Fructose-1,6-bisphosphate aldolase (class II) is the primary site of nickel toxicity in *Escherichia coli*

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Supplemental Data

Table S1. Primers used

Plasmid	Primer sequence
pLEM1	Forward: 5'-ATATCGAATTCTTAACAGAGTTAAGGAGGTGAAGCTATGTCT-AAGATTTTTGATTTCG-3'
	Reverse: 5'-AATCAAGAGCTCTTACAGAACGTCGATCGCGTTCAGTTCCTG-3'
pLEM5	Forward: 5'-AGTATCGGATCCGATGTCTAAGATTTTTGATTTCG-3'
	Reverse: 5'-AGTATCTCGAGATTACAGAACGTCGATCGCGTTCAG-3'
pLEM6	Forward: 5'-CTCTTCTCACATGATCGCGCTGTCTGAAGAATCTC-3''
	Reverse: 5'-GAGATTCTTCAGACAGCGCGATCATGTGAGAAGAG-3'
pLEM7	Forward: 5'-CATGACTCTGGAAATCGCGCTGGGTTGCACCGGTG-3'
	Reverse: 5'-CACCGGTGCAACCCAGCGCGATTTCCAGAGTCAT-3'
pLEM8	Forward: 5'-GAAATCGAACTGGGTGCGACCGGTGGTGAAGAA-3'
	Reverse: 5'-CTTCTTCACCACCGGTCGCACCCAGTTCGATTTC-3'
pLEM9	Forward: 5'-TGGGTTGCACCGGTGGTGCGGAAGACGGCGTGGACAA-3'
	Reverse: 5'-TTGTCCACGCCGTCTTCCGCACCACCGGTGCAACCCA-3'

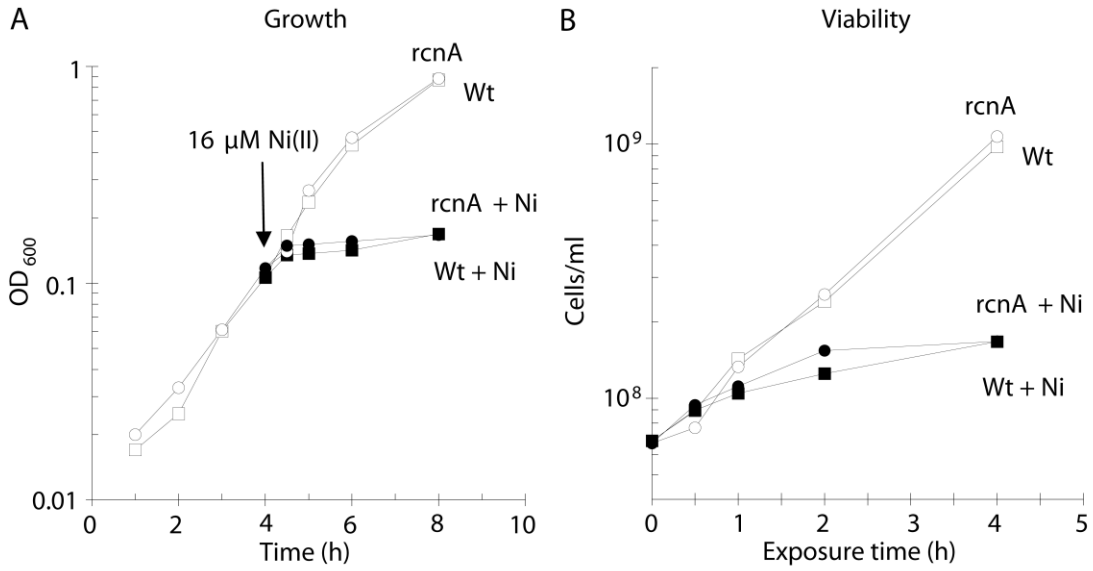

Fig. S1. Nickel toxicity does not decrease cell viability. (*A*) Wild-type cells (MG1655) and *rcnA* mutant cells (LEM201) were grown aerobically in M9 glucose medium at 37 °C. At an OD_{600} of ~0.1 cells were challenged with 0 µM (open symbols) or 16 µM (closed symbols) Ni(II). (*B*) At each nickel exposure time, cell viability was determined by dilution into LB medium, plating onto LB, and incubation aerobically at 37 °C. The data are representative of three independent experiments.

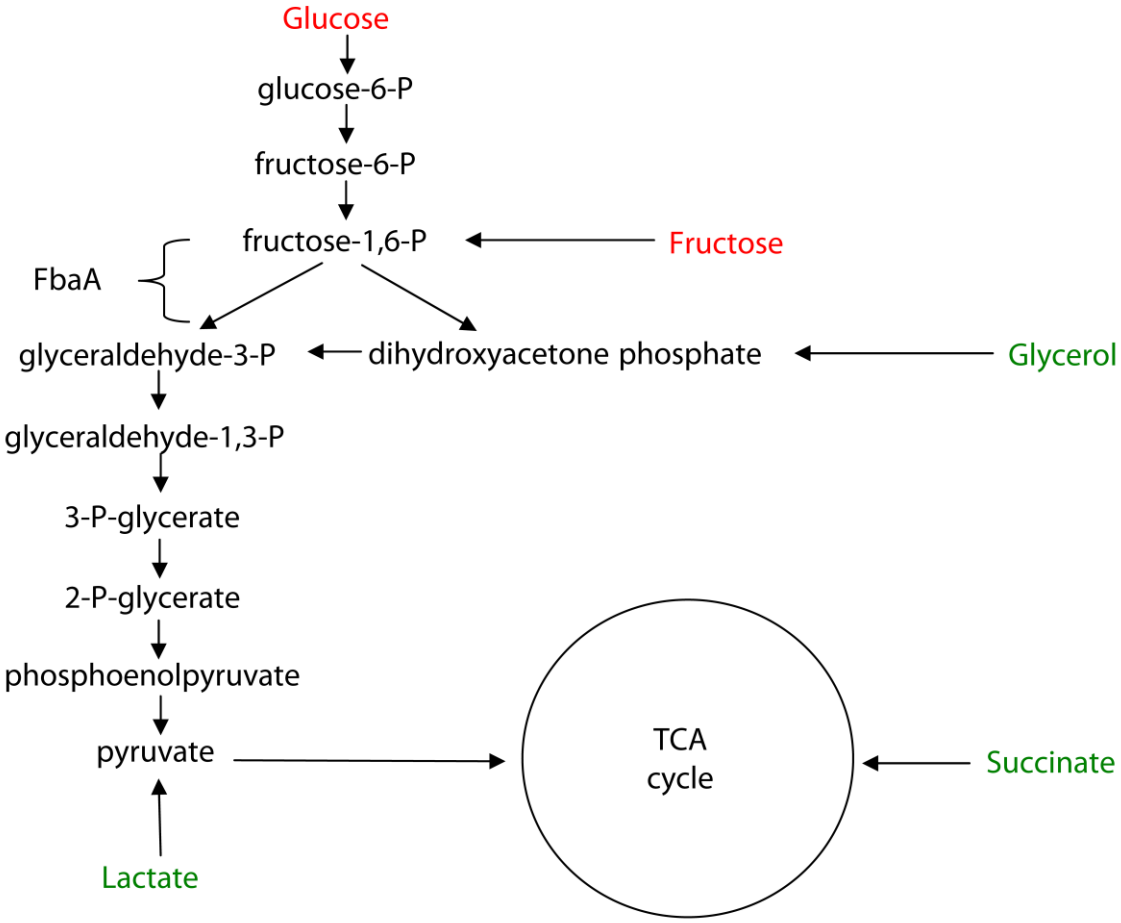

Fig. S2. A schematic of carbon source entrance into central metabolism. *E. coli* cells were sensitive to nickel when growing on glucose or fructose, but they were resistant when growing on glycerol, lactate, or succinate.

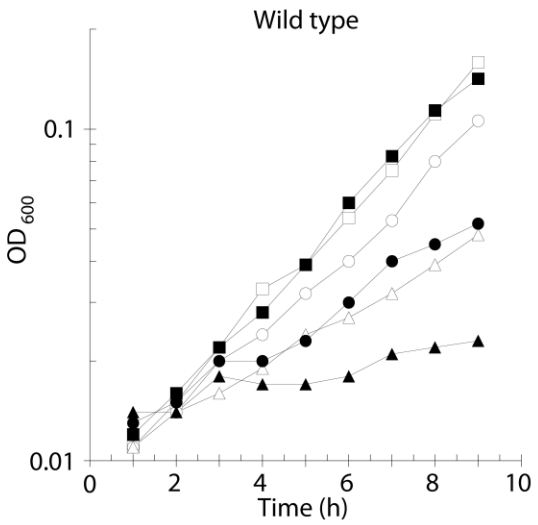

Fig S3. *E. coli* is resistant to nickel when growing on lactate or succinate. Wild-type cells (MG1655) were grown in M9 medium with lactate (open symbols) or succinate (closed symbols) and challenged with 0 µM (squares), 32 µM (circles), or 64 µM (triangles) Ni(II). The data are representative of three independent experiments.

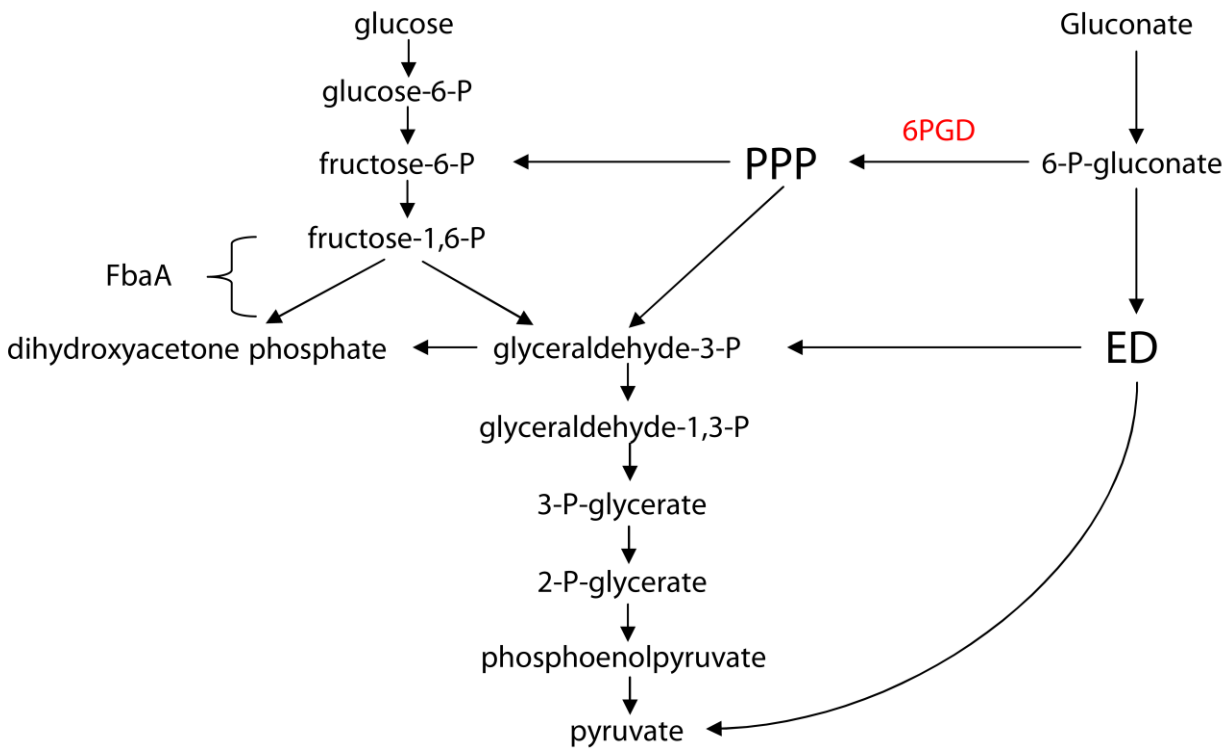

Fig. S4. A schematic of gluconate carbon entrance into central metabolism. Gluconate enters central metabolism primarily through the Entner-Doudoroff pathway (ED); however, 6-P-gluconate can enter the pentose phosphate pathway (PPP) via 6-phosphogluconate dehydrogenase (6PGD). A portion of the gluconate carbon diverted to the pentose phosphate pathway will enter glycolysis as fructose 6-P. *E. coli* cells lacking a functional fructose-1,6-bisphospate aldolase (FbaA) are unable to grow on gluconate due to a buildup of fructose-1,6-bisphosphate (Böck and Neidhardt, 1966; Schneider and Gourse, 2003; Schreyer and Böck, 1973). Elimination of *gnd*, the gene encoding 6PGD, restored growth on gluconate by preventing 6-P-gluconate from entering the PPP, thus preventing the buildup of fructose-1,6-bisphosphate (Schreyer and Böck, 1973).

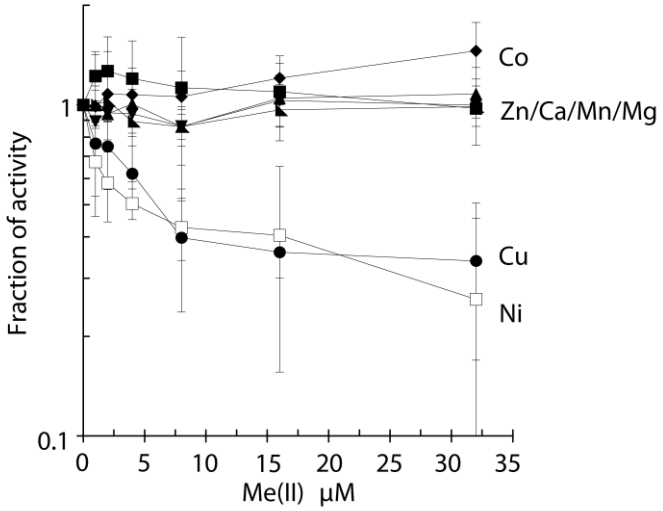

Fig S5. FbaA is resistant to inhibition by divalent cations. FbaA (20 nM) was challenged with Ni(II) (□), Ca(II) (◣), Co(II) (♦), Cu(II) (●), Mg(II) (▼), Mn(II) (▲), and Zn(II) (■) for 5 min aerobically at 37 ^{o}C. Error bars represent the standard deviation of three independent experiments.

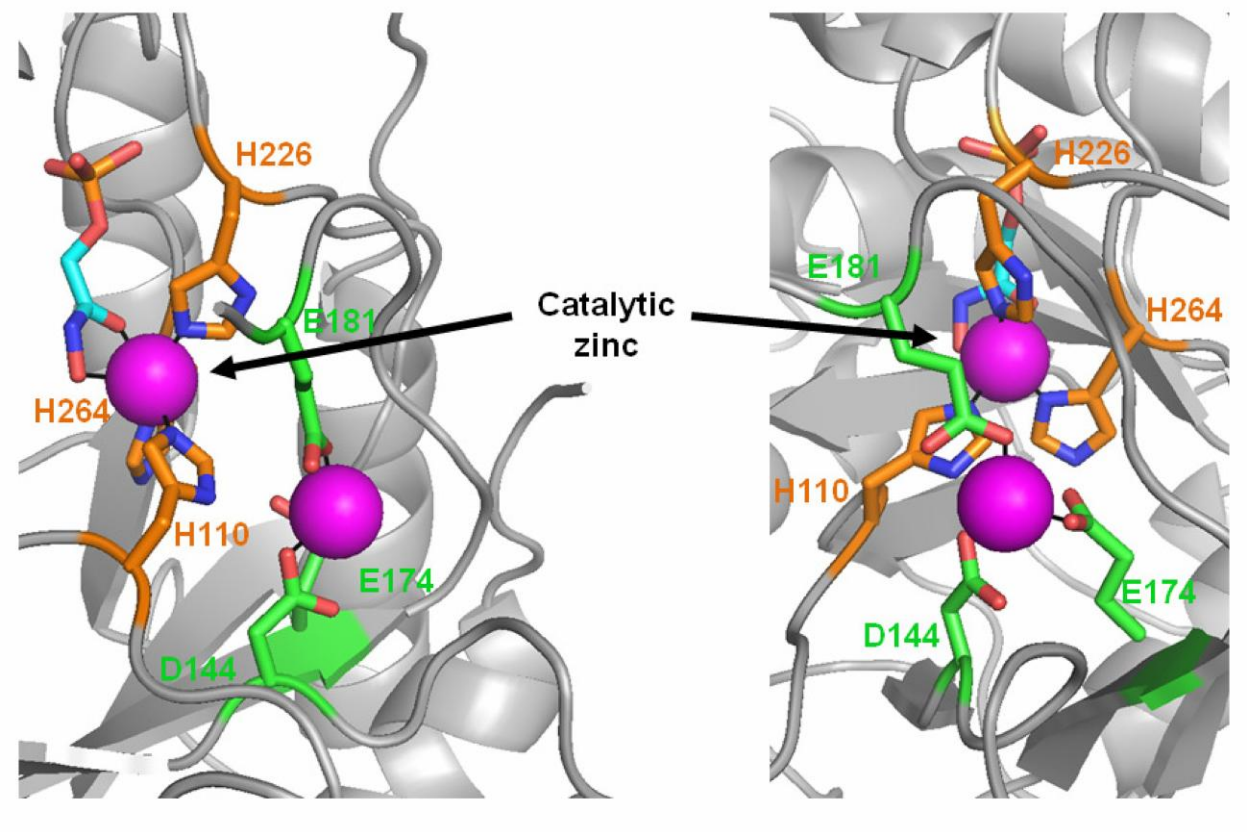

Fig S6. Two views of the *E. coli* FbaA active site. The structure of the protein complex with active site inhibitor phosphoglycolohydroxamate was previously reported (PDB code 1b57), and a portion of the protein is depicted here in cartoon mode in gray using PYMOL. The inhibitor (shown in stick representation with cyan carbons) chelates the required zinc ion, which is coordinated by H110, H226, and H264 (shown as orange sticks). A second zinc ion is bound nearby on the surface of the protein and coordinated by D144, E174, and E181 (green sticks). The two views are rotated by 90 degrees around a vertical axis.

References

Böck, A., and Neidhardt, F.C. (1966) Properties of a mutant of *Escherichia coli* with a temperature-sensitive fructose-1,6-diphosphate aldolase. *J Bacteriol* **92**: 470-476.

Schneider, D.A., and Gourse, R.L. (2003) Changes in the concentrations of guanosine 5'-diphosphate 3'-diphosphate and the initiating nucleoside triphosphate account for inhibition of rRNA transcription in fructose-1,6-diphosphate aldolase (*fda*) mutants. *J Bacteriol* **185**: 6192-6194.

Schreyer, R., and Böck, A. (1973) Phenotypic suppression of a fructose-1,6-diphosphate aldolase mutation in *Escherichia coli*. *J Bacteriol* **115**: 268-276.