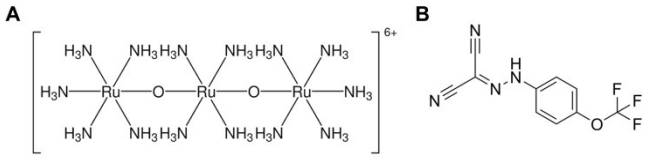

Figure S1. Chemical structures of RuRed and FCCP. **A)** Chemical structure of Ruthenium Red. **B)** Chemical structure of carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP).

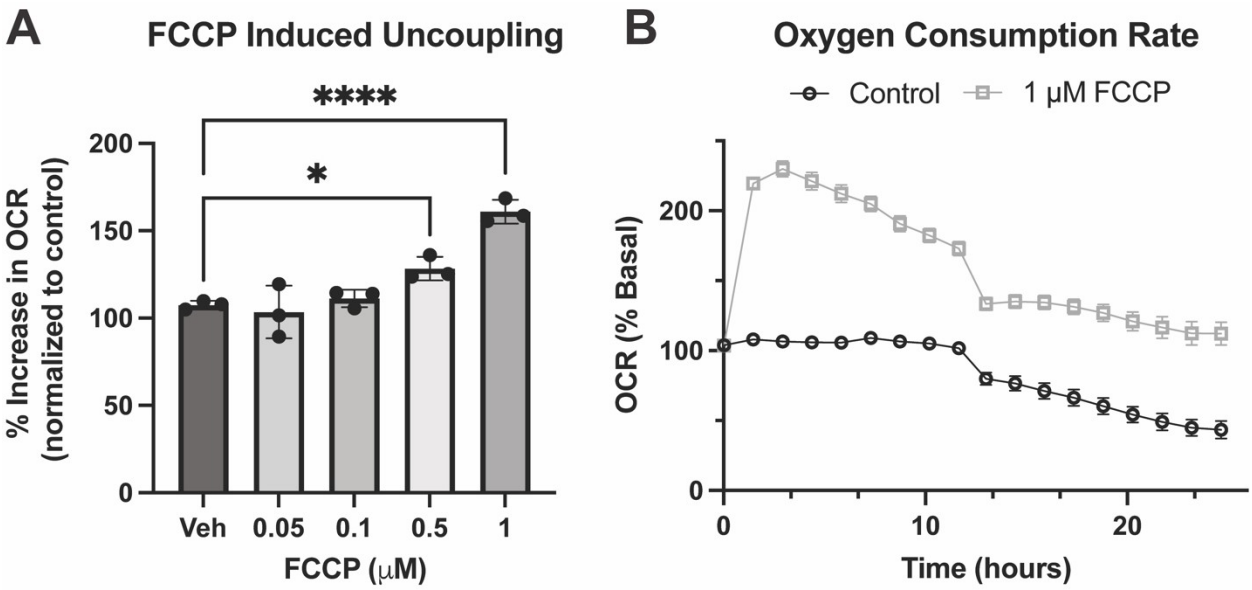

Figure S2. FCCP-induced mitochondrial uncoupling. **A)** Levels of FCCP-induced uncoupling with various FCCP concentrations. The y-axis represents the percentage increase in the oxygen consumption rate (OCR) normalized to the vehicle control. Data was analyzed via one-way ANOVA with a Dunnett's post-hoc test for multiple comparisons. *: $p<0.05$, ****: $p<0.0001$. **B)** Representative OCR traces obtained from a Seahorse XF Analyzer over the course of 24 hours, following an injection of 1 μM FCCP. OCR is represented as % basal OCR, prior to the injection of FCCP. The decrease in both groups observed at the 12-hour time point is associated with stopping and starting the Seahorse instrument, as it is unable to run an assay longer than 12 hours.

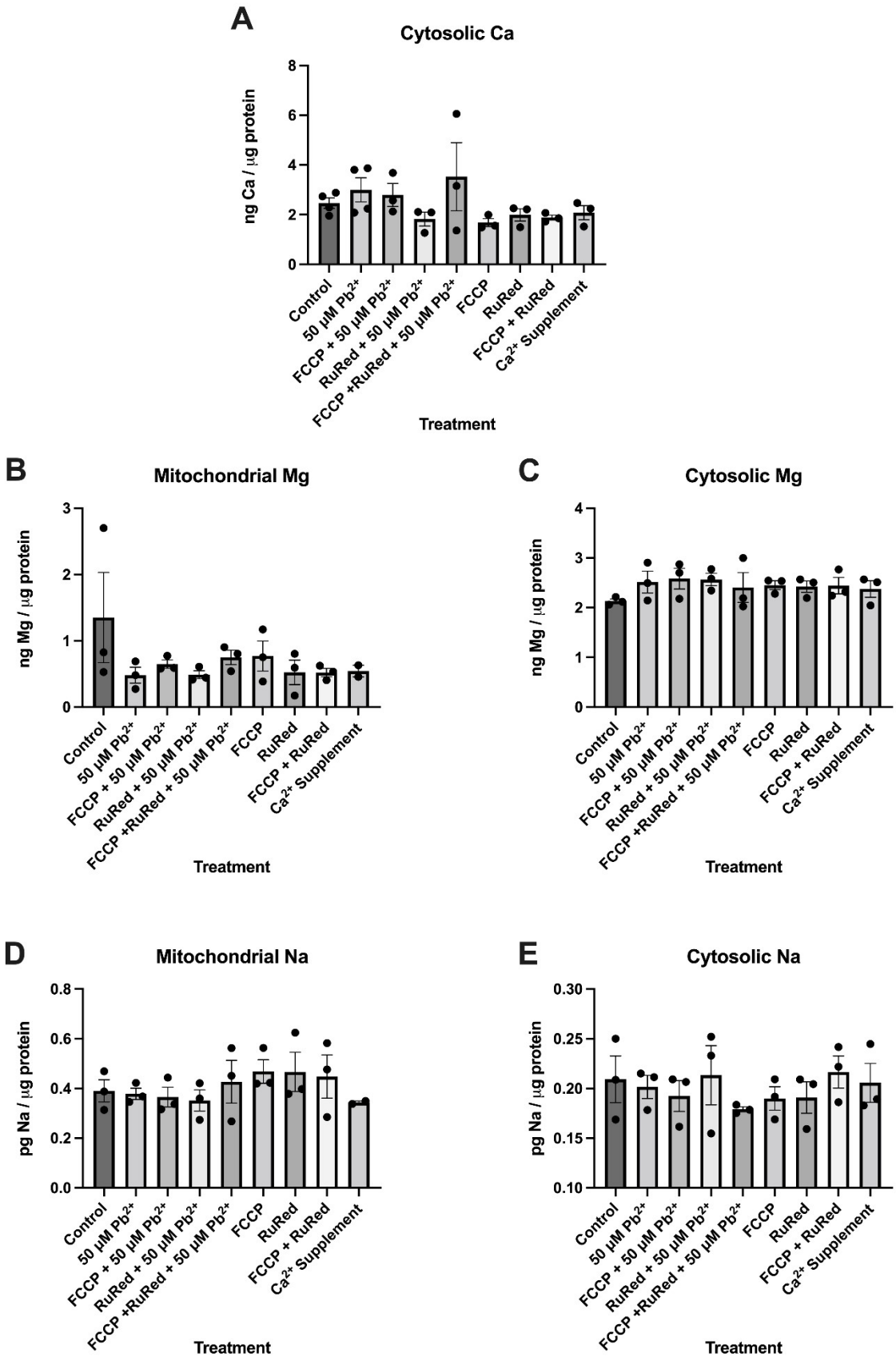
A
Cytosolic Ca
ng Ca / µg protein
0
2
4
6
8
Control
50 µM Pb2+
FCCP + 50 µM Pb2+
RuRed + 50 µM Pb2+
FCCP +RuRed + 50 µM Pb2+
FCCP
RuRed
FCCP + RuRed
Ca2+ Supplement
Treatment
B
Mitochondrial Mg
ng Mg / µg protein
0
1
2
3
Control
50 µM Pb2+
FCCP + 50 µM Pb2+
RuRed + 50 µM Pb2+
FCCP +RuRed + 50 µM Pb2+
FCCP
RuRed
FCCP + RuRed
Ca2+ Supplement
Treatment
C
Cytosolic Mg
ng Mg / µg protein
0
1
2
3
4
Control
50 µM Pb2+
FCCP + 50 µM Pb2+
RuRed + 50 µM Pb2+
FCCP +RuRed + 50 µM Pb2+
FCCP
RuRed
FCCP + RuRed
Ca2+ Supplement
Treatment
D
Mitochondrial Na
pg Na / µg protein
0.0
0.2
0.4
0.6
0.8
Control
50 µM Pb2+
FCCP + 50 µM Pb2+
RuRed + 50 µM Pb2+
FCCP +RuRed + 50 µM Pb2+
FCCP
RuRed
FCCP + RuRed
Ca2+ Supplement
Treatment
E
Cytosolic Na
pg Na / µg protein
0.10
0.15
0.20
0.25
0.30
Control
50 µM Pb2+
FCCP + 50 µM Pb2+
RuRed + 50 µM Pb2+
FCCP +RuRed + 50 µM Pb2+
FCCP
RuRed
FCCP + RuRed
Ca2+ Supplement
Treatment

Figure S3. Analyte levels in mitochondrial and cytosolic fractions across treatment groups. **A)** Levels of cytosolic calcium levels represented as ng of calcium per µg of protein, as determined by a BCA assay. **B)** Levels of mitochondrial magnesium levels represented as ng of magnesium per µg of protein, as determined by a BCA assay. **C)** Levels of cytosolic magnesium levels represented as ng of magnesium per µg of protein, as determined by a BCA assay. **D)** Levels of mitochondrial sodium levels represented as pg of sodium per µg of protein, as determined by a BCA assay. **E)** Levels of cytosolic sodium levels represented as pg of sodium per µg of protein, as determined by a BCA assay. For all figures, data was analyzed via one-way ANOVA.

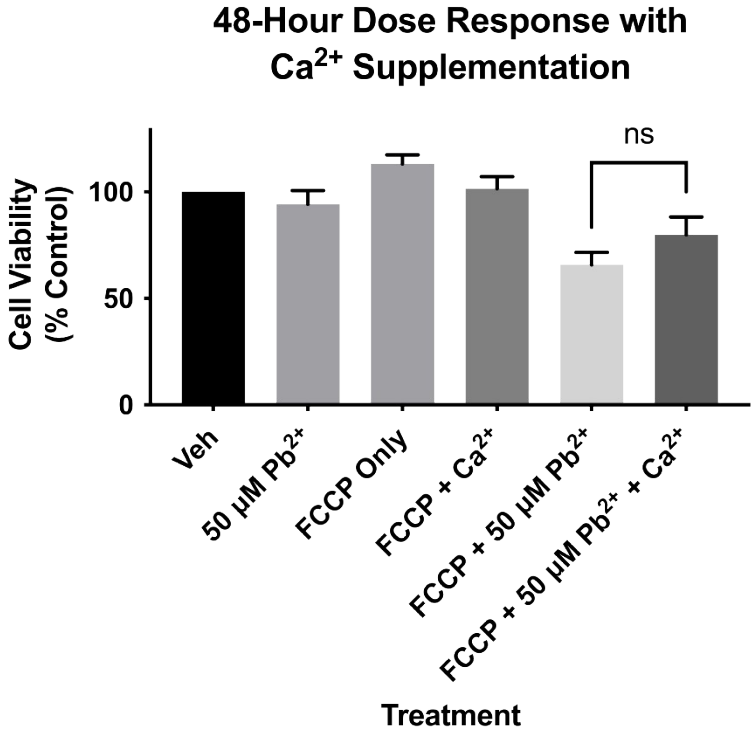

Figure S4. Calcium supplementation does not offer a rescue to the increased cytotoxicity in combination with FCCP. Cell viability following a 48-hour exposure to 50 uM Pb^2 and FCCP is not rescued by Ca^{2+} supplementation. FCCP and 50 µM Pb^{2+} corresponds to a 35% reduction in cell viability. FCCP, 50 µM Pb^{2+}, and Ca^{2+} is associated with a 20% reduction in cell viability. Data is analyzed via one-way ANOVA with a Tukey's post-hoc test for multiple comparisons between FCCP and Pb^{2+} exposure with the FCCP, Pb^{2+}, and Ca^{2+} exposure ($p=0.4184$).

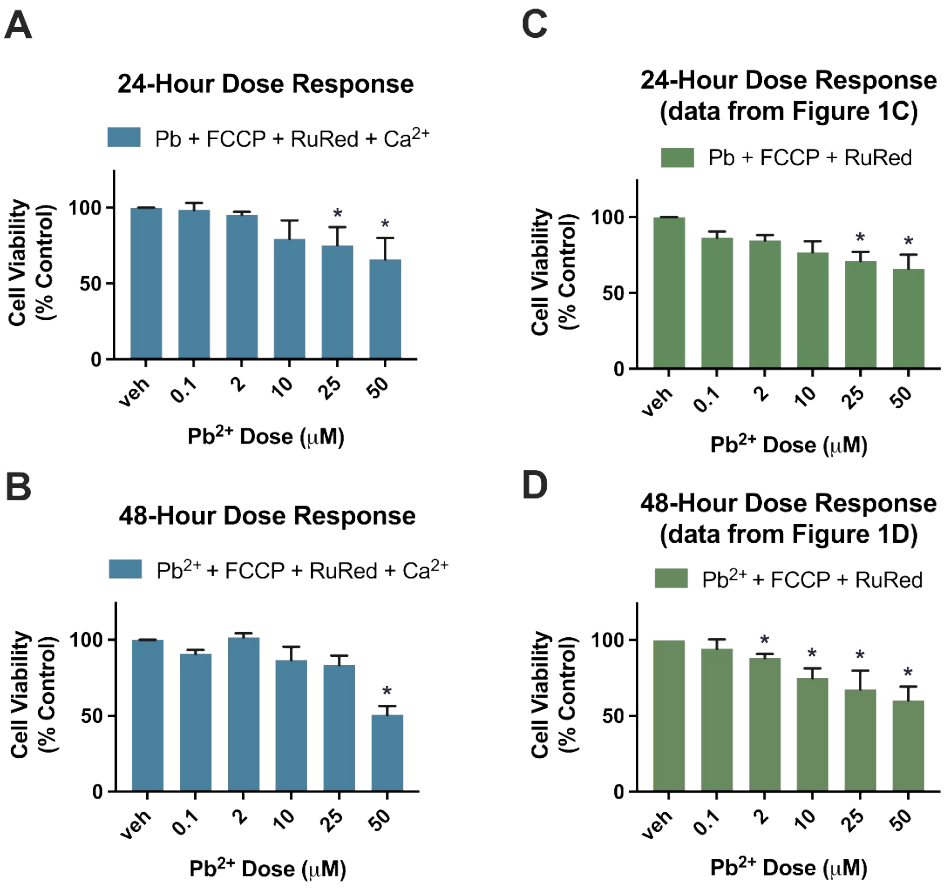

Figure S5. Calcium supplementation does not offer complete rescue of increased cytotoxicity in combination with FCCP and RuRed at 24 hours, but some protection at a 48-hour exposure to low doses of Pb^{2+}. These figures are to be directly compared back to the green columns in Figure 1C and Figure 1D, which represent the original dose response curves performed in the absence of a calcium supplementation and have been reprinted in panels C and D of this figure. **A)** 24-hour dose response curve for a mixture of Pb^{2+}, RuRed, FCCP, and a Ca^{2+} supplementation. Cell viability is significantly decreased at 25 and 50 μM Pb^{2+} doses (25 μM: 25%, p=0.0381 50 μM: 35%, p=0.0399). **B)** 48-hour dose response curve for a mixture of Pb^{2+}, RuRed, FCCP, and a Ca^{2+} supplementation. With Ca^{2+} supplementation, cell viability is no longer significantly decreased at 2, 10, and 25 μM Pb^{2+} doses, however at 50 μM Pb^{2+}, there is still an observed 50% reduction in cell viability ($p<0.001$). Data was analyzed via one-way ANOVA with a Tukey's post-hoc test for multiple comparisons to the control. **C)** 24-hour dose response following exposure to Pb^{2+} and FCCP and RuRed. Exposure to a mixture of all chemicals results in a significant decrease in cell viability at multiple Pb^{2+} concentrations (25 μM: 30%, p=0.0067, 50 μM: 34%, p=0.0256). Data was analyzed via two-way ANOVA with a Dunnett's post-hoc test for multiple comparisons of each group to the control. Pb^{2+} dose: p=0.003, Additional compound: $p<0.0001$, interaction: p=0.3513. **D)** 48-hour dose response following exposure to Pb^{2+} and FCCP and RuRed. Exposure to a mixture of all chemicals results in a significant decrease in cell viability at multiple Pb^{2+} concentrations (2 μM: 12%, p=0.0328, 10 μM: 25%,

p=0.0234, 25 μM: 33%, p=0.0007, 50 μM: 40%, p=0.0008). Data was analyzed via two-way ANOVA with a Dunnett's post-hoc test for multiple comparisons of each group to the control. Pb^{2+} dose: $p<0.0001$, Additional compound: $p<0.0001$, interaction: $p=0.0082$. Fluorescence values recorded by the resazurin assay corresponding to the cell viability have been normalized to the control group and are presented as a percentage of the control on all graphs.