SUPPORTING INFORMATION

Mutational and functional analyses of substrate binding and catalysis of the Listeria monocytogenes EutT ATP:Co(I)rinoid adenosyltransferase

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Running title: EutT substrate-binding residues

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## Supporting Tables

### Table S1. Strains and vectors used in this study.

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*Unless otherwise indicated, plasmids were constructed during the course of this work.*
### Table S2. Primers used in this study.

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SUPPORTING FIGURES

Figure S1. Binding of Mg(II)ATP to the LrPduO active site. Hydrogen bonding interactions and distances are shown in Å. In the intent of this figure is to help the reader visualize how we propose residues in LmEutT may interact with Mg(II)ATP. For additional structural information on LrPduO, The reader is also referred to PDB code 2NT8 (http://www.rcsb.org/structure/2NT8). This figure was originally published in the Journal of Biological Chemistry. St. Maurice, M, Mera, P. E., Taranto, M., Sesma, F., Escalante-Semerena, J. C., and Rayment, I. Structural characterization of the active site of the PduO-type ATP:Co(I)rinoid adenosyltransferase from Lactobacillus reuteri. J. Biol. Chem. 2007; 282:2596-2606. © the American Society for Biochemistry and Molecular Biology.
**Figure S2.** Inter-subunit salt bridge between R128 and D35 in \( LrPduO \). A. Salt bridge formed between two subunits of \( LrPduO \) with ATP bound to the active site (top of the figure). B. A view of the active site of \( LrPduO \) with both substrates bound to the active site, and interactions with the substrates shown by dashed lines. C. Substitutions at positions R128 or D35 negatively affect the catalytic efficiency of the enzyme. The data presented in panels B and C were originally published in the journal *Biochemistry* by Mera, P. E., St. Maurice, M., Rayment, I., and Escalante-Semerena, J. C. Structural and functional analyses of the human-type corrinoid adenosyltransferase (PduO) from *Lactobacillus reiteri*; 46:13829-13836, © 2007, American Chemical Society.

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**Figure S3. Room temperature** CD spectra of Co(II)Cbl in the absence (red) and presence (black) of \( \text{LmEutT}^{\text{W238H}} \).

**REFERENCES**
