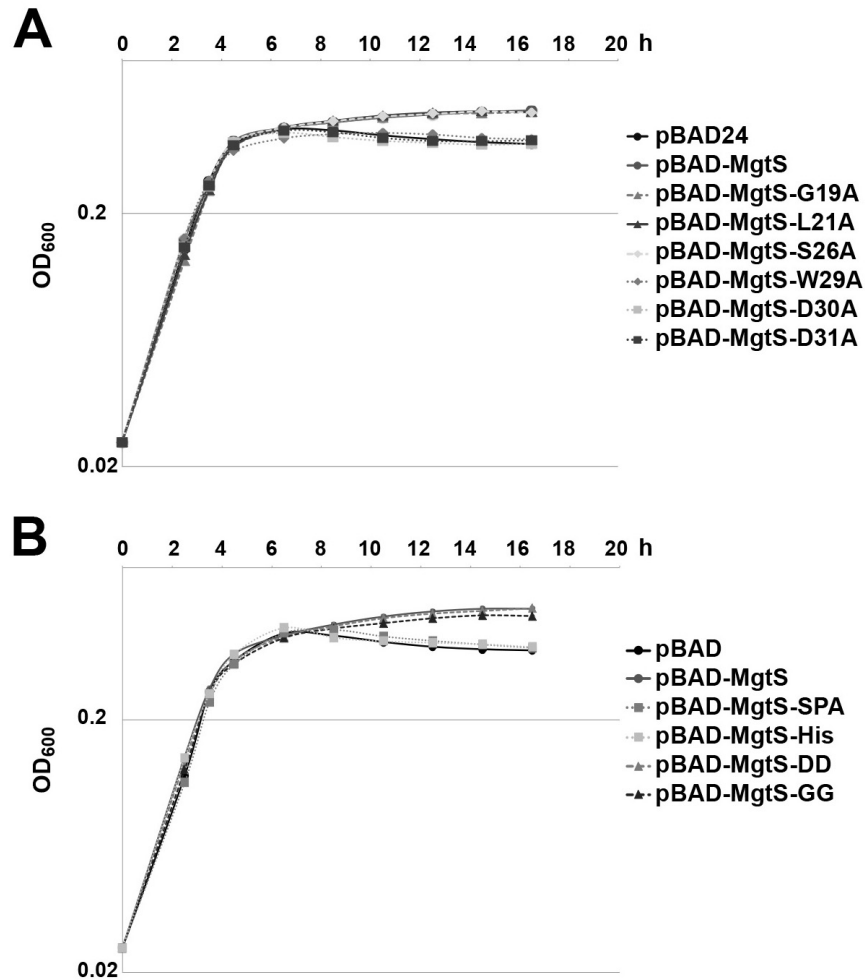


Fig. S2. Mutant derivatives of MgtS vary in ability to complement $\Delta mgtS$. (A) W29A, D30A and D31A derivatives of MgtS are unable to suppress growth defect of $\Delta mgtA$ strain in low Mg^{2+} . Cells from overnight cultures of $\Delta mgtS$ (GSO775) carrying either the pBAD24 vector, pBAD-MgtS or indicated mutants were washed 1X with Mg^{2+} free N-minimal medium and diluted to an $OD_{600} = 0.025$ in N-minimal medium containing $7.5 \mu M Mg^{2+}$. (B) While SPA and His-tagged (Gly_6 -His₆) derivatives of MgtS are unable to suppress growth defect of $\Delta mgtA$ strain in low Mg^{2+} , MgtS derivatives with DD or GG added to the C-terminus are functional. Cells from overnight cultures of $\Delta mgtS$ (GSO775) carrying either the pBAD24 vector, pBAD-MgtS or

indicated tagged derivatives were washed 1X with Mg^{2+} free N-minimal medium and diluted to an $\text{OD}_{600} = 0.025$ in N-minimal medium containing $7.5 \mu\text{M Mg}^{2+}$. For both (*A*) and (*B*), the OD_{600} of the cultures was measured at the indicated times. The average of two cultures is shown.

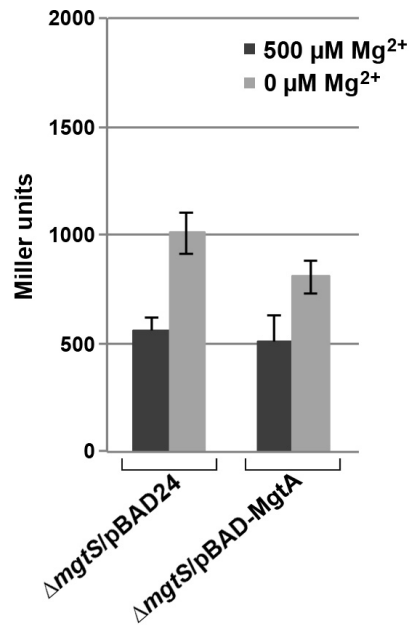


Fig. S3. MgtA overexpression can partially suppress ΔmgtS . Cells from overnight cultures of ΔmgtS (GSO775) cells carrying the chromosomal $P_{lac}\text{-}5'\text{UTR}_{\text{mgtA}}\text{-lacZ}$ fusion and the indicated plasmids were diluted 1:100 into N medium with 500 $\mu\text{M Mg}^{2+}$. Upon reaching $\text{OD}_{600} \sim 0.4\text{-}0.6$, cultures were split; one half was washed 2X with N medium with 500 $\mu\text{M Mg}^{2+}$ and the other half was washed 2X with N medium without added Mg^{2+} . After the addition of IPTG (1 mM final concentration) to induce the P_{lac} promoter, cells were allowed to grow 30 min whereupon cells were collected for β -galactosidase assays. The average of three independent assays is shown, and the error bars represent 1 SD.

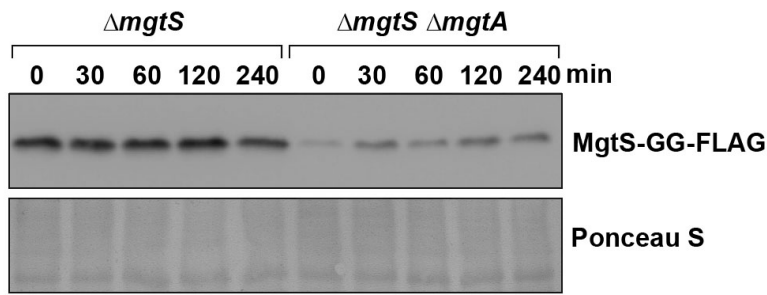


Fig. S4. MgtS is reciprocally stabilized by MgtA. Overnight cultures of $\Delta mgtS$ (GSO775) and $\Delta mgtS \Delta mgtA$ (GSO781) cells carrying pBAD24-MgtS-GG-FLAG were diluted 1:100 into N medium with 1 mM Mg^{2+} . Upon reaching $OD_{600} \sim 0.4-0.6$, the remaining cells were washed 2X with N medium without added Mg^{2+} , resuspended in the same volume of N medium without added Mg^{2+} , and arabinose (0.2%) was added to induce the P_{BAD} promoter. After 60 min in N medium with 0.2% arabinose but lacking Mg^{2+} , the cultures were washed 2X with N medium lacking Mg^{2+} without arabinose and then incubated in the same medium. Aliquots taken at the indicated time points after the wash were pelleted, resuspended to $OD_{600} \sim 50$ in SDS loading buffer, and separated by SDS-PAGE for immunoblot analysis using anti-FLAG antibodies.