

## **Supplemental Figure Legends**

### **Supplemental Figure 1. Hydroxyl Radical Inhibitors do not Alter Survival of *Acinetobacter***

***baumannii***. (diamonds) *A. baumannii* ATCC 17978, colistin-sensitive MDR strains of *A. baumannii* (circles) CI-2, and (squares) CI-3, as well as a colistin-resistant PDR strain (triangles) CI-4, were treated with (open) 600mM thiourea or (filled) 600mM dipyridyl. At 0, 15, and 30 minutes, cultures were plated and colony forming units enumerated. Data are representative of two independent experiments. Points represent the mean and bars the standard deviation of triplicate samples.

### **Supplemental Figure 2. Lysis is not Sufficient for Increased HPF Fluorescence. *A.***

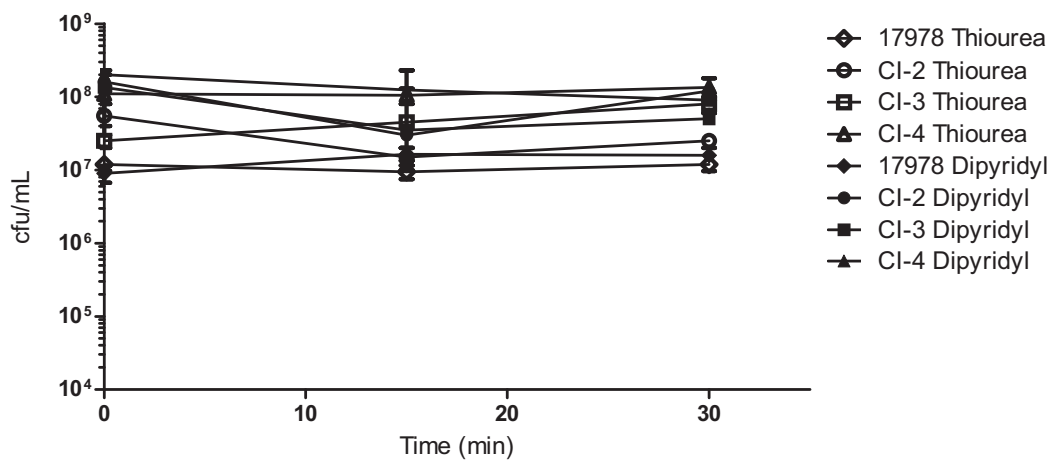
***baumannii*** cultures were untreated or sonicated. The hydroxyl radical specific fluorescent dye 3'-*(p-hydroxyphenyl) fluorescein* was added and fluorescence measured (490nm / 515nm). Bars represent the mean and standard deviation of triplicate samples.

### **Supplemental Figure 3. Colistin Killing of Diverse Gram-Negatives is Delayed by Hydroxyl**

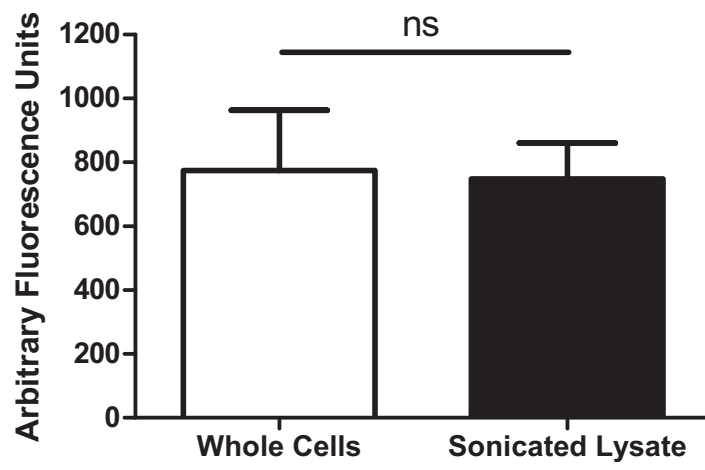
**Radical Quenching.** (A) *E. coli* or (B) *F. novicida* cultures were treated with 2 $\mu$ g/mL or 400 $\mu$ g/mL colistin, respectively, (◆) alone or in combination with either (■) 600mM thiourea or (●) 600 $\mu$ M dipyridyl, or (Δ) left untreated in culture media. At 0, 15, and 30 minutes, cultures were plated and colony forming units enumerated. Data are representative of two independent experiments. Points represent the mean and bars the standard deviation of triplicate samples. \*\*\*,  $P < 0.0001$ ; \*,  $P < 0.05$ .

**Supplemental Figure 4. Colistin Induces Hydroxyl Radical Production in Diverse Gram-Negative Bacteria.** *E. coli* cultures were treated with (A) 5µg/mL kanamycin, (B) 2µg/mL colistin, or left untreated for 30 minutes. *F. novicida* cultures were treated with (C) 5µg/mL kanamycin, (D) 400µg/mL colistin, or left untreated for 30 minutes. Following treatment, the hydroxyl radical specific fluorescent dye 3'-(p-hydroxyphenyl) fluorescein was added and fluorescence measured (490nm / 515nm). Data are representative of two independent experiments. Bars represent the mean and standard deviation of triplicate samples. \*,  $P < 0.05$ ; \*\*\*,  $P < 0.0001$ .

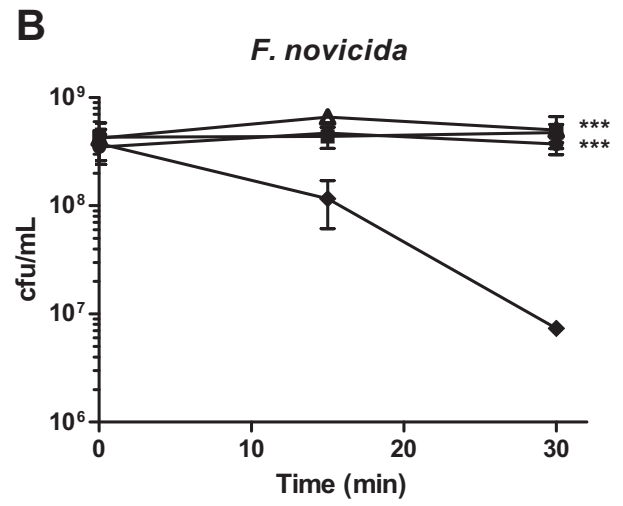
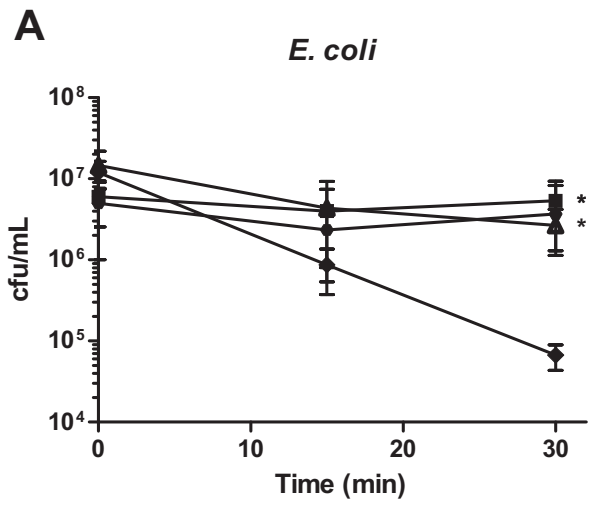
**Supplemental Figure 5. Colistin-Mediated Hydroxyl Radical Production is Inhibited by Thiourea and Dipyridyl.** (A) *A. baumannii*, (B) *E. coli*, or (C) *F. novicida* cultures were treated with colistin alone, or in combination with 600mM thiourea or 600µM dipyridyl for 30 minutes. Following treatment, the hydroxyl radical specific fluorescent dye 3'-(p-hydroxyphenyl) fluorescein was added and fluorescence measured (490nm / 515nm). Data are representative of two independent experiments. Bars represent the mean and standard deviation of triplicate samples. \*\*,  $P < 0.005$ ; \*\*\*,  $P < 0.0001$



Supplemental Figure 1.

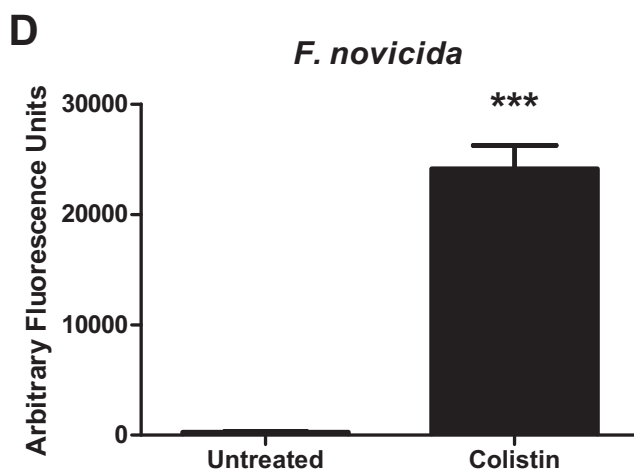
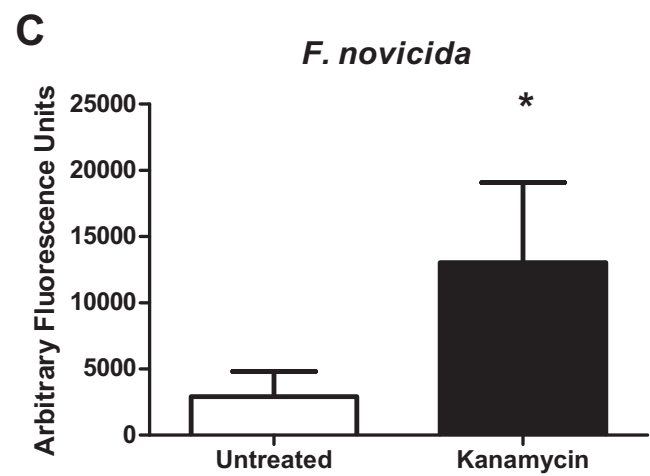
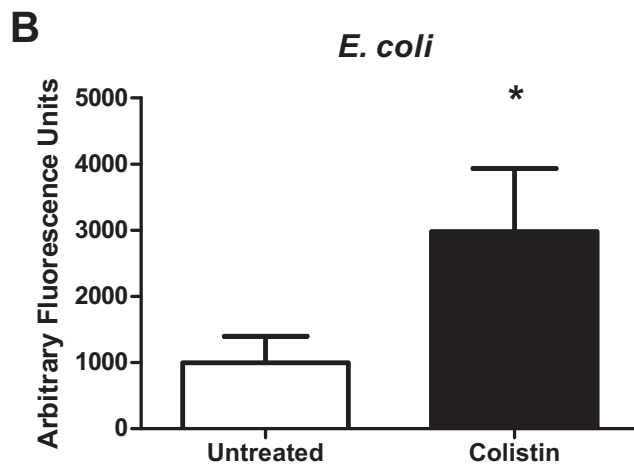
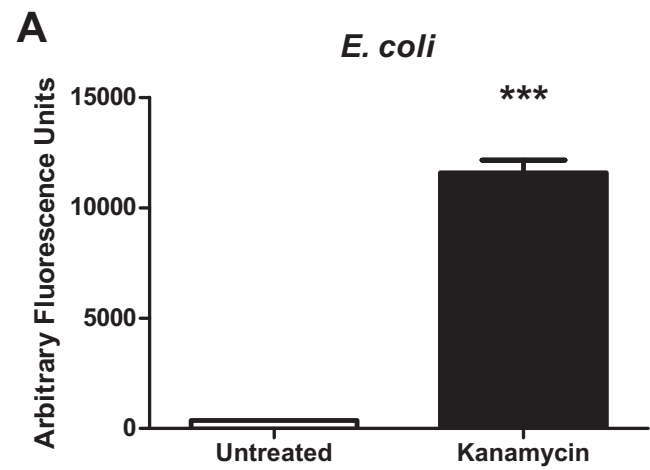


**Supplemental Figure 2.**

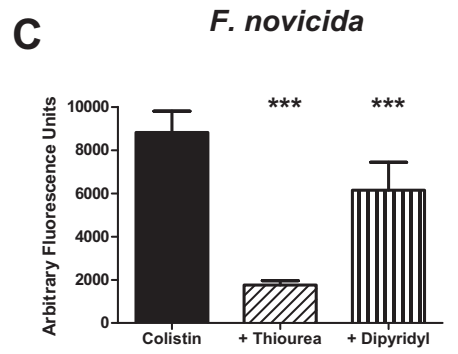
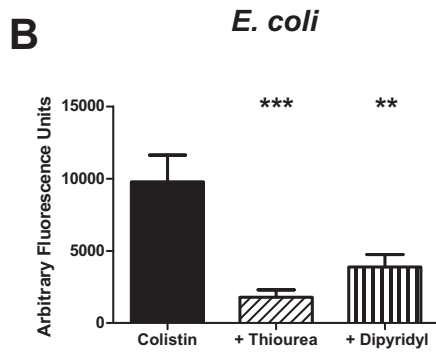
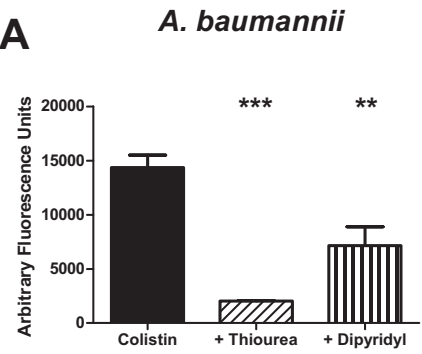


- ▲ Untreated
- Dipyridyl
- Thiourea
- ◆ Colistin Only

Supplemental Figure 3.



Supplemental Figure 4.



Supplemental Figure 5.