Supplementary method and materials

Protein electrophoresis and immunoblotting

Expression of γ-glutamyl transferase (GGT), 5-oxoproline (OPLAH) and γ-glutamyl cysteine ligase catalytic unit (GCLC) in HT22, PC12 and primary cortical neuron cells was determined by immunoblotting. Cells were washed in PBS, scraped into phosphate lysis buffer with 0.5% N-lauroylsarcosine, sonicated, and cleared by centrifugation. Protein concentrations in the cell extracts were determined Bradford assay. 25 ug of protein were solubilized in 1x NuPAGE LDS sample buffer (Invitrogen), electrophoresed on NuPAGE 4-12% Bis-Tris Gel and transferred to immobilon-P PVDF membrane (Millipore). The primary antibodies used were anti-GGT1 (Sigma WH0002678M1; 1:1000), anti-OPLAH (Abcam ab85215; 1:200), anti-GCLC (Abcam ab41463; 1:1000) and anti-human β-actin (Cell Signaling 4967; 1:1000). Secondary antibodies included horseradish peroxidase conjugated (HRP) anti-rabbit IgG (GE Healthcare NA934; 1:5000) for anti-OPLAH, anti-GCLC and anti-human β-actin, and HRP anti-mouse IgG (GE Healthcare NA931; 1:5000) for anti-GGT1. Blots were treated with SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific) and visualized with ImageQuant LAS-4000 mini (GE Healthcare). Protein expression was quantified with Image-J software (NIH, http://rsb.info.nih.gov/ij/) using β-actin as a control for normalization.

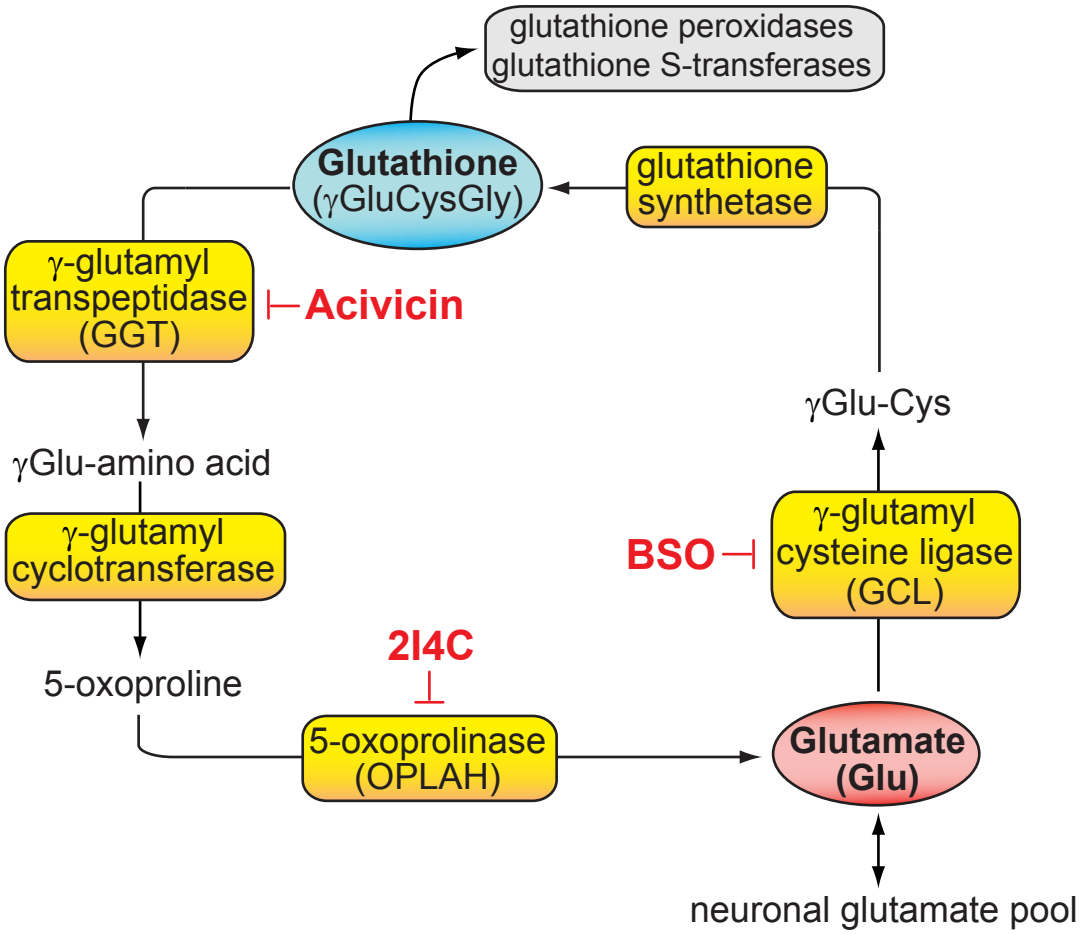
Supplementary figure legends

Supplementary Fig. 1. The γ-glutamyl cycle (glutathione cycle). Glutamate is liberated from the cycle via OPLAH, and used as a substrate for glutathione synthesis by GCL. Inhibitor drugs (acivicin, 2I4C, and BSO) are shown with their respective targets. OPLAH, 5-oxoprolinase; GCL, γ-glutamyl cysteine ligase; 2I4C, 2-imidazolidone-4-carboxylate; BSO, buthionine sulfoximine.

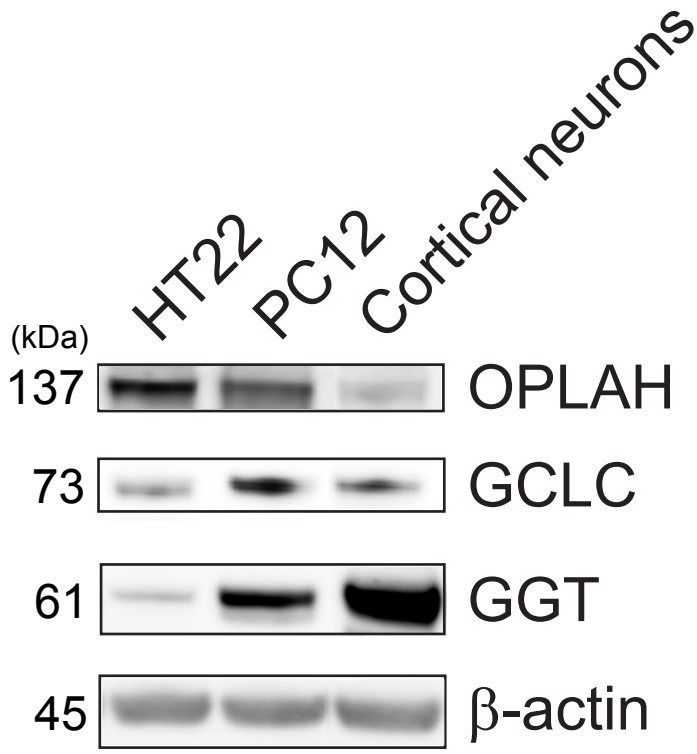
Supplementary Fig. 2. Expression of γ-glutamyl transferase (GGT), 5-oxoprolinase (OPLAH) and γ–glutamyl cysteine ligase catalytic subunit (GCLC) in HT22 and PC12 cells. 25 ug of protein was immunoblotted for each sample.

Supplementary Fig. 3. Acivicin increases γ–glutamyl cysteine ligase catalytic subunit (GCLC) expression in PC12 cells. (A,B) Immunoblots of GCLC and β-actin expression in HT22 and PC12 cells treated with acivicin for 24 hours. 25 ug of protein was loaded in each lane, and blots were probed with anti-GCLC (73kDa) and anti-β-actin (45kDa) antibodies. (C,D) Acivicin increases GCLC expression in PC12 cells. Relative expression was determined by normalization of density of GCLC protein bands to that of β-actin of the same blot. Data represent mean ± standard error of triplicates, representative of 3 independent experiments. *, $p<0.05$ by 1-way ANOVA with Tukey-Kramer post hoc test.

Supplementary Fig. 1 - Glutathione cycle and inhibitors



Supplementary Fig. 2 - Expression of glutathione cycle enzymes in HT22 and PC12 cells



Supplementary Fig. 3 - Acivicin increases GCLC expression in PC12 cells