SUPPLEMENTARY FIGURES

Hippocampal neurons require a large pool of glutathione to sustain dendrite integrity and cognitive function

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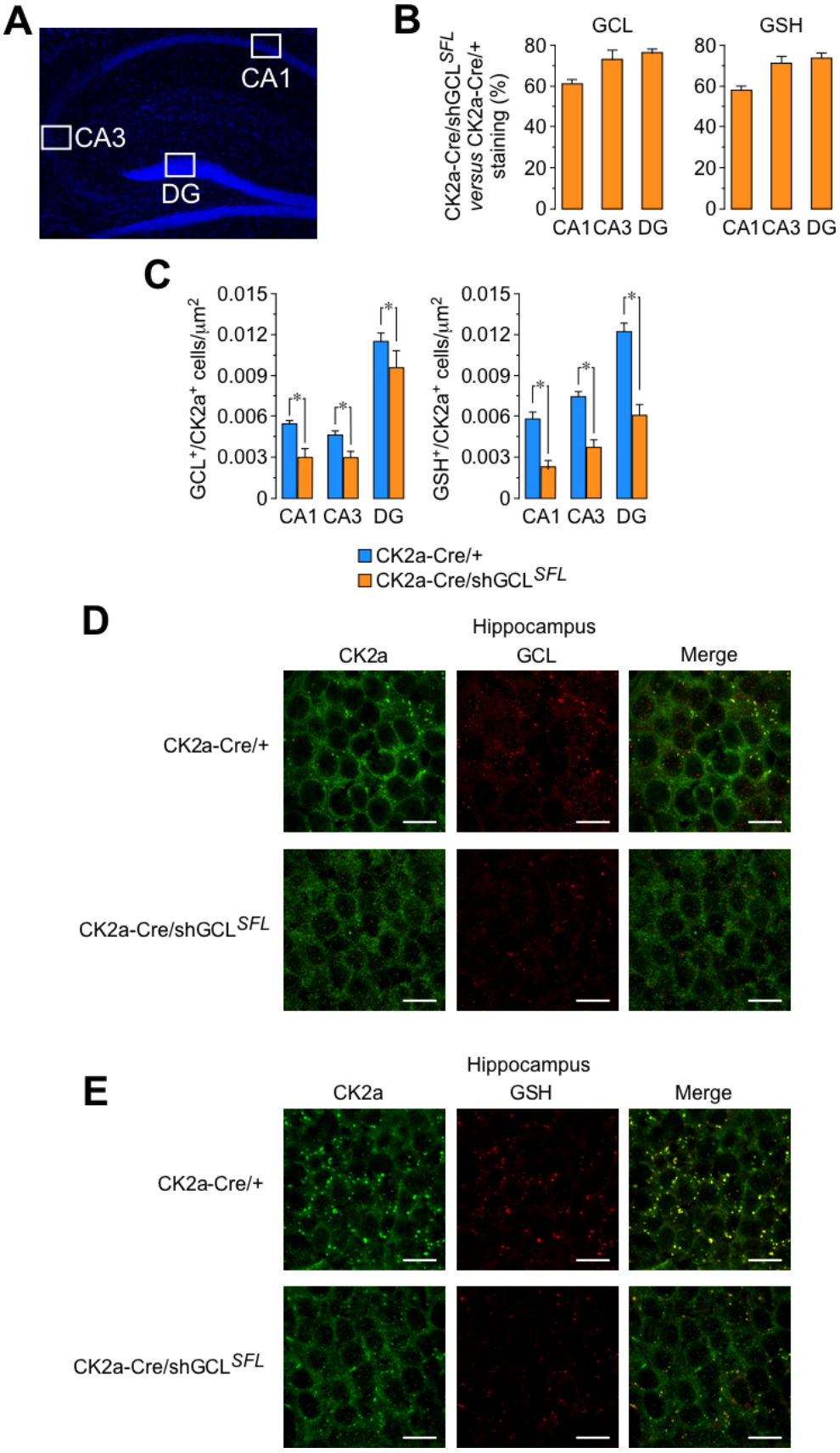
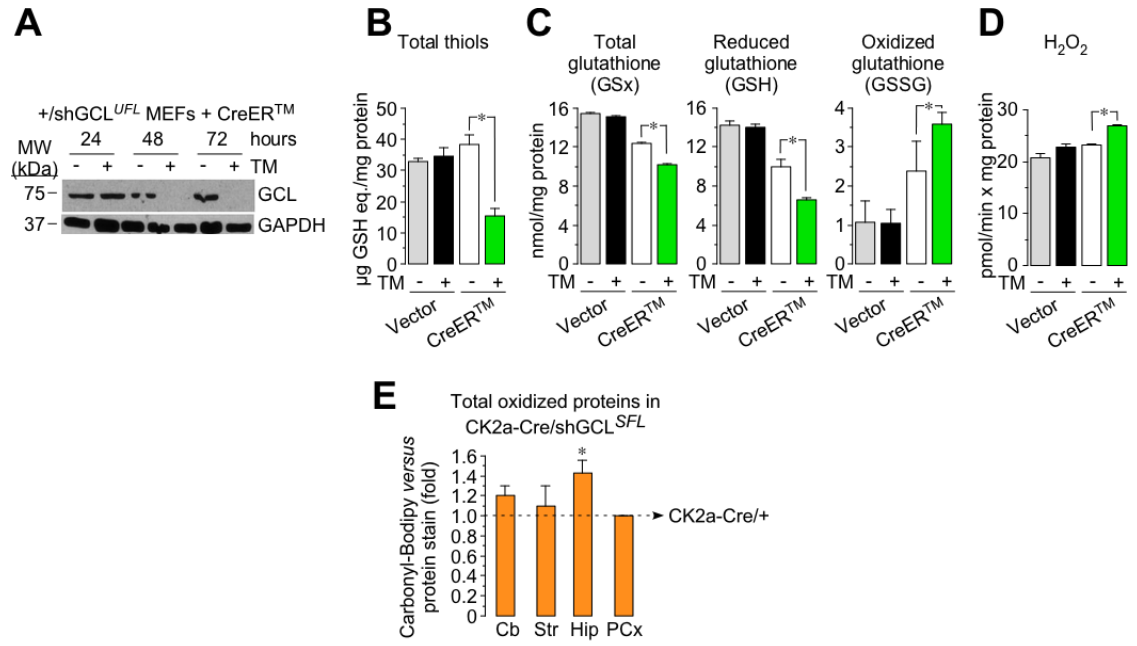


Supplementary Figure S1 GCL and GSH staining data in the hippocampus of the CK2a-Cre/shGCL^{SFL} mice *in vivo*. (**A**) Immunofluorescence DAPI signal showing the selected

hippocampal areas analyzed (CA1, CA3 DG). (**B**) GCL and GSH relative abundances expressed as the ratio CK2a-Cre/shGCL^{SFL} versus littermate controls (CK2a-Cre/+). (**C**) Number of GCL^{+}/CK2a^{+} and GSH^{+}/CK2a^{+} cells in CK2a-Cre/shGCL^{SFL} and CK2a-Cre/+ mice. Data are mean ± SEM values (n=4). *p<0.05 (Student's *t* test). (**D,E**) Representative images for GCL (D) and GSH (E) immunostaining in the hippocampus of the 9 months-old CK2a-Cre/shGCLSFL male mice and their littermates controls (CK2a-Cre/+). Scale bars, 50 μm.



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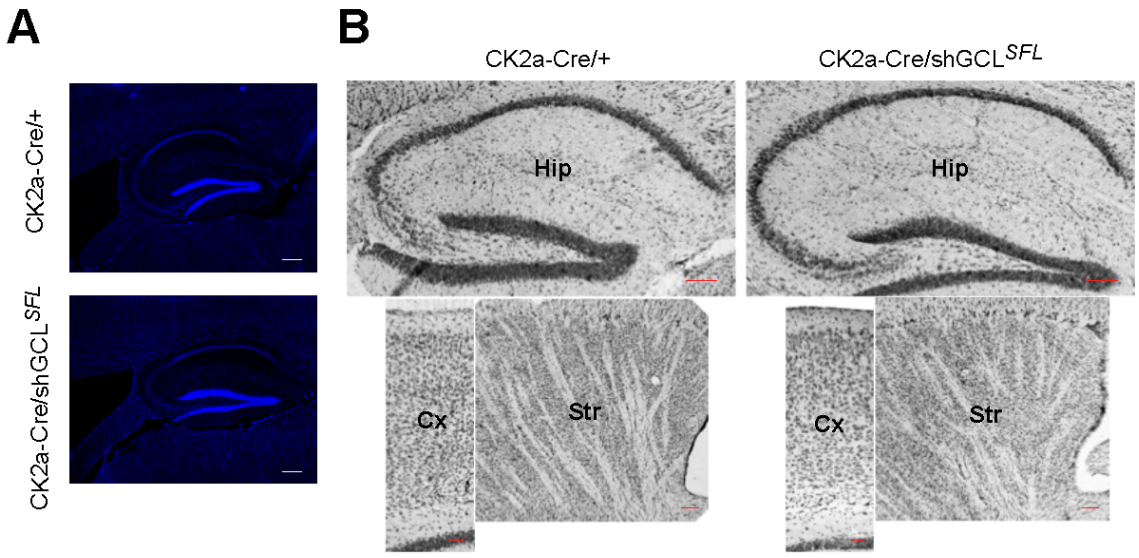
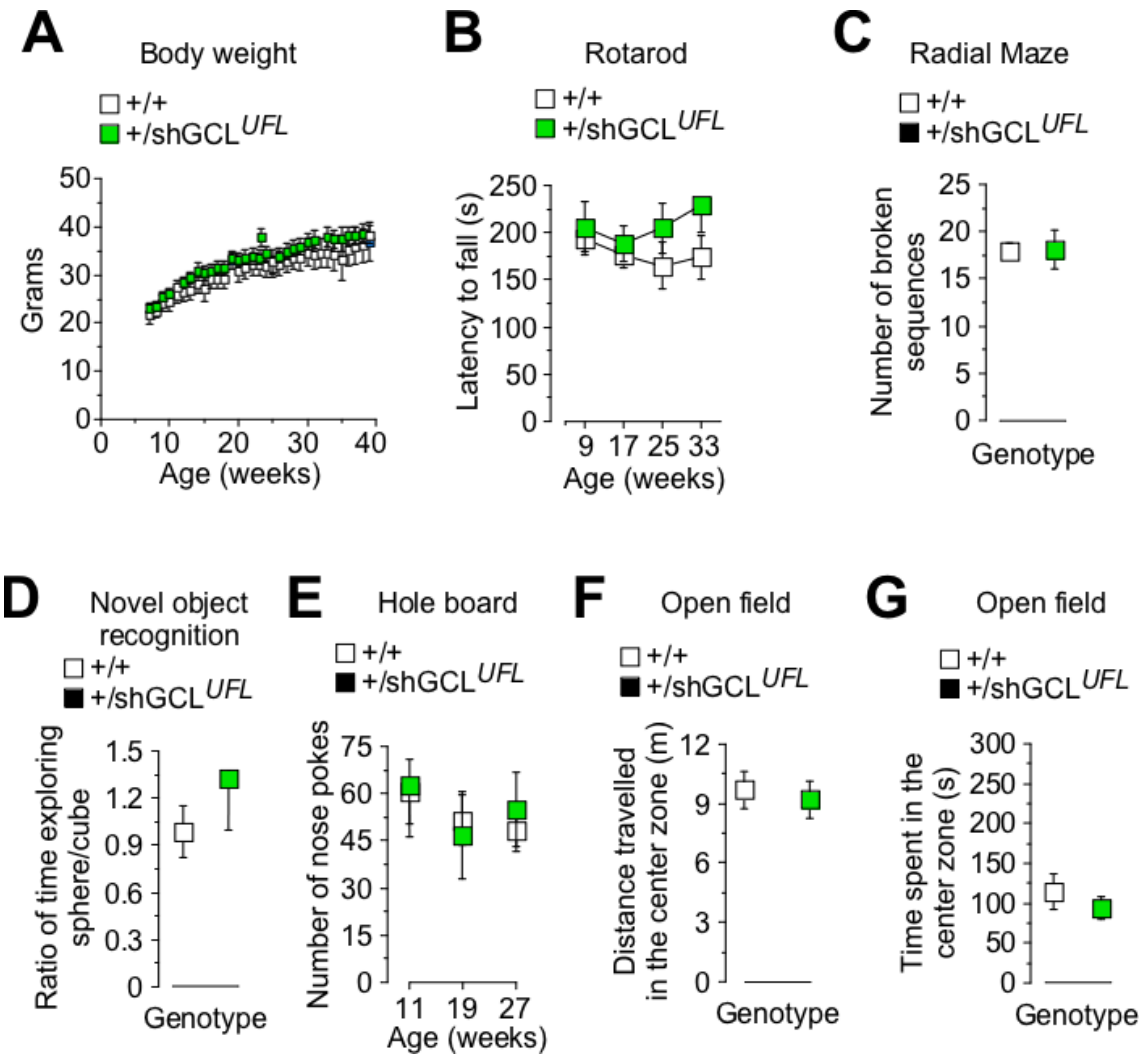
Spot	Carbonyl-Bodipy: CK2a-Cre/+	Carbonyl-Bodipy: CK2a-Cre/shGCL^{SFL}	Protein stain: CK2a-Cre/+	Protein stain: CK2a-Cre/shGCL^{SFL}	Protein acronym	Accession number	Score	Coverage (%)	Matched peptides	Oxidation fold
1					VATA	P50516	199	7	4	2.64
2					VATB	P62814	215	40	20	2.61
3					DPYL2	O08553	306	62	30	1.84
4					ENOG	P17183	284	61	28	3.04
5/6					ENOA	P17182	230/252	56/59	23/29	1.42/1.97
7					PDHE1	Q9D051	99	42	13	3.50
8/9					MDH	P14152	145/141	31/39	14/16	2.20/2.42
10					GAPDH	P16858	166	45	15	2.20
11					KCRB	Q04447	139	45	20	2.93
12					PGAM1	Q9DBJ1	127	60	18	2.56
13					EF1A1	P10126	149	25	14	2.60

Supplementary Figure S2 Protein carbonylation and identification from the brain of the CK2a-Cre/shGCL^{SFL} mouse. (**A**) Western blotting in MEFs obtained from the +/shGCL^{UFL} mice shows efficient GCL protein knockdown when cells were transfected with a plasmid vector expressing a tamoxifen-dependent Cre recombinase (CreER^{TM}), only in the presence of tamoxifen (TM). A representative western blot is shown. (**B**) Tamoxifen induced a reduction in total thiols after transfecting +/shGCL^{UFL} MEFs with the plasmid vector expressing CreER^{TM} recombinase, but not when cells were transfected with the empty vector (*p<0.05; n=3; Student's *t* test) (**C**) Tamoxifen induced a reduction in total and reduced (GSH) glutathione, and an increase in oxidized glutathione (GSSG), after transfecting +/shGCL^{UFL} MEFs with the plasmid vector expressing CreER^{TM} recombinase, but not when cells were transfected with the empty vector (*p<0.05; n=3; Student's *t* test). (**D**) Tamoxifen induced ROS, as assessed by H_2O_2 measurement, in +/shGCL^{UFL} MEFs transfected with the plasmid vector expressing CreER^{TM} recombinase, but not when cells were transfected with the empty vector (*p<0.05; n=3; Student's *t* test). (**E**)

Quantification of total protein carbonylation in different brain regions of the CK2a-Cre/+ and the adult CK2a-Cre/shGCL^{SFL} male mice. Data are mean ± SEM values. *p<0.05 *versus* CK2a-Cre/+ (n=3) (Student's *t* test). (**F**) 2D-PAGE selected areas of the 13 proteins found to be most carbonylated, indicating the spots by arrows. These spots were purified and subjected to mass spectrometry identification. The accession number corresponds to Swissprot database. Proteins with score >50 were considered statistically significant according to the Probability based Mowse Score. Coverage represents the percentage of the protein sequence that was covered by the identified peptides. The oxidation fold is the mean values of fold increase in carbonyl levels in the CK2a-Cre/shGCL^{SFL} *versus* CK2a-Cre/+ mice (n=3 mice per group; 3 independent biological samples run in independent gels). Cb, cerebellum; Str, striatum, Hip, hippocampus; PCx, pre-frontal cortex. VATA and VATB, V-type proton ATPase A and B subunits, respectively; DPYL2, dihydropyriminidase-related protein 2; ENOG, neuron-specific enolase-γ; ENOA, enolase-α; GAPDH, glyceraldehyde-3-phophate dehydrogenase; PGAM1, phosphoglycerate mutase-2; pyruvate dehydrogenase E1 subunit, PDHE1; MDH1, malate dehydrogenase-1; KCRB, creatine kinase B; EF1A1, elongation factor 1α.



Supplementary Figure S3 Morphological analysis of the CK2a-Cre/shGCL^{SFL} adult male brain. (**A**) Nuclear staining of the hippocampus shows no gross morphological differences in the hippocampus structure between the CK2a-Cre/shGCL^{SFL} and CK2a-Cre/+ male mice (scale bars, 200 µm). (**B**) No major structural changes were observed in the width of the DG, CA1 and CA3 regions of the hippocampus (Hip; scale bars, 50 µm), length of the cortex layers (Cx; Hip; scale bars, 50 µm), or cell density in the striatum (Str; Hip; scale bars, 100 µm) in the CK2a-Cre/shGCL^{SFL} when compared with the CK2a-Cre/+ male mice.



Supplementary Figure S4 No behavioral alterations in the shGCL^{UFL}/+ mice. (**A**) Unaltered body weight in the shGCL^{UFL}/+ male mice when compared with the wild type (+/+) male mice. (**B**) Unaltered performance of the shGCL^{UFL}/+ mice at the rotarod test. (**C**) Unaltered number of broken sequences of the shGCL^{UFL}/+ mice at the radial arm maze test. (**D**) Unaltered performance of the shGCL^{UFL}/+ mice at the novel object recognition test. (**E**) Unaltered number of nose pokes of the shGCL^{UFL}/+ mice at the Hole board test. (**F, G**) Unaltered distance travelled and time spent in the central zone of the shGCL^{UFL}/+ mice at the Open field test. Data are mean ± SEM values (n=6) (Student's *t* test).