Additional Methods used in Supplemental Data

Anaerobic Growth in Anoxomat

Plates were pre-incubated in an Anoxomat chamber (Advanced Instruments), which creates an anaerobic environment in roughly 5 minutes. Bacteria were grown to an O.D. of 0.1, serially diluted onto the pre-incubated plates, and incubated in the Anoxomat chamber under anaerobic conditions. CFUs were counted after 18 hours of growth.

Modeling

The structure of NrdF from *E. coli* (pdb: 3N37) was aligned with a Phyre 2 model of NrdF from TIGR4 by using MacPyMOL.54
Supplemental Figure Legends

**Supplemental Figure 1. Copper decreases nucleotide synthesis.** (A) Aerobic nucleotide synthesis genes increase during copper stress. \( p < .05 \) compared to \( \Delta \text{copA} \) alone by using Student’s \( t \)-test. (B) CFUs of \( \Delta \text{nrdD} \) mutant in aerobic, anaerobic GasPak jar, and Anoxomat chamber conditions. Aerobically grown bacteria at O.D. 0.3; bacteria at 620 nm were plated on blood agar plates in aerobic, anaerobic GasPak jar, or Anoxomat conditions \( n = 4 \) (C) Additional transition metals do not change the growth of \( \Delta \text{nrdD} \) mutants in aerobic and anaerobic zone-of–growth inhibition assays. (D) Titration of hydroxyurea in the \( \Delta \text{nrdD} \) mutant. O.D. was measured 8 hours after inoculation. Error bars represent SD. * Student’s \( t \)-test indicates \( p < .01 \) compared to wild-type TIGR4 treated with the same concentration of hydroxyurea (HU) \( n = 3 \). (E) Manganese increases replication of the \( \Delta \text{nrdD} \) mutants but cannot rescue them from hydroxyurea stress. Replication measured 8 hours after inoculation and normalized to that of the \( \Delta \text{nrdD} \) mutant without 1 mM of HU and/or 250 µM of manganese. Error bars represent SEM \( n = 3 \). * Student’s \( t \)-test indicates \( p < .01 \) compared to the \( \Delta \text{nrdD} \) mutant without metal stress; ND = Student’s \( t \)-test indicates no difference.

**Supplemental Figure 2. Potential binding configuration for copper in NrdF** (A) The structural alignment of *E. coli* NrdF (pdb: 3N37) (cyan) to a Phyre model of TIGR4 *S. pneumoniae* NrdF (green) bound to manganese (magenta), as predicted by using PyMOL (Bennett-Lovsey et al., 2008; Boal et al., 2010). (B) Binding affinities of specific amino acids for various metals (Furia, 1972).
Supplemental Figure 1

A

<table>
<thead>
<tr>
<th>TIGR4 Gene</th>
<th>Real Time Fold Increase ΔcopA w/Cu vs. ΔcopA w/o Cu</th>
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</thead>
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<tr>
<td>SP_1179 (nrBH)</td>
<td>2.33</td>
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<tr>
<td>SP_1713 (nrDR)</td>
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</tbody>
</table>

B

![Graph showing CFU/mL for different conditions]

C

![Bar graph showing zone of inhibition in mm]

D

![Bar graph showing fraction of growth compared to no copper added]

E

![Bar graph showing ΔnrID growth with different treatments]
Supplemental Figure 2

(E. Coli NrdF, Modeled S.Pneumoniae NrdF)
Manganese metal cofactor