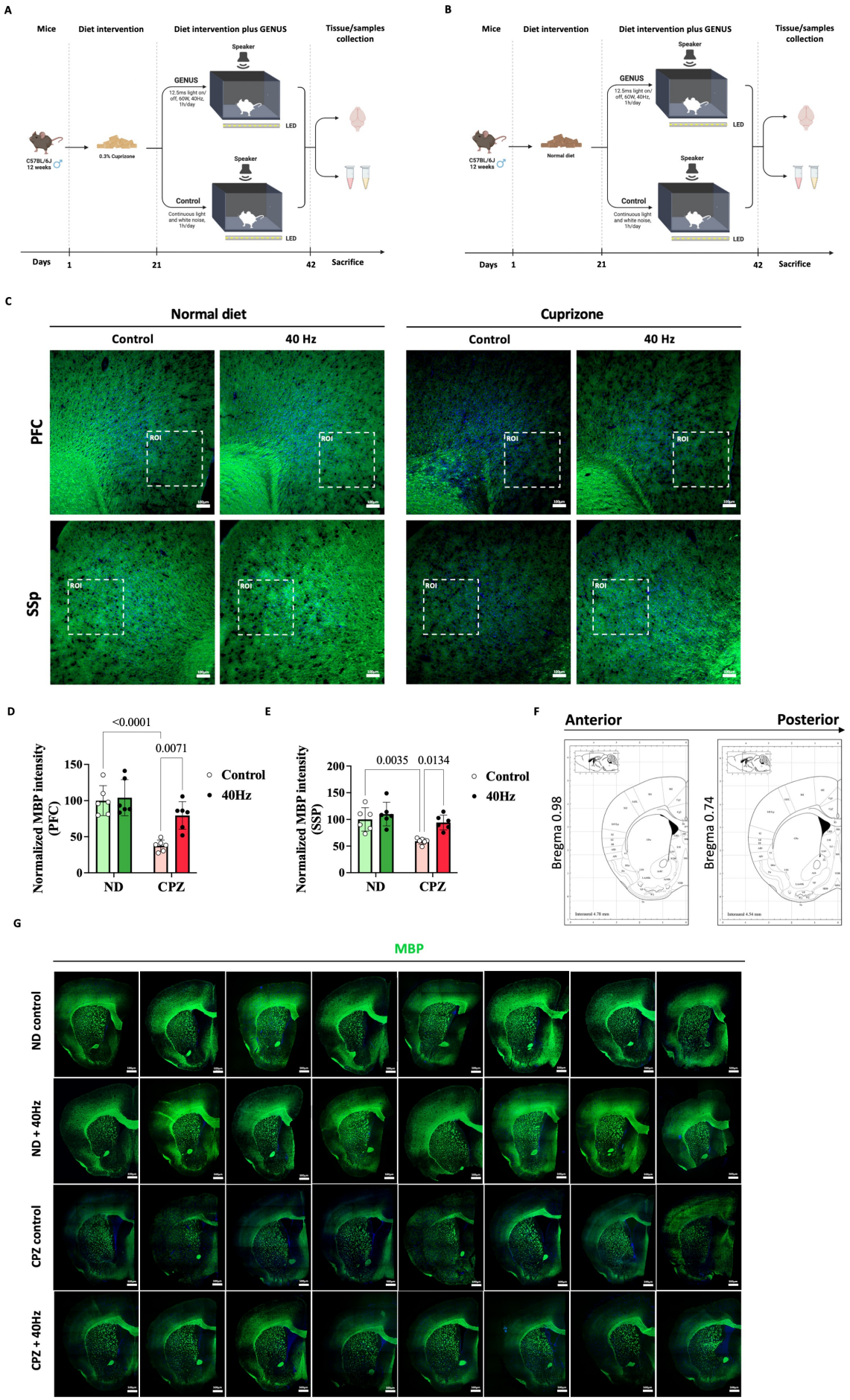
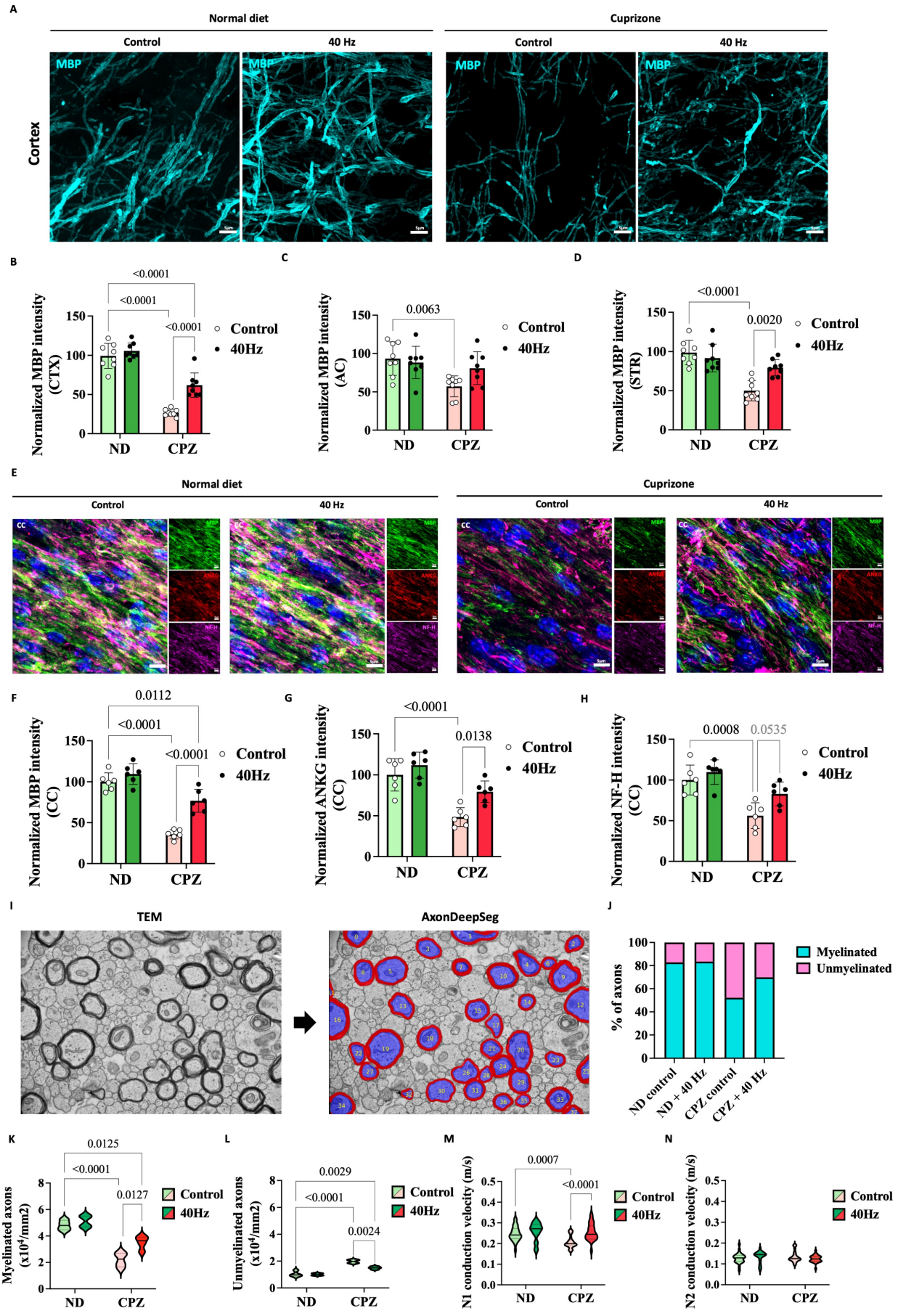
Supplementary Figures S1



Supplementary Figure S1: Study paradigm and complementary analyses of the myelination process.

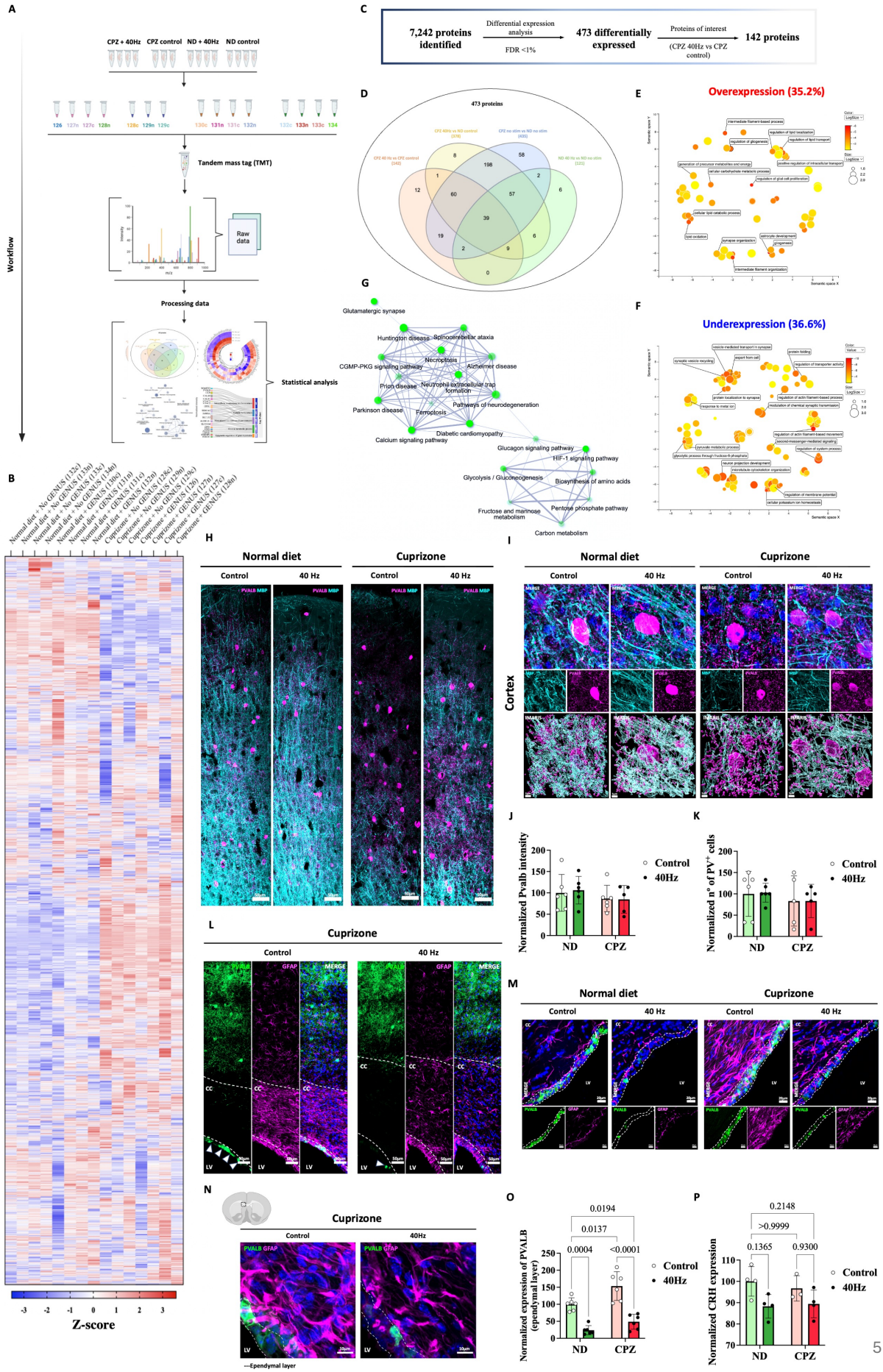
(A) Experimental paradigm: 12-week-old male C57BL/6J mice were exposed to either GENUS or control light and sound conditions duration of 3 weeks, in addition to being fed either a CPZ during 6 weeks. (B) Experimental paradigm: 12-week-old male C57BL/6J mice were exposed to either GENUS or control light and sound conditions duration of 3 weeks, in addition to being fed either a ND for 6 weeks. (C) Power spectral density was analyzed in the PFC and SSp using LFP recordings at two different time points(weeks 3 and 6), with 4 mice under a CPZ diet with (GENUS) or without stimulation (baseline). (D) Power analysis comparing baseline to GENUS in the PFC for the 1º LFP recording and 2º LFP recording. $F(1,3)=28.69$; $p=0.0127$. (E) Power analysis comparing baseline to GENUS in the PFC for the 1º LFP recording and 2º LFP recording. $F(1,3)=1.112$; $p=0.3690$. (F-H) IHC of PFC and SSp areas using MBP (green) antibody. A ROI area was drawn to analyze the expression of MBP (6 mice per group, one technical replicate, scale bar- 100µm). (G) Normalized fluorescence intensity of MBP in the PFC. $F(1,20)=5.791$; $p=0.0259$. (H) Normalized fluorescence intensity of MBP in the SSp. $F(1,20)=3.119$; $p=0.0926$. (I) Schematic representation of coronal sections analyzed aligned with the coordinates: Interaural: 4.78-4.54 mm, Bregma: 0.98-0.74 mm (Paxinos, G., & Franklin, K. B. J. (2001). (J) IHC of CC using MBP antibody (green). The image illustrates the CC of 32 animals (8 mice per group), highlighting the noticeable reduction in the CC size in the CPZ control group. All P-values were calculated using two-way ANOVA followed by Bonferroni's multiple comparisons test (Supplementary Data S14). Schematic panels A and B were created with BioRender. Source data are provided as a Source Data file.

Supplementary Figures S2



Supplementary Figure S2: Myelination and functional dynamics.
(A) IHC of the cortex using MBP antibody (cyan) at 63x. The image demonstrates complete disruption of myelin fibers in the cortex of the CPZ non-stimulated group. (B) Normalized fluorescence intensity of MBP in the cortex (n=8 per group, one technical replicate). $F(1,28)=10.73$; $p=0.0028$. (C) Normalized fluorescence intensity of MBP in the anterior commissure(n=8 per group, one technical replicate). $F(1,28)=4.232$; $p=0.0491$. (D) Normalized fluorescence intensity of MBP in the striatum(n=8 per group, one technical replicate). $F(1,28)=12.77$; $p=0.0013$. (E-H) IHC of the CC using MBP (green), ANKG (red) and NFH (magenta) antibodies (n=6, one technical replicate, tile scan, scale bar- 50μm). (F) Normalized intensity of MBP in the CC. $F(1,28)=11.76$; $p=0.0027$). (G) Normalized intensity of ANKG in the CC. $F(1,20)=2.329$; $p=0.1426$. (H) Normalized intensity of NFH in the CC. $F(1,20)=1.704$; $p=0.2066$. (I) Transmission electron microscopy image of CC and its analysis using AxonDeepSeg. (J) Violin graph showing the myelinated axons per mm^2 across all groups. $F(1,12)=4.598$; $p=0.0532$. (K) Violin graph showing all groups' unmyelinated axons per mm2. $F(1,12)=10.78$; $p=0.0065$. (L) Bar graph showing the percentage of myelinated and unmyelinated axons per group. (M) Violin graph showing N1 conduction velocity across all groups. $F(1,104)=7.438$; $p=0.0075$. (N) Violin graph showing N2 conduction velocity across all groups. $F(1,104)=2.741$; $p=0.1008$. All P-values were calculated using two-way ANOVA followed by Bonferroni's multiple comparisons test (Supplementary Data S14). Source data are provided as a Source Data file.

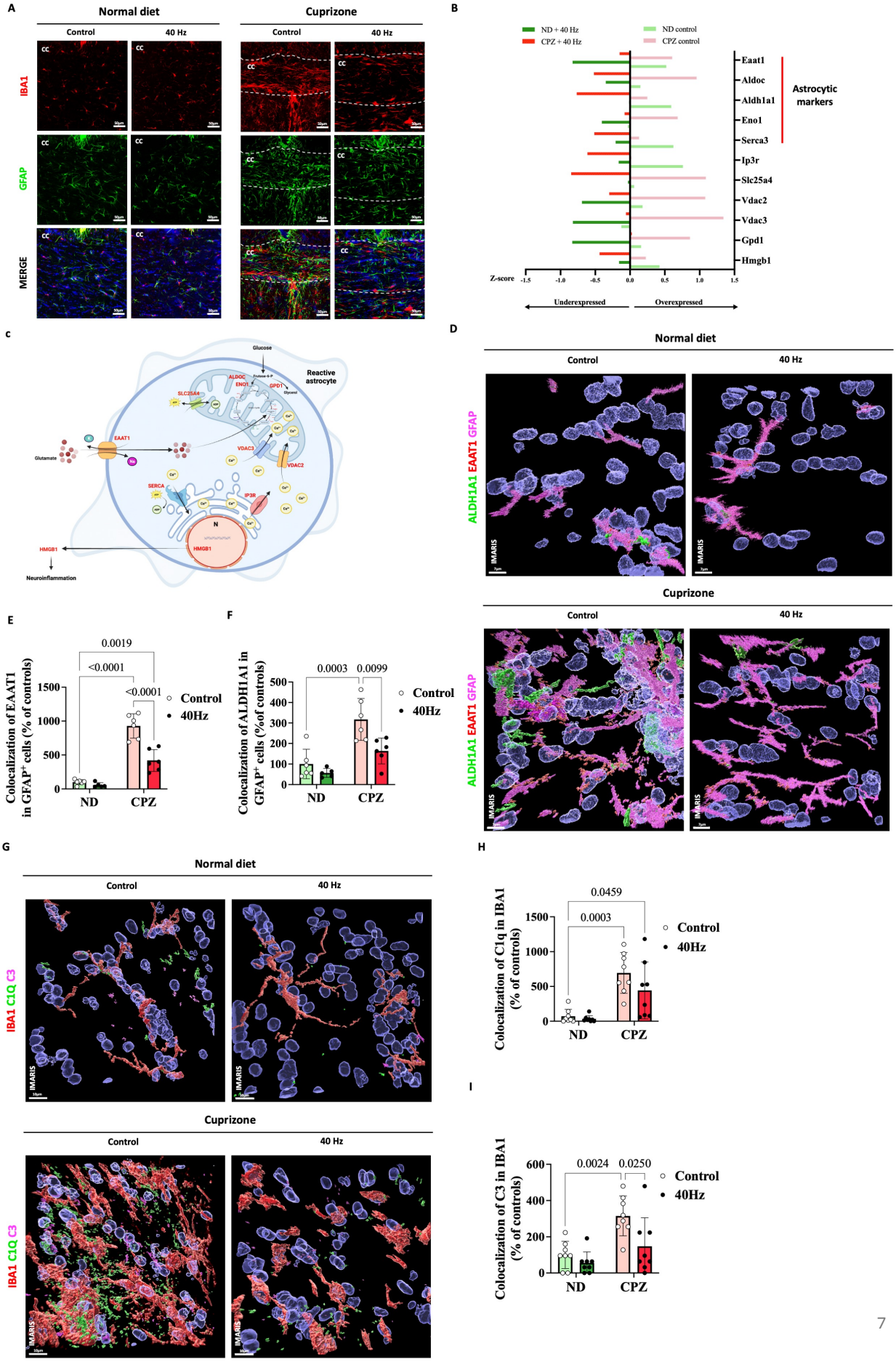
Supplementary Figures S3



Supplementary Figure S3: Proteomics workflow and complementary analyses of the brain proteome.

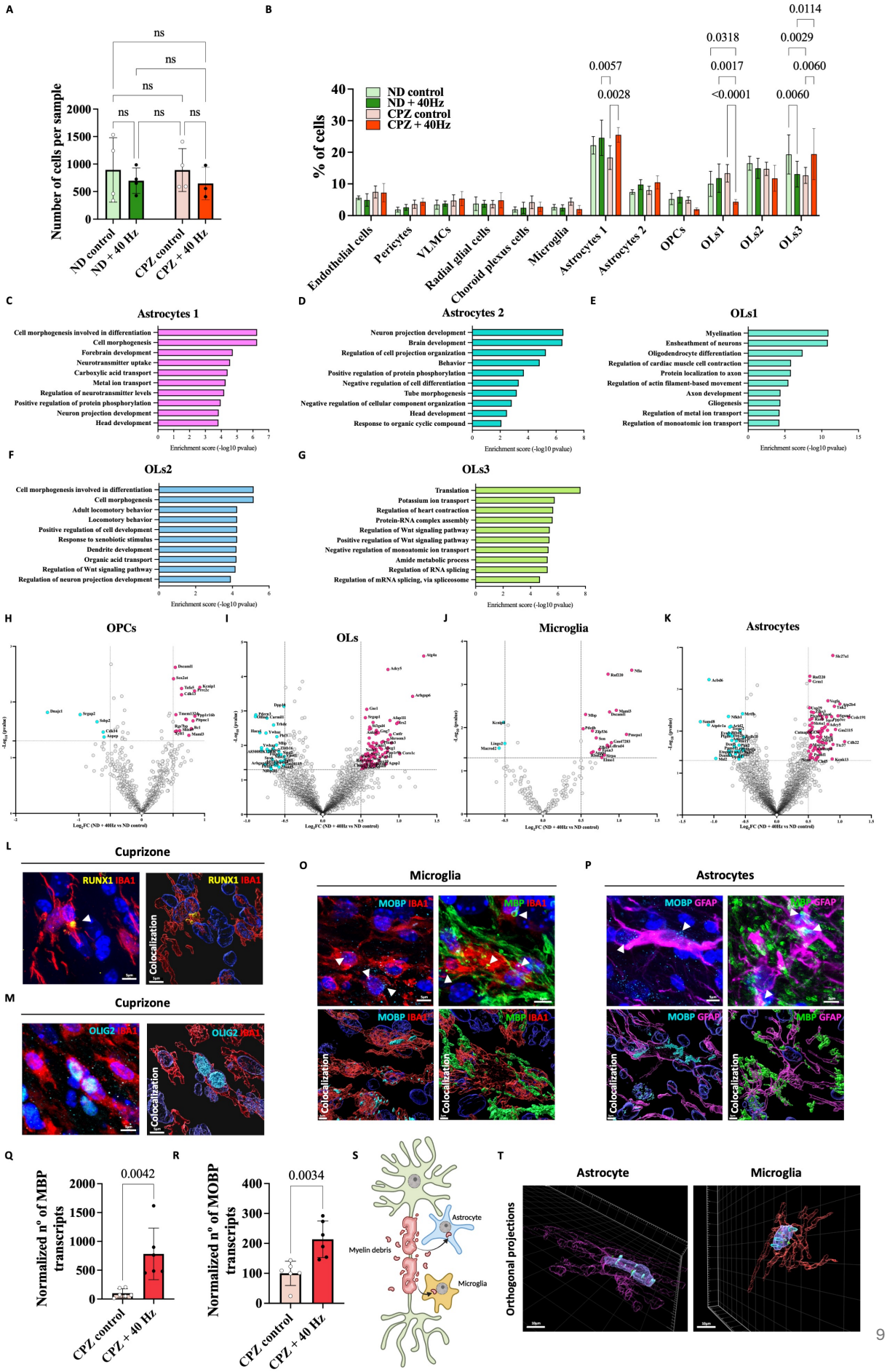
(A) Workflow for brain proteome analysis using a TMT approach (one technical replicate). (B) Heatmap of 7,242 proteins identified. (C) Schematic representation of target proteins filtered. (D) Venn diagram of the statistically significant proteins overlapped or unique proteins across the 4 combinations of interest. (E) Scatterplot showing representative clusters after semantic reduction of enriched GO terms of overexpressed proteins after statistical analysis ($p<0.05$). (F) Scatterplot showing representative clusters after semantic reduction of enriched GO terms of underexpressed proteins after statistical analysis ($p<0.05$). (G) Hierarchical clustering tree of the enriched protein sets (Panel I, II, III). (H-K) IHC of the cortex using MBP (cyan) and PVALB (magenta) antibodies (n=6, except CPZ+40Hz n=5, one technical replicate, tile scan, scale bar- 50μm). (I) IHC of the cortex was performed using MBP (cyan) and PVALB (magenta) antibodies at high magnification (63X) with superresolution Airyscan imaging. Image reconstruction was carried out using Imaris to show myelin fibers and PV neurons network in the cortex. (J) Normalized number of $PVALB^+$ cells in the cortex. $F(1,18)=0.0052$; $p=0.9432$. (K) Normalized intensity of PVALB in the cortex. $F(1,18)=0.0789$; $p=0.7817$. (L) IHC using PVALB (green) and GFAP (cyan) antibodies to analyze the expression of PVALB protein in different cells (tile scan, scale bar- 50μm) in the CPZ cohort. (M) Detection of PVALB expression in the ependymal layer of the third ventricle. (N) IHC using PVALB (green) and GFAP (cyan) antibodies focused on the ependymal layer (40X objective). (O) Quantification of PVALB expression in the ependymal layer (n=6 per group, one technical replicate). ROI was drawn and PVALB expression was normalized by area. $F(1,20)=1.753$; $p=0.2004$. (P) Normalized CRH expression showing absence of stress response when comparing the groups ($p>0.05$). All P-values were calculated using two-way ANOVA followed by Bonferroni's multiple comparisons test (Supplementary Data S14). Source data are provided as a Source Data file.

Supplementary Figures S4



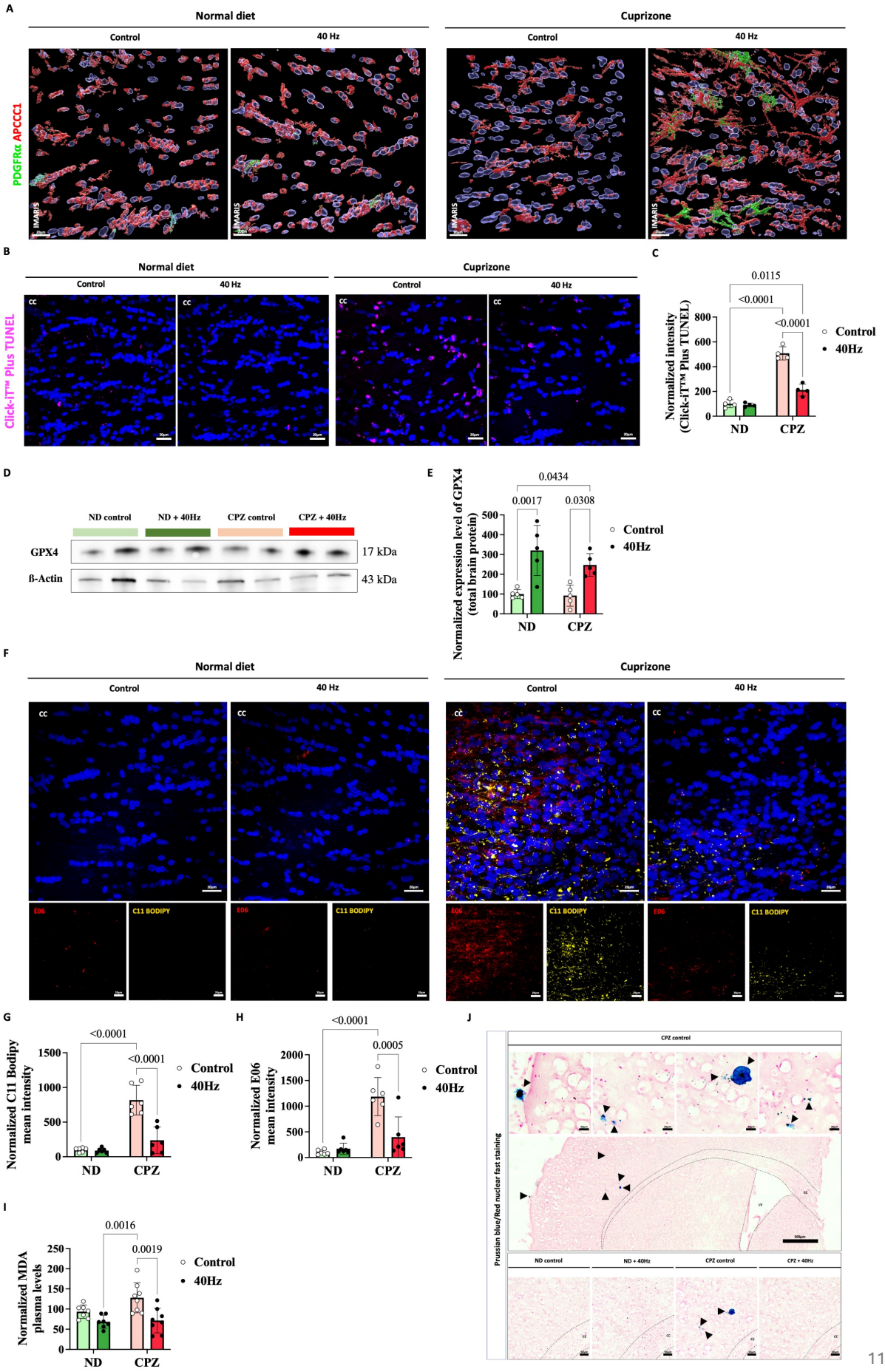
Supplementary Figure S4: Microgliosis and astrogliosis in the cuprizone mouse model.
(A) IHC of IBA1 (red) and GFAP (green). Tile scans at 40x were acquired to quantify the number of cells and volume of cells in the CC. (B) Bar graph of over and under-expressed proteins based on their Z-score, associated with astrocytic activity. (C) Representation of an astrocyte and its involvement in producing proinflammatory molecules, such as HMGB1. (D) Imaris reconstruction of IHC images stained for GFAP (magenta), ALDH1A1 (green) and EAAT1 (red). (E) Normalized colocalization of EAAT1 $GFAP^+$ cells in the CC area (n=6 mice per group, one technical replicate, scale bar- 10µm). F (1,19)=19.59; p=0.0003. (F) Normalized colocalization of ALDH1A1 $GFAP^+$ cells in the CC area (n=6 mice per group, one technical replicate, scale bar- 10µm). F (1,19)=3.294; p=0.0854. (G) Imaris reconstruction of IHC images stained for IBA1 (red), C1Q (green) and C3 (magenta). (H) Normalized colocalization of C1Q $IBA1^+$ cells in the CC area (n=6 mice per group, one technical replicate, scale bar- 10µm). F (1,28)=1.377; p=0.2505. (I) Normalized colocalization of C3 $IBA1^+$ cells in the CC area (n=6 mice per group, one technical replicate, scale bar- 10µm). F(1,28)=2.652; p=0.1146. All P-values were calculated using two-way ANOVA followed by Bonferroni's multiple comparisons test (Supplementary Data S14). Schematic panel C was created with BioRender. Source data are provided as a Source Data file.

Supplementary Figures S5



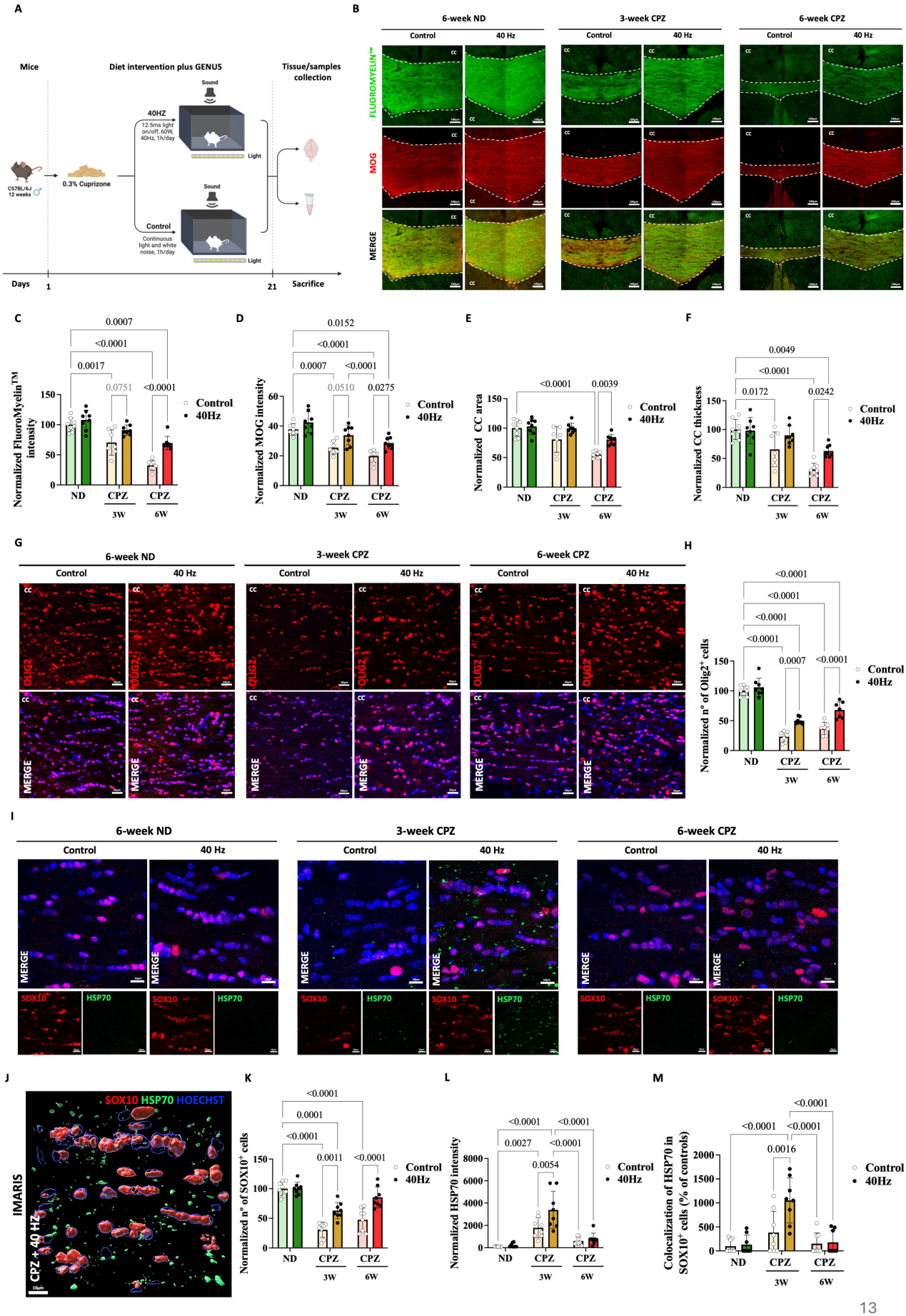
Supplementary Figure S5: Cellular composition and DEGs analysis.
(A) Number of cells per sample. P-value was calculated using two-way ANOVA followed by Bonferroni's multiple comparisons test (Supplementary Data S14). (B) Bar graph showing the percentage of cells per cluster. Ordinary two-way ANOVA followed the post hoc Bonferroni multiple comparison test. (C-G) Bar plot of significantly enriched biological pathways of top-50 upregulated genes in Astrocytes1, Astrocytes2, OLs1, OLs2 and OLs3, respectively. (H-K) Volcano plot showing the top differentially expressed genes within OPCs, OLs, microglia and astrocytes, respectively. (L) IHC of RUNX1 (yellow) and IBA1 (red) and 3D reconstruction of the images showing colocalization. (M) IHC of OLIG2 (cyan) and IBA1 (red) and 3D reconstruction of the images showing colocalization. (O) IHC of MOBP (cyan) and IBA1 (red) and MBP (green) and IBA1 (red) and 3D reconstruction of the image showing colocalization. (P) IHC of MOBP (cyan) and GFAP (magenta) and MBP (green) and GFAP (magenta) and 3D reconstruction of the image showing colocalization. (Q) Normalized number of MBP transcripts per image (n=6 CPZ + 40Hz and n=6 CPZ control, one technical replicate, scale bar- 20µm, CC area). The graph displays normalized values to the CPZ control group in percentage using an unpaired two-tailed Student's *t*-test. (R) Normalized number of MOBP transcripts per image (n=6 CPZ + 40Hz and n=6 CPZ control, one technical replicate, scale bar- 20µm, CC area). The graph displays normalized values to the CPZ control group in percentage using an unpaired two-tailed Student's *t*-test. (S) Schematic representation of the presence of OL-derived transcripts in microglia and astrocytes. (T) Imaris orthogonal views. Schematic panel S was created with BioRender. OPCs – oligodendrocytes progenitor cells, OLs – oligodendrocytes. NS – non-significant. Source data are provided as a Source Data file.

Supplementary Figures S6



Supplementary Figure S6: Oligodendrocytes and ferroptosis.
(A) Imaris reconstruction of IHC images stained for immature OLs (PDGFRα) and mature OLs (APCCC1). (B) Click-iT TUNEL Alexa Fluor 647 (C) Normalized mean intensity of Tunel 647 (n=4 per group, one technical replicate). $F(1,28)=51.80$; $p<0.0001$. (D) Western blot analysis of GPX4 using protein isolated from the left hemisphere of the brain (two animals per group, one technical replicate). (E) Normalized expression level of GPX4 in the brain (n=5 per group, one technical replicate). $F(1,16)=0.9434$; $p=0.3459$. (F-H) IHC of C11 Bodipy (yellow) and E06 (red) of the CC of 12-week WT mice under CPZ and ND after 6 weeks of diet intervention and 3 weeks of 1 h/day of GENUS or control (n=6 mice per group, one technical replicate, scale bar-20μm). (G) Normalized mean intensity of C11 Bodipy in the CC. $F(1,20)=22.97$; $p=0.0001$. (H) Normalized mean intensity of E06 in the CC. $F(1,20)=14.55$; $p=0.0011$. (I) Normalized levels of MDA in plasma (n=8 in CPZ groups and n=7 in ND groups, one technical replicate). $F(1,26)=2.502$; $p=0.1258$. (J) Prussian blue staining revealed the presence of Fe^{3+} in the CPZ non-stimulated group. All P-values were calculated using two-way ANOVA followed by Bonferroni's multiple comparisons test (Supplementary Data S14). Source data are provided as a Source Data file.

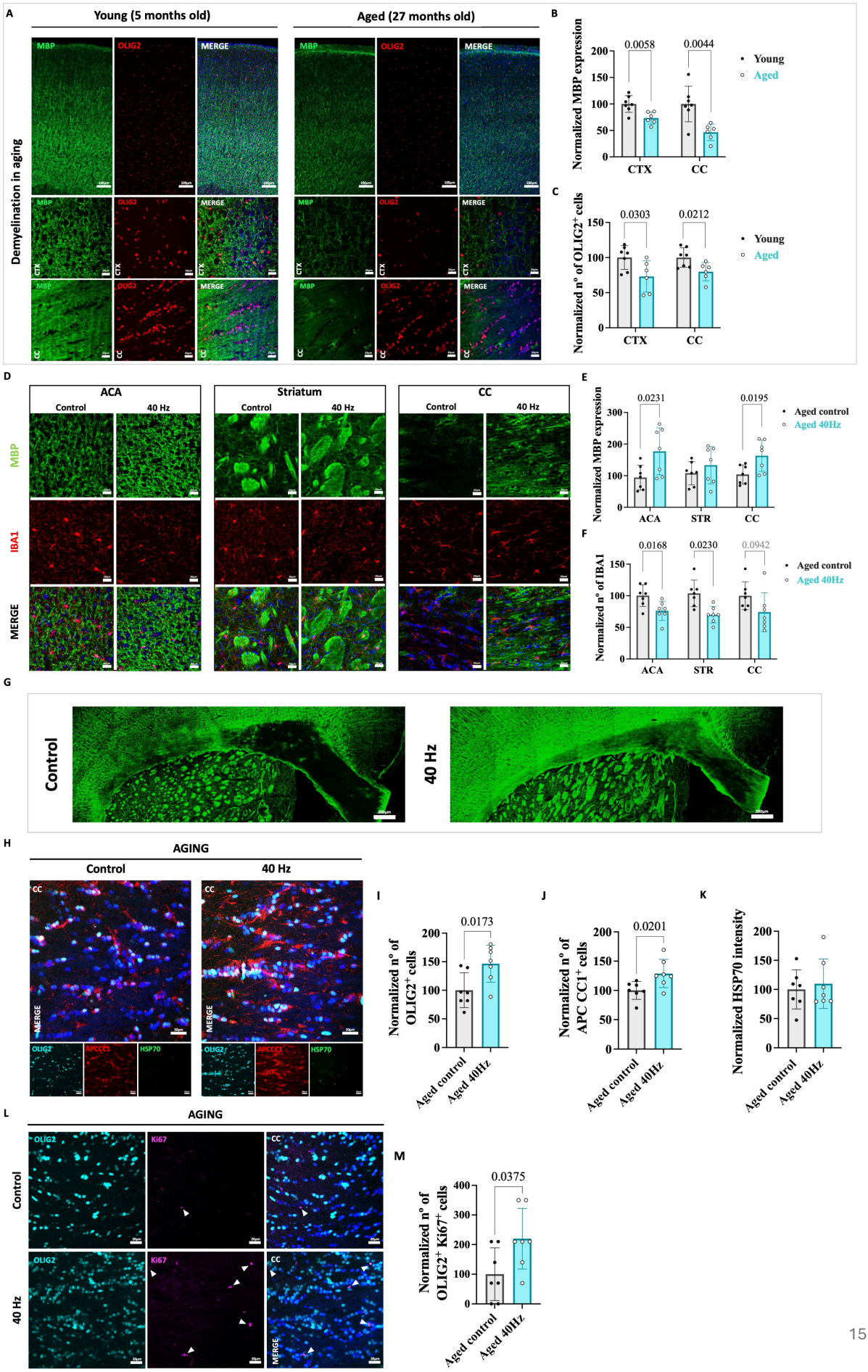
Supplementary Figures S7



Supplementary Figure S7: Temporal dynamics of CPZ-Induced demyelination across different time points.

(A) Experimental paradigm: 12-week-old male C57BL/6J mice were simultaneously exposed to either GENUS or control light and sound conditions and a CPZ diet for a duration of 3 weeks. (B-F) IHC depicting Fluoromyelin (green) and MOG antibody (red) staining in the CC of 12-week-old WT mice exposed to both CPZ and ND after 3 or 6 weeks of diet intervention. The mice additionally underwent 3 weeks of daily 1-hour exposure to either GENUS or control conditions (n=8 mice per group, except for the 3-week CPZ control group where n=7, each dot corresponds to a technical replicate, tile scan, scale bar-100µm). (C) Normalized fluorescence intensity of Fluoromyelin® in the CC area. $F(2,41)=4.820$; $p=0.0132$. (D) Normalized fluorescence intensity of MOG in the CC area. $F(2,41)=0.7728$; $p=0.4683$. (E) Morphometric analysis of CC area. $F(2,41)=3.345$; $p=0.0451$. (F) Morphometric analysis of CC thickness. $F(2,41)=3.489$; $p=0.0399$. (G) IHC of OLIG2 (red) of the CC of of 12-week-old WT mice exposed to both CPZ and ND after 3 or 6 weeks of diet intervention. The mice additionally underwent 3 weeks of daily 1-hour exposure to either GENUS or control conditions (n=8 mice per group under ND, n=7 mice per group under CPZ diet, each dot corresponds to a technical replicate, tile scan, scale bar-100µm). (H) Normalized number of $OLIG2^+$ cells in the CC area. $F(2,38)=5.731$; $p=0.0067$. (I-M) IHC of the CC using SOX10 (red) and HSP70 (green) antibodies (n=8, one technical replicate, tile scan, scale bar- 20µm). (J) Imaris reconstruction of CPZ + 40 Hz confocal image serves as a representative of the analysis performed. (K) Normalized number of $SOX10^+$ cells in the CC area. $F(2,42)=7.607$; $p=0.0015$. (L) Normalized intensity of HSP70 in the CC area. $F(2,42)=4.172$; $p=0.0223$. (M) Colocalization analysis of HSP70 in $SOX10^+$ cells in the CC area. $F(2,42)=5.554$; $p=0.0072$. All P-values were calculated using two-way ANOVA followed by Bonferroni's multiple comparisons test (Supplementary Data S14). Schematic panel A was created with BioRender. Source data are provided as a Source Data file.

Supplementary Figures S8



Supplementary Figure S8: Validation of GENUS effects on myelination and oligodendrogenesis in an advanced aging model.
(A-C) IHC revealing MBP (green) and OLIG2 (red) in 5-month-old (young) and 2-month-old (aged) WT mice comparing CTX and CC regions (n=7 for young, n=6 for aged; each dot represents a technical replicate). (B) The graph displays normalized MBP expression values across CTX and CC using an unpaired two-tailed Student's t-test. (C) The graph displays normalized the number of $OLIG2^+$ cells across CTX and CC using an unpaired two-tailed Student's t-test. (D-F) IHC showing MBP (green) and IBA1 antibody (red) staining in the ACA, STR, and CC of 27-months-old WT mice exposed to either GENUS or control conditions for 4 weeks with daily 1-hour exposure (n=7 mice per group; each dot represents a technical replicate, scale bar=20μm). (E) The graph displays normalized MBP expression values across ACA, STR, and CC areas using an unpaired two-tailed Student's t-test (calculated separately for each area). (F) Normalized nº of $IBA1^+$ cells across ACA, STR and CC areas. The graph displays normalized values, using an unpaired two-tailed Student's t-test. (G) Representative tile scan comparing aging-40Hz conditions to aging-control. (H-K) IHC of OLIG2 (cyan), APCCC1 antibody (red) and HSP70 of the CC of 25-month-old WT mice and 3 weeks of 1 h/day of GENUS or control (n=7 mice per group, each dot corresponds to a technical replicate, tile scan, scale bar-20μm). (I) Normalized nº of $OLIG2^+$ cells in the CC. The graph displays normalized values using an unpaired two-tailed Student's t-test (J) Normalized nº of $APCCC1^+$ cells in the CC. The graph displays normalized values using an unpaired two-tailed Student's t-test (K) Normalized intensity of HSP70 in the CC. The graph displays normalized values using an unpaired two-tailed Student's t-test (L-M) IHC of OLIG2 (cyan) and Ki67 antibody (magenta) of the CC of 25-month-old WT mice and 4 weeks of 1 h/day of GENUS or control (control) (n=7 mice per group, each dot corresponds to a technical replicate, tile scan, scale bar-20μm). (M) Normalized nº of $OLIG2^+Ki67^+$ cells in the CC. The graph displays normalized values using an unpaired two-tailed Student's t-test. Source data are provided as a Source Data file.

Supplementary Data S1-S14
(Excel spreadsheets)

Supplementary Data S1
Legend: TMT labeling.

Supplementary Data S2
Legend: Brain proteome.

Supplementary Data S3
Legend: Normalized summed signal-to-noise.

Supplementary Data S4
Legend: Statistically significant proteins.

Supplementary Data S5
Legend: Differentially expressed proteins across CPZ groups.

Supplementary Data S6
Legend: Single-nucleus RNA sequencing data processing (1º filter).

Supplementary Data S7
Legend: Single-nucleus RNA sequencing data processing (2º filter).

Supplementary Data S8
Legend: Top 50 differential expressed genes.

Supplementary Data S9
Legend: GO terms – OPCs.

Supplementary Data S10
Legend: GO terms – oligodendrocytes.

Supplementary Data S11
Legend: GO terms – microglia.

Supplementary Data S12
Legend: GO terms – astrocytes.

Supplementary Data S13
Legend: Percentage of cells per sample.

Supplementary Data S14
Legend: Two-way ANOVA results.

Additional methods

Western blot

The brain proteins previously isolated from both the CPZ and ND cohorts were mixed with a 2X volume of Laemmli buffer (Bio-Rad, California), comprising 950 µl of Laemmli buffer and 50 µl of β-mercaptoethanol, and subsequently boiled at 95 °C for 5 minutes. The quantification of total proteins was accomplished using the bicinchoninic acid (BCA) method (ThermoFisher Scientific, catalog number 23225), following the manufacturer's instructions. This enabled the determination of the necessary protein amount for each sample, set at 10 µg of protein. The samples were loaded into Mini-PROTEAN® TGX™ Precast Protein Gels, 4–20% (Biorad, catalog number 4561093), and electrophoresis was conducted using a PowerPac™ universal power supply (Bio-Rad, California) at 120 V for a duration of 60 minutes. Subsequently, the proteins were immediately transferred onto Trans-Blot Turbo Midi 0.2 µm PVDF membranes (Bio-Rad, catalog number 1704157). To block nonspecific binding, the membranes were incubated with 5% milk in a tris-buffered saline solution containing Tween (TBST) for 20 minutes, followed by three washes with the same TBST solution. For primary antibody incubation, the membranes were placed overnight at 4°C on stirrers with either the anti-GPX4 rabbit monoclonal antibody (ab25066, Abcam) at a dilution of 1:1,000 or the anti–β-actin mouse monoclonal antibody (66009-1-Ig, Proteintech) at a dilution of 1:1,000. Following three washes with TBST, the membranes were exposed to the appropriate secondary antibody (GE Healthcare Life Sciences) at a dilution of 1:10,000 for 60 minutes over a stirrer. After two washes with TBST and one wash with TBS, the ChemiDoc system (Bio-Rad) was employed to analyze membrane chemiluminescence using WesternBright® Quantum® substrate (Advansta, catalog number K-12042-C20). For analysis of acquired blot images, Image Lab 6.0.1 software (Bio-Rad) was utilized, and densitometric band quantification was carried out using Fiji software (National Institutes of Health).

Lipid peroxidation measurement

Detection of malondialdehyde (MDA) was performed using a commercial kit (orb219867, Biobyrt), following manufacturer instructions. Standards and plasma previously collected and stored at 80ºC were added to a 96-well plate (10µl). Next, 100µl of reaction buffer I was added to each well and carefully mixed in a stirring plate. Reaction buffer II (10µl) was added to each well followed by the dye reagent working solution (100µl). The plate was incubated at 90ºC for 30 min. Finally, when the plate reached the RT, absorbance was measured at 532nm using the EnSpire Multimode Reader Plate system (PerkinElmer). The detection of malondialdehyde (MDA) was conducted using a commercial kit (219867, Biobyrt), following the instructions provided by the

manufacturer. In this procedure, standards and plasma that had been previously collected and stored at -80ºC were added to a 96-well plate (10 µl volume). Subsequently, 100 µl of reaction buffer I was added to each well, and thorough mixing was achieved using a stirring plate. This was followed by the addition of 10 µl of reaction buffer II to each well, and then 100 µl of the dye reagent working solution was added. The plate was subjected to incubation at 90ºC for a duration of 30 minutes. Upon reaching room temperature (RT), the absorbance was measured at 532nm using the EnSpire Multimode Reader Plate system (PerkinElmer).

Prussian blue staining

The detection of Fe^{3+} was performed using a commercial kit (ab150674, Abcam). After mixing an equal volume of hydrochloric acid solution and potassium ferrocyanide solution, the working solution was added to brain tissue sections, for 20 minutes at RT. Slides were rinsed in distilled water and immediately incubated with the nuclear fast red solution for 5 minutes at RT. Following this step, the slides *were dehydrated* in two successive changes of 95% ethanol, transitioning to absolute ethanol. Finally, the slides were mounted using Permount™ mounting medium. The acquisition of bright-field images was carried out using a Zeiss LSM 900 confocal microscope.

TUNEL fluorometric assay

To assess cell death in the brain floating sections, we performed the TUNEL assay using the Click-iT™ TUNEL Alexa Fluor™ 647 (C10247, Thermo Fisher) according to the manufacturer's instructions. Briefly, free-floating sections mounted in slides were permeabilized with Protein K solution and incubated for 15 minutes at RT. After washes using 1X PBS, slides were incubated with 100 µl of terminal deoxynucleotidyl transferase (TdT) reaction buffer for 10 minutes at 37ºC. Previous solution was removed carefully and 50 µl of the TdT reaction mixture was added to each slide and incubated for 1 hour at 37ºC. Slides were rinsed with H_2Od and washed in a solution of 3% BSA, 0.1% Triton and 1X PBS for 5 minutes at RT. Then, Click-iT™ plus reaction solution was prepared following the protocol and 50 µl were added to each slide and incubated for 30 minutes at 37ºC, protecting from the light. Followed this time, slides were washed with 3% BSA, 0.1% Triton and 1X PBS for 5 minutes, following a wash with 1X PBS. Lastly, slides were incubated with Hoechst 1:10,000 for 10 minutes in the dark. After wash, slides were mounted using Permount™ mounting medium.