## Supporting Information

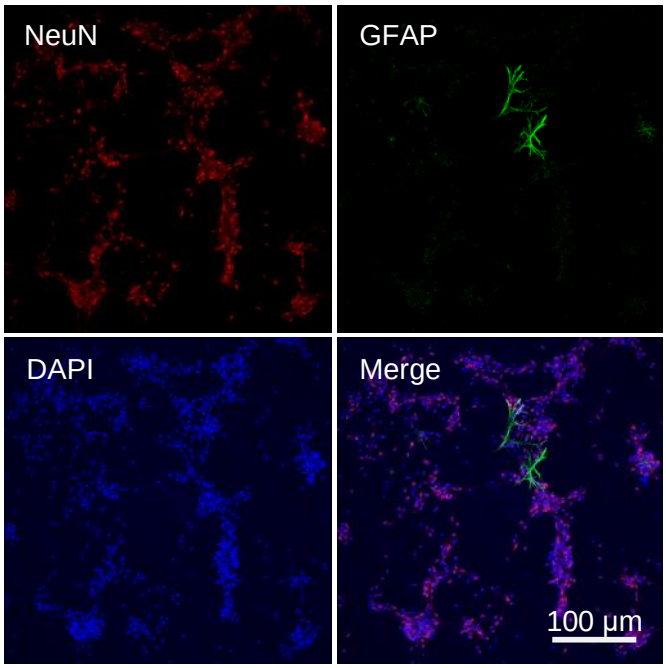


**Figure S1** Primary cortical cultures were predominantly neuronal. Primary cortical cells were grown on poly-D lysine coated 12 mm glass coverslips until DIV9 and prepared for immunocytochemistry as previously described (Xu, Perreau et al., 2016). Anti-neuron-specific nuclear protein (1:200) and anti-mouse Alexa 488 (1:400, ThermoFisher Scientific, Scoresby, Australia) were used to identify neurons (red). Anti-glial fibrillary acidic protein (GFAP, 1:400) and anti-guinea pig Alexa 647 (1:400, ThermoFisher Scientific) were used to identify astrocytes (green). DAPI (300 nM, ThermoFisher Scientific) was used to detect nuclei (blue). Images were recorded at 20X magnification using an SP8 confocal microscope with Leica Application Suite X software (Leica Microsystems, Macquarie Park, Australia). Images at each wavelength were captured sequentially and multi-colour XY images were prepared using Image J (Schneider, Rasband et al., 2012). XY images are shown as maximum brightness stacked images. Representative images are shown.

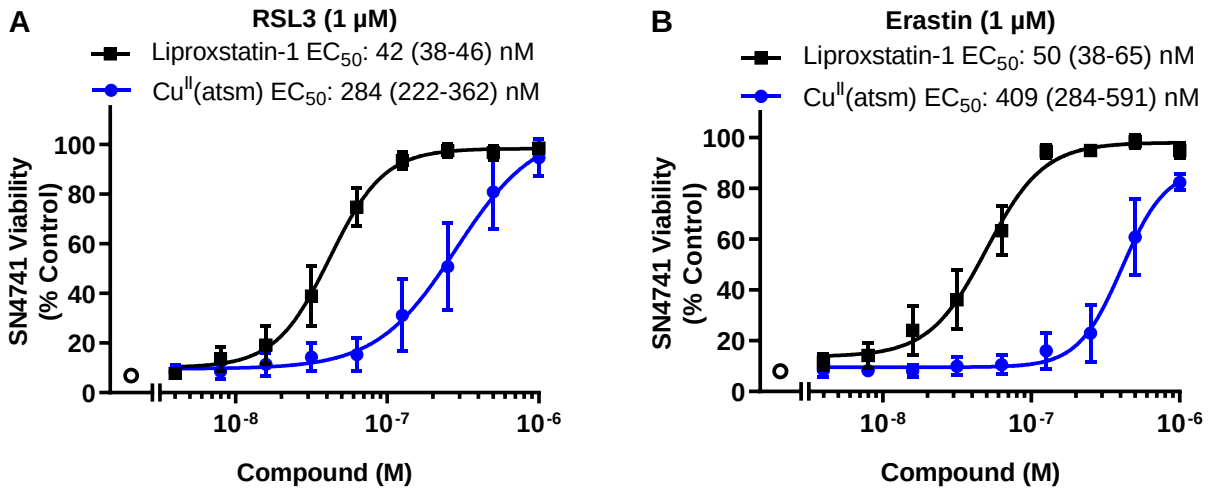


**Figure S2** $Cu^{II}$(atsm) prevents ferroptosis in SN4741 cells. Cells were derived from E13.5 mouse substantia nigra tissue (Son, Chun et al., 1999) and cultured as per N27 cells. Viability of cells treated with RSL3 (1 μM, A) or erastin (1 μM, B) ± liproxstatin-1 or $Cu^{II}$(atsm) for 24 hr. Viability was measured with MTT and expressed as a percentage of control cells. Data are means ± SEM, *n*=5 independent experiments. $EC_{50}$ was determined by nonlinear regression, with 95% CI shown in parenthesis.

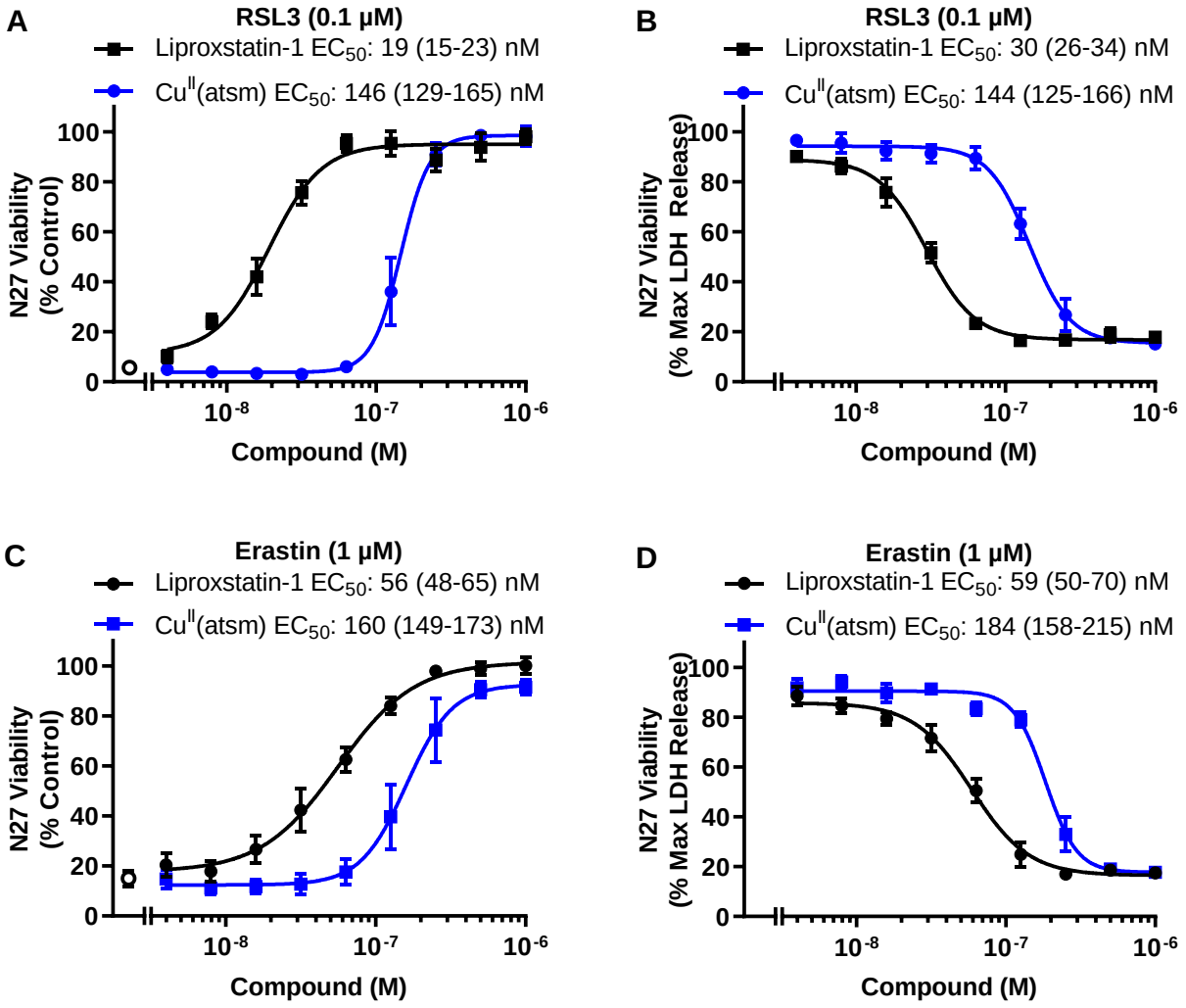


**Figure S3** $Cu^{II}$(atsm) prevents ferroptosis in N27 cells. Viability of cells treated with RSL3 (0.1 µM, A-B) or erastin (1 µM, C-D) ± liproxstatin-1 or $Cu^{II}$(atsm) for 24 hr. (A and C) Viability was measured with PrestoBlue according to the manufacturer's instructions (ThermoFisher Scientific) and expressed as a percentage of control cells. (B and D) Viability measured with the Cytotoxicity Detection Kit (LDH) according to the manufacturer's instructions (Merck, Bayswater, Australia) and expressed as a percentage of maximum lactate dehydrogenase (LDH) release. Data are means ± SEM, *n*=5 independent experiments. $EC_{50}$ was determined by nonlinear regression, with 95% CI shown in parenthesis.

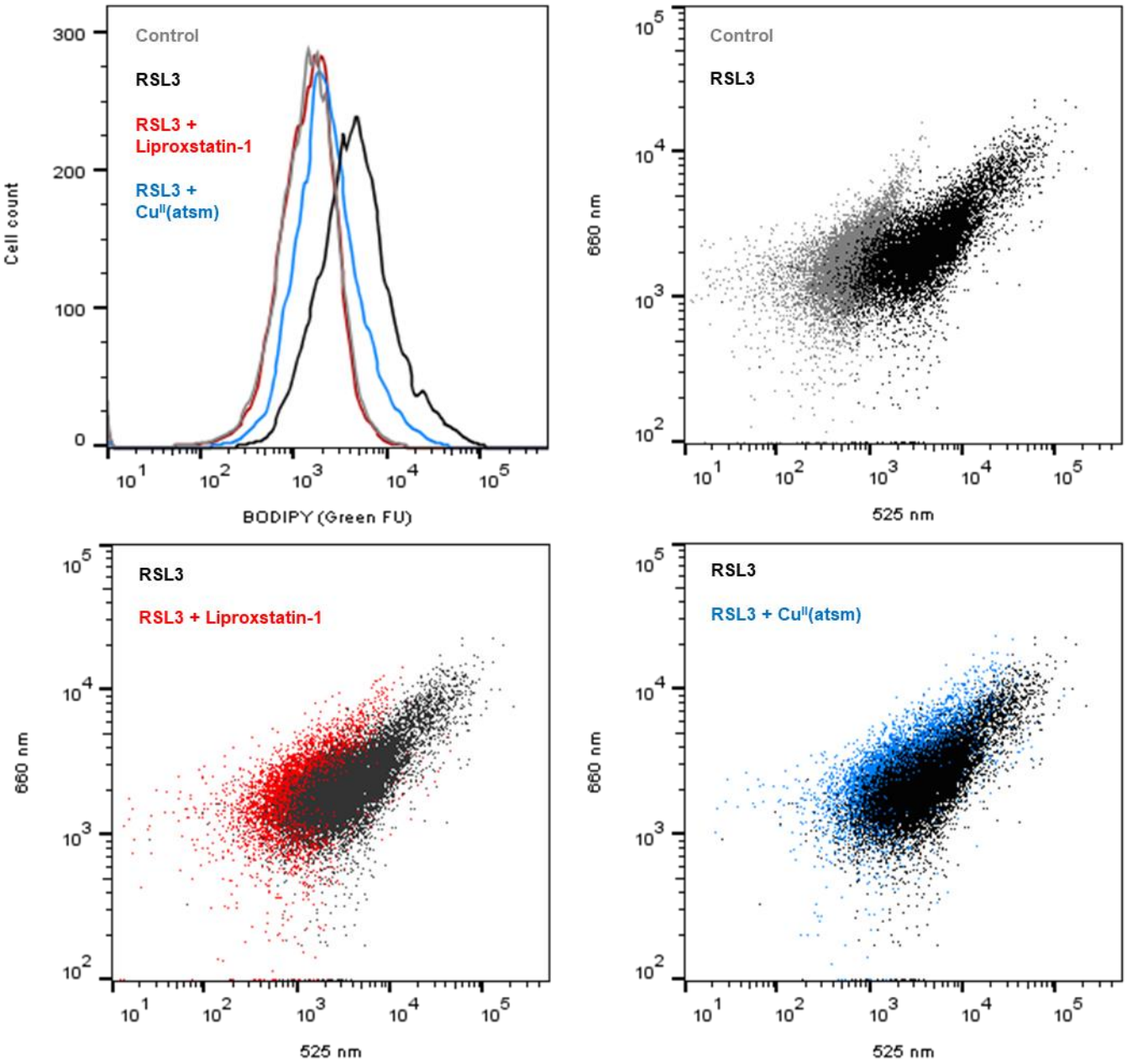


**Figure S4** Cu$^{II}$(atsm) prevents lipid peroxidation in N27 cells. Cells were cultured in 6 well plates and treated in growth media with RSL3 (50 nM) ± liproxstatin-1 or Cu$^{II}$(atsm) (1 µM) for 4 hr. Cells were washed in PBS and incubated in growth media with C11-BODIPY(581/591) (125 nM) for 1 hr. Cells were harvested with trypsin and resuspended in PBS containing: EDTA 1 mM, HEPES 25 mM, foetal calf serum 1%, penicillin and streptomycin. Cells were strained through a 35 µm cell strainer (Corning, Mulgrave, Australia) and green fluorescence (excitation 488 nm, emission 525 nm) and red fluorescence (excitation 633 nm, emission 660 nm) were detected using a CytoFLEX S flow cytometer (Beckman Coulter, Mount Waverly, Australia). A minimum of 10000 cells were analysed per condition. Representative data from one of three experiments is shown.

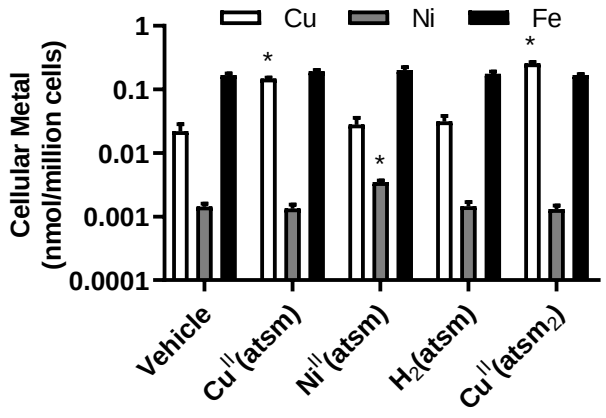


**Figure S5** Ni$^{II}$(atsm) does not promote Cu uptake in N27 cells. Cells were treated with Cu$^{II}$(atsm), Cu$^{II}$(atsm$_2$) or Ni$^{II}$(atsm) (1 µM) for 24 h. Cellular metal levels were measured with inductively coupled plasma mass spectrometry analysis using an Agilent 7700x (Agilent Technologies, Santa Clara, USA) as reported previously (Bica, Liddell et al., 2014). Data are means ± SEM, *n*=5 independent experiments. *P* values were calculated using one-way ANOVA corrected for multiple comparisons with Dunnett's post-hoc test. A Brown-Forsythe test confirmed variance was not significantly different between groups and a Kolmogorov-Smirnov test confirmed residuals were normally distributed. *$P$<0.05 compared to vehicle treated cells.


**Figure S6** Cu$^{II}$(atsm) does not elevate GPX4 levels in N27 cells. Cells treated with erastin (1 µM) or RSL3 (0.1 µM) ± liproxstatin-1 or Cu$^{II}$(atsm) (1 µM) for 24 hr. Samples were lysed in RIPA buffer (Merck) and proteins (15 µg) were resolved on NuPAGE 4-12% Bis-Tris gels and transferred to PVDF membranes with an iBlot2 for western immuno-blotting (ThermoFisher Scientific). Primary antibodies used were rabbit anti-GPX4 (1:5000, Abcam, Cambridge, USA) and mouse anti-actin (1:10000, Merck). Horseradish peroxidase coupled secondary antibodies were rabbit anti-mouse and goat anti-rabbit (1:7000, Agilent Technologies). Chemiluminescence was detected using ECL (GE Healthcare, Parramatta, Australia) and images were captured with a Fujifilm LAS-3000 (Fujifilm, Campbellfield, Australia).

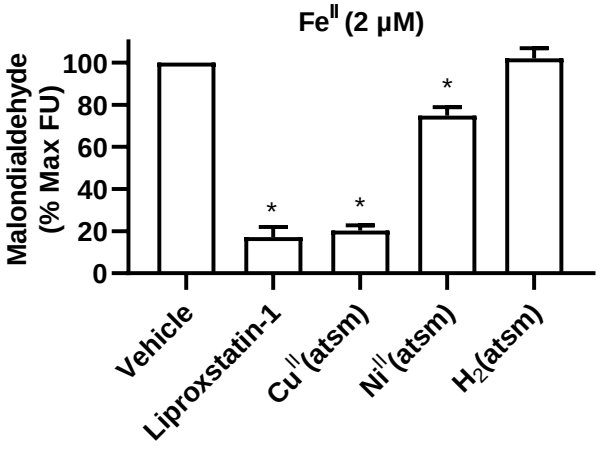


**Figure S7** Cu$^{II}$(atsm) prevents lipid peroxidation induced by Fe$^{II}$. Locke's buffer with arachidonic acid (50 µM) treated with Fe$^{II}$ (2 µM) ± liproxstatin-1, Cu$^{II}$(atsm), Ni$^{II}$(atsm) or $H_2$(atsm) (10 µM) for 30 min. Thiobarbituric Acid was used to detect malondialdehyde accumulation with the Lipid Peroxidation (MDA) Assay kit (Abcam) according to the manufacturer's instructions. Fluorescence (excitation 532 nm, emission 553 nm) was measured with a FlexStation 3 multi-mode microplate reader (Molecular Devices). Lipid peroxidation reported by malondialdehyde was normalised to the maximum signal induced by Fe. Data are means ± SEM, $n$=6 independent experiments. $P$ values were calculated using the Kruskal-Wallis test corrected for multiple comparisons by controlling the false discovery rate with the Benjamini, Krieger, and Yekutieli test. *$P$<0.05 compared to vehicle treated cells.

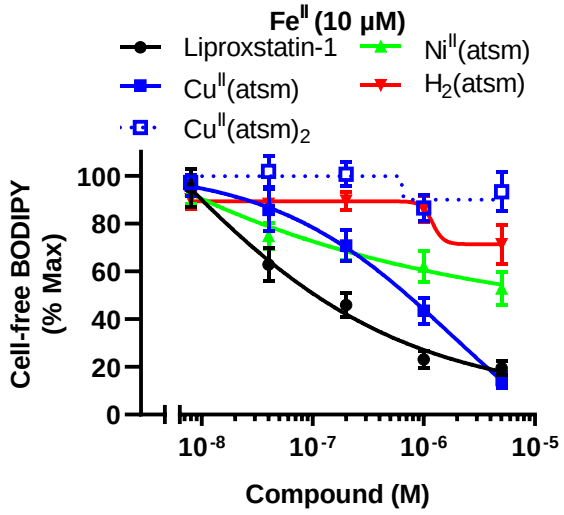


**Figure S8.** Cu$^{II}$(atsm) prevents Fe$^{II}$-induced peroxidation of cellular-derived lipids. Cellular-derived lipids were prepared from 10 million N27 cells snap frozen, resuspended in 10 ml Locke's buffer, and sonicated in a water bath for 30 min. C11-BODIPY(581/591) (2.5 µM) was added to the homogenate (100 µl) which was treated with Fe$^{II}$ (10 µM) ± liproxstatin-1, Cu$^{II}$(atsm), Ni$^{II}$(atsm), $H_2$(atsm) or Cu$^{II}$(atsm$_2$) for 30 min. C11-BODIPY(581/591) fluorescence was measured with a FlexStation 3 multi-mode microplate reader and expressed as the ratio of green to red fluorescence. Lipid peroxidation reported by C11-BODIPY(581/591) was normalised to the maximum signal induced by Fe. Data are means ± SEM, *n*=6 independent experiments.

## References

Bica L, Liddell JR, Donnelly PS, Duncan C, Caragounis A, Volitakis I, . . . White AR (2014). Neuroprotective copper bis(thiosemicarbazonato) complexes promote neurite elongation. PLoS One 9**:** e90070.

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Son JH, Chun HS, Joh TH, Cho S, Conti B, & Lee JW (1999). Neuroprotection and neuronal differentiation studies using substantia nigra dopaminergic cells derived from transgenic mouse embryos. J Neurosci 19**:** 10-20.

Xu H, Perreau VM, Dent KA, Bush AI, Finkelstein DI, & Adlard PA (2016). Iron Regulates Apolipoprotein E Expression and Secretion in Neurons and Astrocytes. J Alzheimers Dis 51**:** 471-487.