Copper induces neuron-sparing, ferrodoxin 1-independent astrocyte toxicity mediated by oxidative stress

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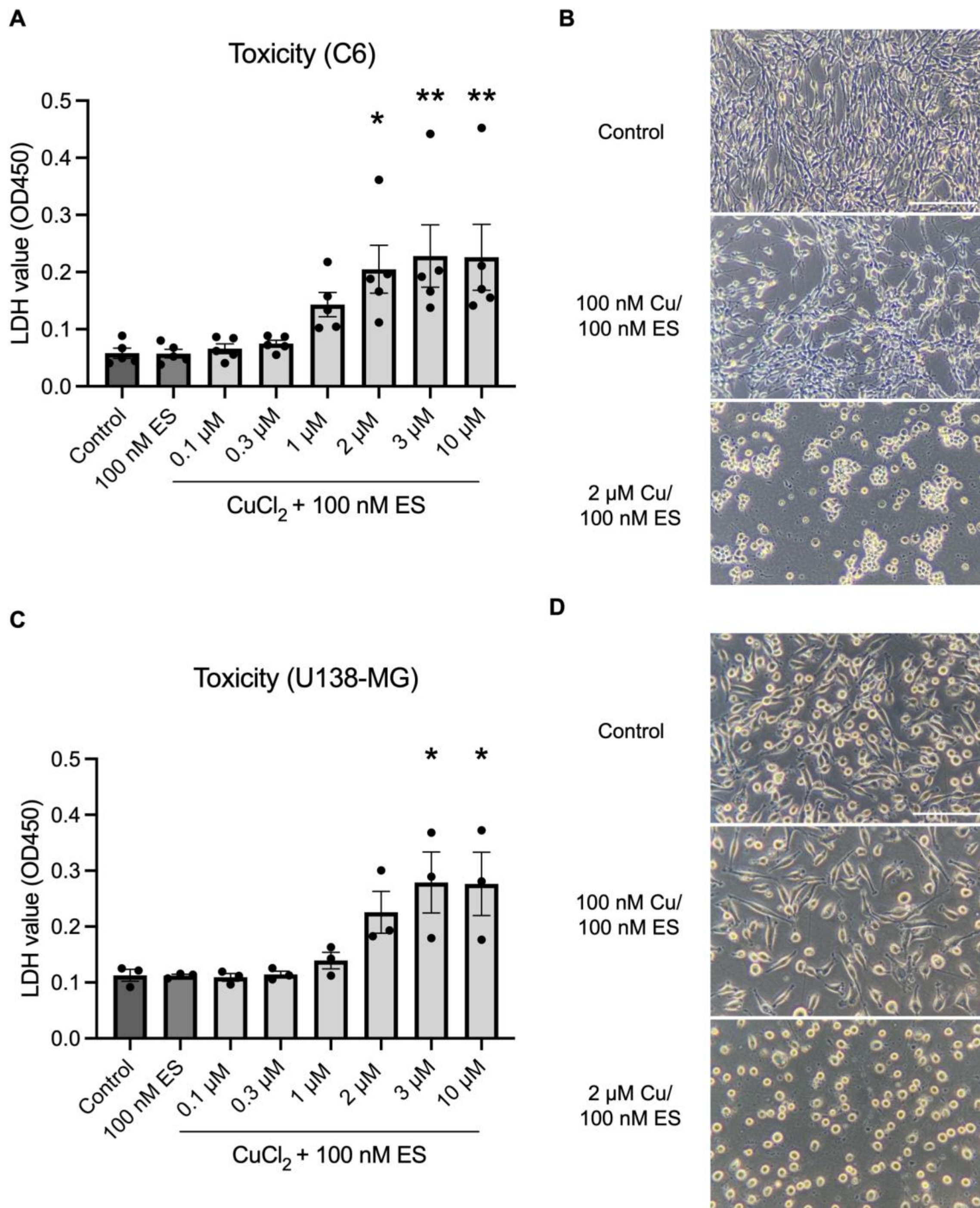

Figure S1. Glioma cells are susceptible to copper ionophore induced cell death (A) LDH assay of C6 glioma cells showing dose dependent copper toxicity in the presence of 100 nM elesclomol ($F(7,32)=5.509$, one-way ANOVA, $p=0.0003$, Dunnet post-hoc, control vs 2 µM CuEs, $p=0.0205$; control vs 3 µM CuEs, $p=0.0058$; control vs 10 µM CuEs, $p=0.0065$). (**B)** Phase contrast images of C6 glioma cells after overnight treatment with CuEls demonstrating reduced proliferation and altered cell morphology. **(C)** LDH assay of U138-MG glioma cells showing dose dependent copper toxicity in the presence of 100 nM elesclomol ($F(7,16)=5.781$,

one-way ANOVA, $p=0.0018$, Dunnet post-hoc, control vs 3 μM CuES, $p=0.0105$; control vs 10 μM CuES, $p=0.0116$). (**D)** Phase contrast images of U-138-MG glioma cells after overnight treatment with CuEls demonstrating reduced proliferation and altered cell morphology. Scale bar is 100 μM.

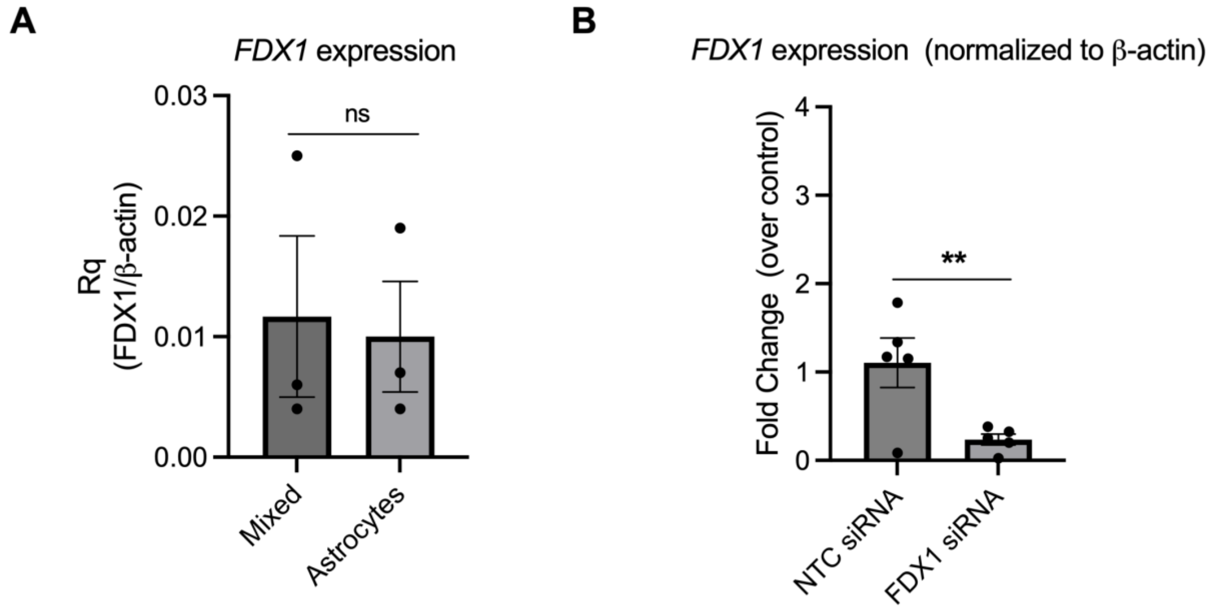

Figure S2. ***FDX1* is expressed in primary cortical cultures and can be knocked down.** All experiments utilized 2 μM Cu^{2+}. Experiments conducted in astrocyte cultures utilized 200 nM ES while those conducted in cultures containing neurons and astrocytes utilized 300 nM ES. **(A)** *FDX1* is expressed at similar levels in astrocytes cultures and cultures containing both neurons and astrocytes (mixed) ($t=0.7625$, paired two tailed t-test, $p=0.5254$) and **(B)** can be knocked down in our cultures 48 hours after siRNA treatment ($t(4)=8.113$, ratio paired two-tailed t-test, $p=0.0013$, NTC=non-targeting control).

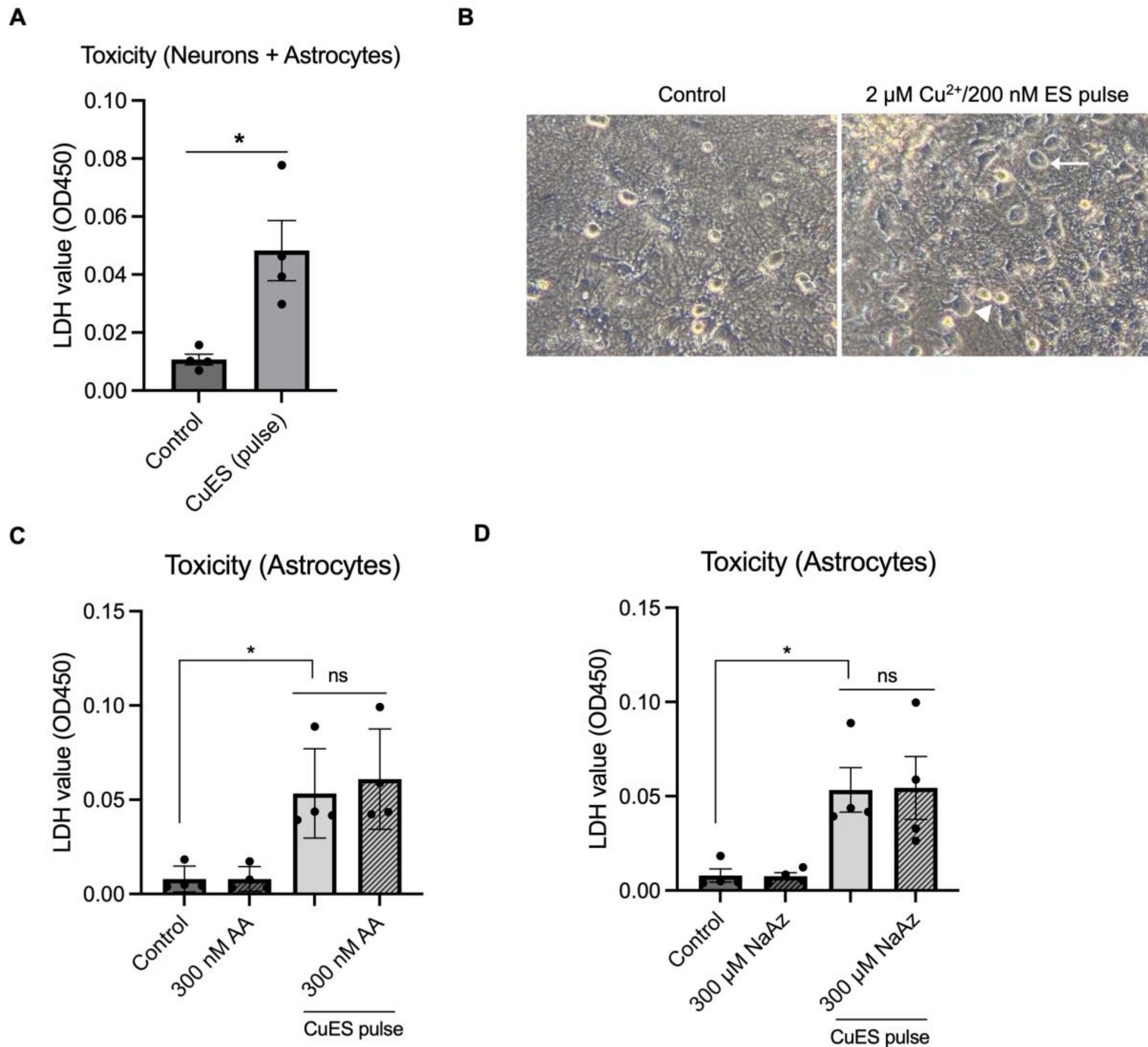

Figure S3. A CuES pulse is sufficient to induce selective astrocyte damage, which cannot be rescued by inhibition of mitochondrial respiration. (A) A two-hour exposure to 2 µM Cu^{2+} in the presence of 300 nM elesclomol is sufficient to induce significant LDH release 18-24 hours later in mixed cortical cultures (paired t-test, p=0.0405, n=4 biological replicates). **(B)** This treatment causes astrocytic swelling (arrow) while healthy phase bright neurons (arrowhead) are readily apparent by phase contrast microscopy. CuES toxicity cannot be rescued by inhibition of oxidative phosphorylation with **(C)** 300 nM antimycin A (F(1.087, 3.260)=26.27, repeated-measures one-way ANOVA, p=0.0114, Sidak post-hoc, control vs. CuES pulse, p=0.0249; CuES pulse vs CuES pulse + 300 nM antimycin A, p=0.1815, n=4 biological replicates) nor **(D)** with 300 µM sodium azide (F(1.139, 3.416)=13.05, repeated measures one-way ANOVA, p=0.0285, Sidak post-hoc, control vs CuES pulse, p=0.0249; CuES pulse vs. CuES pulse + 300 µM sodium azide, p=0.9886, n=4 biological replicates). Scale bar is 100 µM.

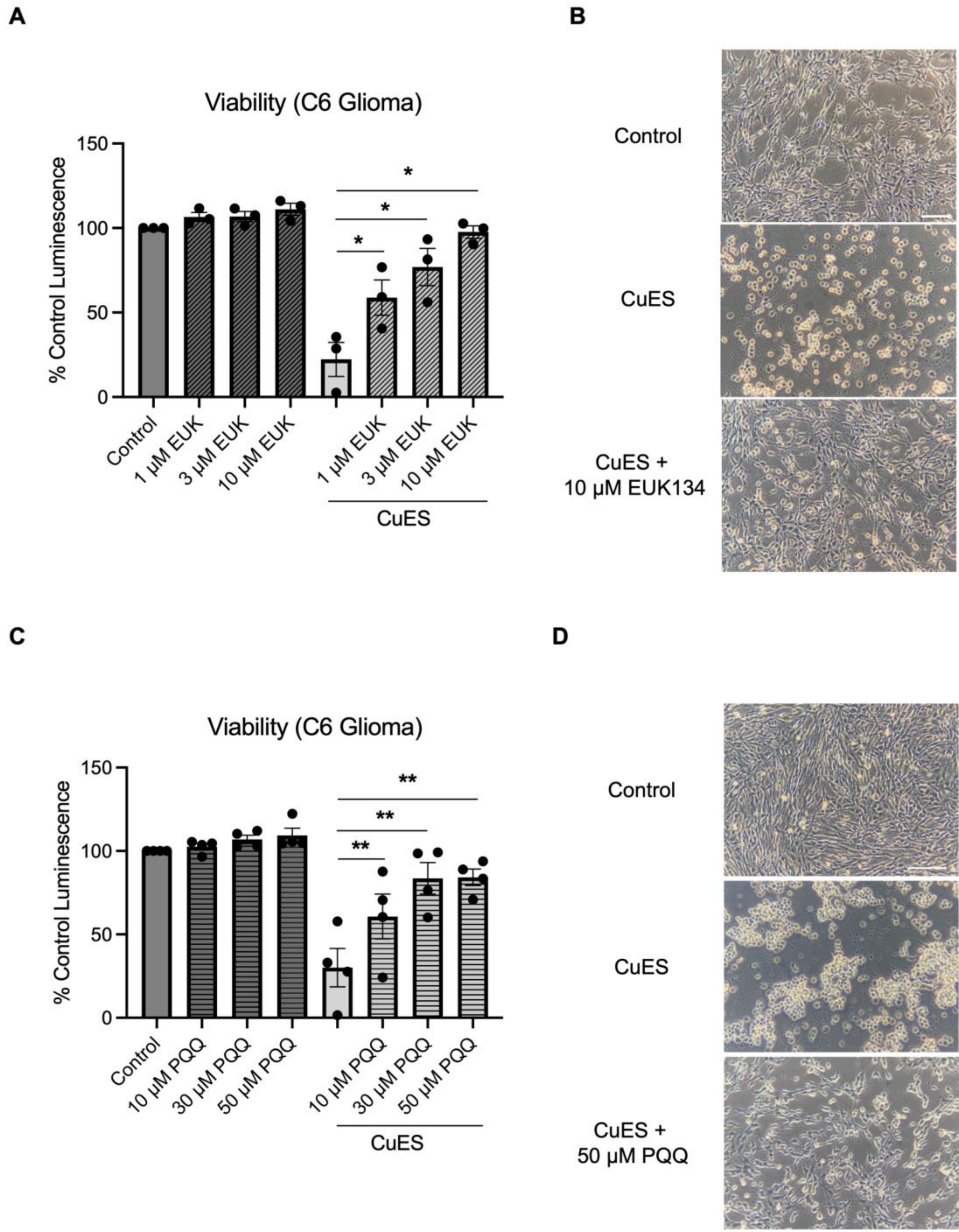

Figure S4. Antioxidants rescue C6 glioma cells from CuES induced cell death. Treatment with increasing concentrations of the antioxidant EUK-134 **(A)** rescued viability to C6 glioma cells following treatment with 2 µM Cu^{2+}/100 nM ES ($F(1.954, 3.908)=45.72$, repeated measures one-way ANOVA, $p=0.0020$, Sidak post hoc, CuES vs CuES + 1 µM EUK134, $p=0.0142$; CuES vs CuES + 3 µM EUK134, $p=0.0188$; CuES vs CuES + 10 µM EUK134, $p=0.0137$, n=3 biological replicates). (**B**) EUK134 (10 µM) improved the morphology of C6 glioma cells, as evidenced by phase contrast microscopy. Treatment with PQQ, another antioxidant and free

radical scavenger also rescued C6 glioma cells from CuES induced toxicity in a concentration dependent manner as evidenced by **(C)** increased viability ($F(1.578,4.733)=36.07$, repeated measures one way ANOVA, $p=0.0016$, Sidak post hoc, CuES vs CuES + 10 µM PQQ, $p=00048$; CuES vs CuES + 30 µM PQQ, $p=0.0051$, CuES vs CuES + 50 µM, $p=0.0090$, n=4 biological replicates) and **(D)** improved cell morphology. Scale bar is 50 µm.