Supplemental data

The mechanism of S-sulfocysteine-mediated neurodegeneration in molybdenum cofactor deficiency

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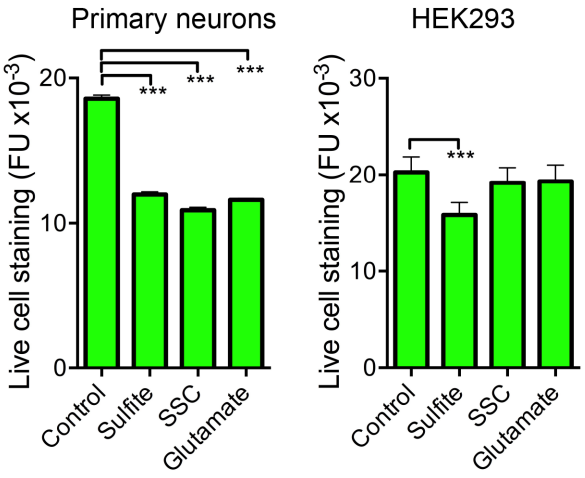
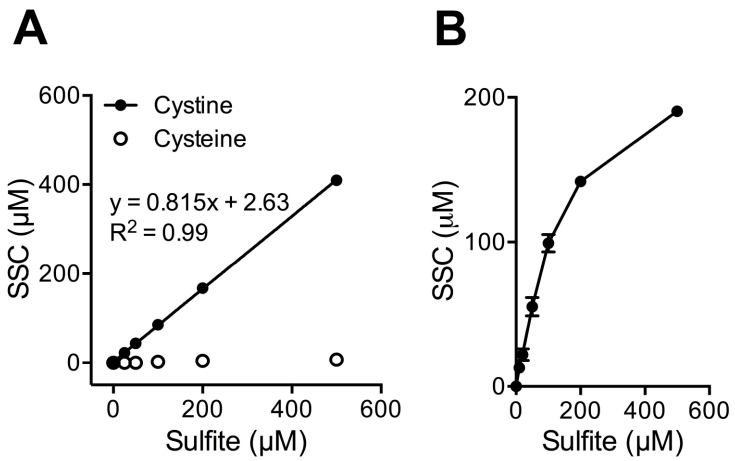
Supplemental figures and figure legends



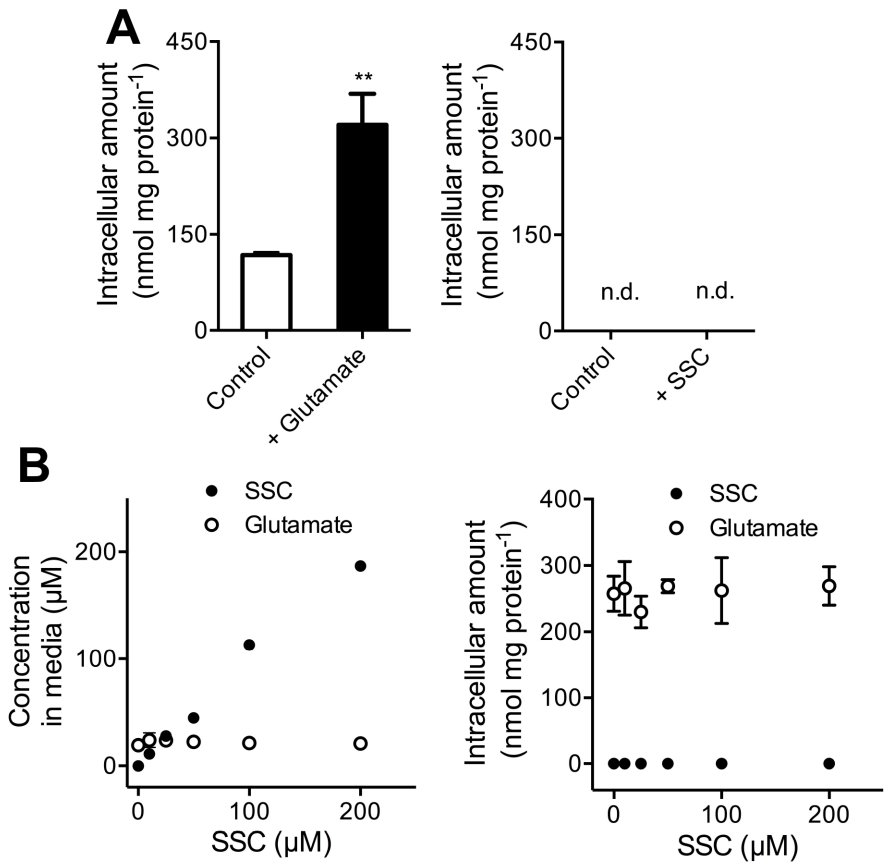
Supplemental Figure 1. SSC induces toxicity in primary neurons and not in HEK293 cells.

The quantification of live cells from the live/dead staining of mouse primary cortical neurons and HEK293 cells was performed using a commercial kit by measuring the fluorescence of calcein (n = 6 for each condition) after treatment with sulfite, SSC, glutamate and under control conditions. Data are presented as mean ± s. e. m., (n = 6 for each condition), $^{**}p < 0.01$; $^{***}P < 0.001$, Two-Way ANOVA, Dunnet multiple comparisons.



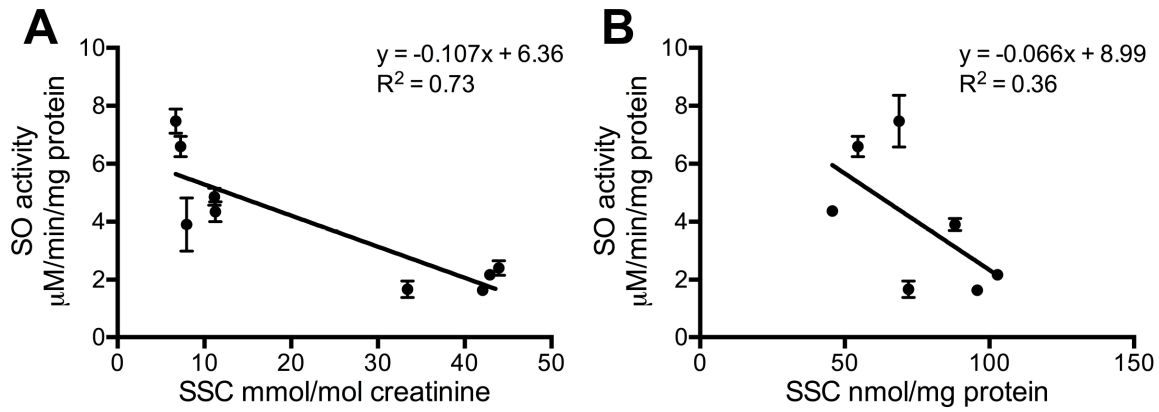
Supplemental Figure 2. SSC is formed stoichiometrically by the reaction of sulfite and cystine.

(**A**) SSC *in vitro* synthesis from the reaction of sulfite (0-500 µM) with either 2 mM cysteine (empty circles) or 2 mM cystine (filled circles), the linear fitting corresponding to SSC formation from cystine and sulfite is highlighted with the corresponding slope. (**B**) SSC formation in neuronal culture media after addition of sulfite (0-500 µM). Data are presented as mean (n=3) ± s.d.



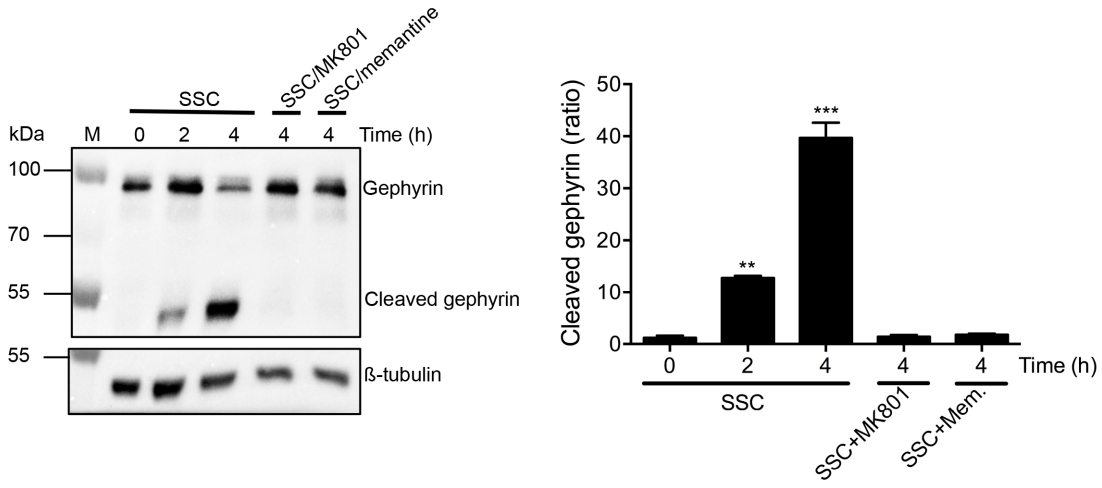
Supplemental Figure 3. SSC is not transported into primary mouse cortical neurons.

(**A**) Uptake capacities of cortical neurons for glutamate and SSC were assessed after incubation of cultured cortical neurons with 100 μM of either metabolite, normalized to total extracted proteins (n.d.: not detected). (**B**) Different concentrations of SSC (0 to 200 μM) were co-added with glutamate (100 μM) into culture media of primary mouse cortical neurons and SSC as well as glutamate were quantified in the culture media (left panel) and inside the neurons (right panel) after 1h treatment to evaluate transport capacity of neurons for each metabolite. Data are presented as mean (n=3) ± s.d. $^{**}P < 0.01$, Two-tailed unpaired Student's t-test.



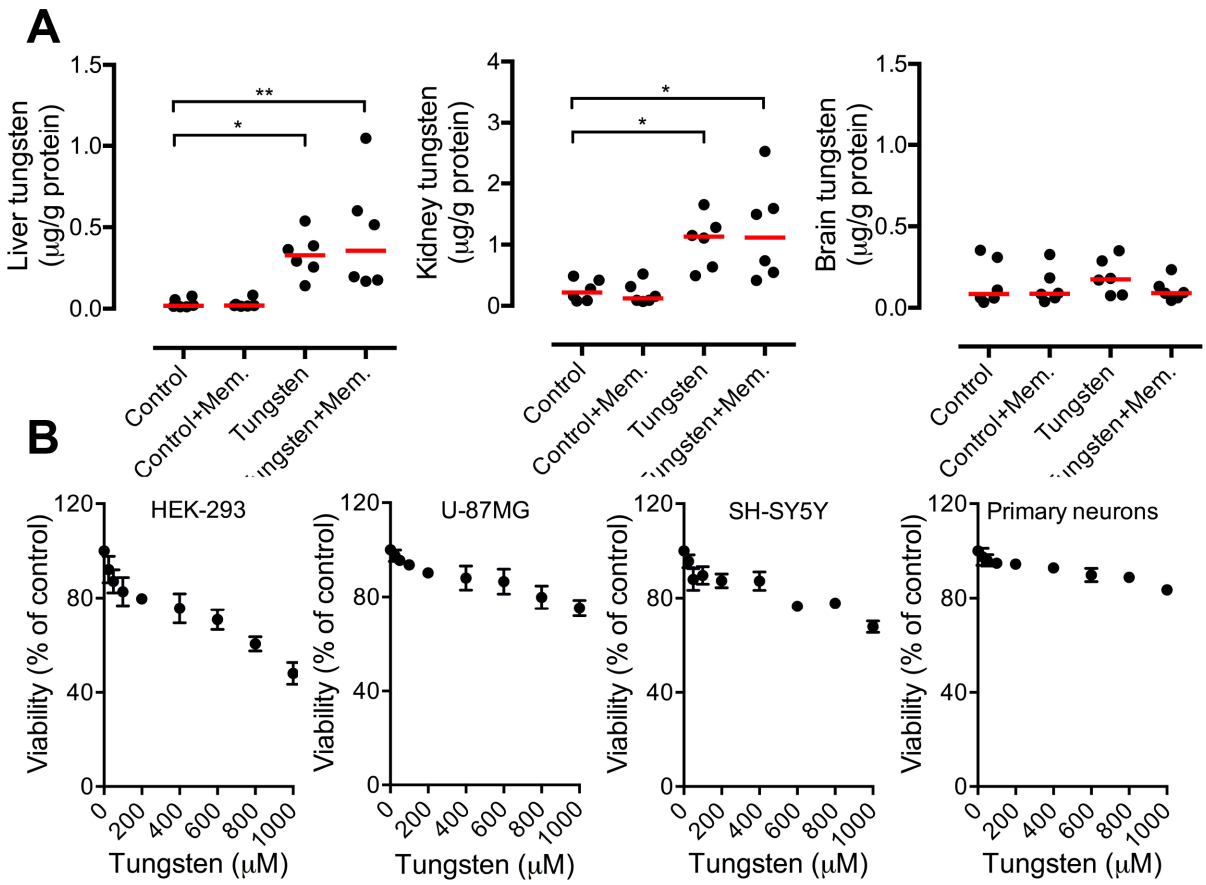
Supplemental Figure 4. SSC levels in urine and brain correlates with decrease in liver SO activity.

Sulfite oxidase activities were measured in tungsten treated mice and correlated with SSC levels in urine (**A**) or brain extracts (**B**). The linear fitting corresponding to the correlation between SO activities and SSC levels is highlighted in each panel with the corresponding slope. Data are presented as mean ± s.d., SO activities are measured in duplicates and each data point corresponds to a different mouse.



Supplemental Figure 5. Memantine prevents SSC-dependent cleavage in primary neurons.

Representative western blot of gephyrin in hippocampal neurons treated with SSC alone (100 μM), with MK801 (1 μM) or memantine (10 μM) at different time points (left panel). Quantification of band intensities of cleaved gephyrin is shown in the right panel. Data are presented as mean ± s. e. m., (n = 3), $^{**}p < 0.01$; $^{***}P < 0.001$, Two-Way ANOVA, Dunnet multiple comparisons.



Supplemental Figure 6. Tungsten does not induce direct toxicity in the brain.

(**A**) Tungsten total content in crude extracts of liver, kidney and brain of the different mice groups of the Memantine (Mem.) study was measured using inductively coupled plasma mass spectrometry, (n = 7 for each group). (**B**) Dose-dependent toxicity of tungsten in human kidney cells (HEK293); human glial cell line (U-87MG); human neuroblastoma cells (SH-SY5Y) and mouse primary neurons (n = 5 for each group). Toxicity was determined using the MTT assay and the calculated LD50 are HEK293: 1082 ± 20 µM; U-87MG: 3083 ± 31 µM; SH-SY5Y: 2180 ± 40 µM and primary neurons: 5282 ± 62 µM. Data are presented as mean ± s.d., horizontal line indicate median value. *$p < 0.05$; ***$P < 0.001$, Two-Way ANOVA, Dunnet multiple comparisons.

Supplementary Videos:

Supplemental Video 1: Control mouse after 4-week treatment

Supplemental Video 2: Tungsten-treated mouse after 4-week treatment