Supplementary information:

Methods:

Inductively-coupled plasma mass spectrometry (ICP-MS): Inductively-coupled plasma mass spectrometry (ICP-MS) of metal levels was performed as reported previously (Price et al., 2011). Cell pellets collected for metal analysis by ICP-MS were re-suspended in 50 µL of concentrated nitric acid (Aristar, BDH) and left to digest overnight. Samples were then heated for 20 min at 90°C to complete the digestion. The volume of each sample was reduced to approximately 40-50 µL after digestion, then 1 mL of 1% (v/v) nitric acid diluent was added to each cell sample. Measurements were made using an Agilent ICPMS 7700 x series ICPMS instrument under operating conditions suitable for routine multi-element analysis. The instrument was calibrated using 0, 5, 10, 50, 100 and 500 parts per billion (ppb) of certified multi-element ICPMS standard calibration solutions (ICP-MS-CAL2-1, ICP-MS-CAL-3 and ICP-MS-CAL-4, Accustandard) for a range of elements prepared in 1% (v/v) nitric acid for Cu, Fe, Zn and Mn. A certified internal standard solution containing 200 ppb of Yttrium (Y89) was used as an internal control (ICP-MS-IS-MIX1-1, Accustandard). Results were expressed as micromole per litre concentrations of metal (µmol/L). The concentrations of Cu and other metals were calculated as µg of metal per mg of protein based on the protein concentration of parallel cultures.

Sucrose density gradient fractionation: Sucrose density gradients were prepared using the method of Saegusa et al. (Saegusa et al., 2002). Briefly, treated cells were scraped into media and centrifuged at 720g for 3 min. The cell pellet was re-suspended in 1 mL of homogenization buffer (containing 300 mM sucrose, 5 mM EDTA, 5 mM HEPES, 1 µM phenylmethanesulfonylfluoride (PMSF) and protease inhibitors, pH 7.4), passed through a Dounce homogenizer 10 times and aspirated through a 25G needle 10 times before centrifugation at 10,000g for 10 min (4^{o}C). The supernatants were collected and adjusted to the same protein concentration with sucrose gradient buffer (containing 150 mM NaCl, 10 mM HEPES, 1 mM EDTA, 100 µM $MgCl_2$ and protease inhibitors, pH 7.4). Sucrose

gradients were constructed by layering 2.2 mL each of 10-50% w/v sucrose (in gradient buffer) into 12.4 mL centrifuge tubes. Samples in sucrose gradient buffer were loaded onto the gradient and centrifuged at 100,000g for 16 hr in a Beckman Ultracentrifuge (SW41Ti rotor). Fractions of 500 µL each were progressively collected using a 1 mL micropipette from the centrifuge tube starting from the top of the gradient to total 24 fractions. Fraction 1 therefore represented material which had equilibrated at a sucrose density of ~10%, and fraction 24 represented material which had equilibrated at a sucrose density of ~50%. Fractions collected from the sucrose density gradient were stored at -20°C prior to analysis by Western blot or ICP-MS. Each sample was weighed to confirm the expected density. Close concordance was observed between actual and predicted density of fractions.

qRT-PCR analysis of metallothionein expression:

All RNA isolation, cDNA synthesis and qRT-PCR reagents were purchased from Life Technologies, Mulgrave, Victoria, Australia. RNA from PC12 cells was isolated and DNAse-treated using the MagMAX™-96 Total RNA Isolation Kit, according to manufacturer's instructions. RNA concentrations were quantified using the Qubit® 2.0 Fluorometer. RNA (400 ng) was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit.

TaqMan Gene Expression assays for rat *MT1A*, *MT2A*, *MT3* and *GAPDH* were purchased from Life Technologies (Rn00821759_g1, Rn01536589_g1, Rn00588658_g1 and Rn01775763_g1, respectively). Each 10 µl reaction mix consisted of 0.5 µl of Taqman Gene Expression assays containing FAM-labelled probes, 5 µl of 2x TaqMan Fast Advanced Master mix and 4 µl of cDNA template (diluted 1:8). Duplicate reactions were performed using the LightCycler 480 (Roche) and the following conditions: 2 min at 50°C, 20 s at 95°C, followed by 45 cycles of 3 s at 95°C, and 60°C for 30 s. Cycle threshold (Ct) values were calculated as the lowest cycle number producing an exponential increase in PCR product amplification. The Delta-Ct method was used to normalise target gene expression in each sample to expression of the housekeeping gene, *GAPDH,* and are expressed as fold induction compared to DMSO-treated cells.

References:

Price. K.A., Crouch, P.J., Volitakis, I., Paterson, B.M., Lim, S.C., et al. (2011). Mechanisms controlling cellular accumulation of copper-*bis*(thiosemicarbazone) complexes. *Inorg. Chem*. **50,** 9594-9605**.**

Saegusa, C., Fukuda, M., Mikoshiba, K. (2002). Synaptotagmin V is targeted to dense-core vesicles that undergo calcium-dependent exocytosis in PC12 cells. *J. Biol. Chem.* **277,** 24499-24505.