Supplementary Material

Supplementary Figure Legends

Supplementary Fig. S1: Immuno co-localization experiments using anti-NeuN and anti-glycogen antibodies. Lateral sections of hippocampus and cerebellum from *Epm2b-/-* mice of 12 months of age were immunodetected using a combination of anti-NeuN (neuronal marker) and anti-glycogen antibodies, and secondary anti-rabbit AlexaFluor (green) 488 and anti-IgM AlexaFluor (red) 633 conjugated antibodies, respectively. Sections were then stained with DAPI to visualize nuclei. Images were acquired with a Leica DM RXA2 microscope. Representative images are presented. Scale bars: 100 μm and 10 μm. DG: hippocampus dentate gyrus, GL: cerebellar granular layer.

Supplementary Fig. S2: Immuno co-localization experiments using anti-GlnS or anti-GFAP and anti-glycogen antibodies. Lateral sections of cerebellum from *Epm2b-/-* mice of 12 months of age were immunodetected using a combination of anti-GlnS (glutamine synthetase; astrocytic marker) (A) or anti-GFAP (glial fibrillary acidic protein; astrocytic marker) (C), anti-glycogen antibodies, and secondary anti-rabbit AlexaFluor (green) 488 and anti-IgM AlexaFluor (red) 633 conjugated antibodies, respectively. Sections were then stained with DAPI to visualize nuclei. Images were acquired with a Leica DM RXA2 microscope. Representative images are presented. Scale bars: 10 μm. GL: cerebellar granular layer. White arrows indicate examples of astrocytes containing glycogen inclusions. Images were also acquired from similar preparations using a Leica TCS SP8 confocal microscope (B, anti-GlnS; D, anti-GFAP). Four consecutives images from the Z-stacks are shown.

Supplementary Fig. S3: Immuno co-localization experiments using anti-Iba1 and anti-glycogen antibodies. A) Lateral sections of cerebellum from *Epm2b-/-* mice of 12 months of age were immunodetected using a combination of anti-Iba1 (microglia marker) and anti-glycogen antibodies, and secondary AlexaFluor (green) 488 and anti-IgM AlexaFluor (red) 633 conjugated antibodies respectively. Sections were then stained with DAPI to visualize nuclei. Images were acquired with a Leica DM RXA2 microscope. Representative images

are presented. Scale bars: 10 μm. GL: cerebellar granular layer. B) Images were also acquired from similar preparations using a Leica TCS SP8 confocal microscope. Four consecutives images from the Z-stacks are shown.

Supplementary Fig. S4: Immuno co-localization experiments using anti-Tuj1, anti-NeuN, anti-GlnS or anti-GFAP and anti-glycogen antibodies in samples from *Epm2a-/-* mice. Lateral sections of hippocampus from *Epm2a-/-* mice of 12 months of age were immunodetected using a combination of anti-Tuj1 (neuronal marker) (A), anti-NeuN (neuronal marker) (B), anti-GlnS (glutamine synthetase; astrocytic marker) (C) or anti-GFAP (glial fibrillary acidic protein; astrocytic marker) (D) and anti-glycogen antibodies, and secondary anti-rabbit AlexaFluor (green) 488 and anti-IgM AlexaFluor (red) 633 conjugated antibodies, respectively. Sections were then stained with DAPI to visualize nuclei. Images were acquired with a Leica DM RXA2 microscope. Representative images are presented. Scale bars: 10 μm.

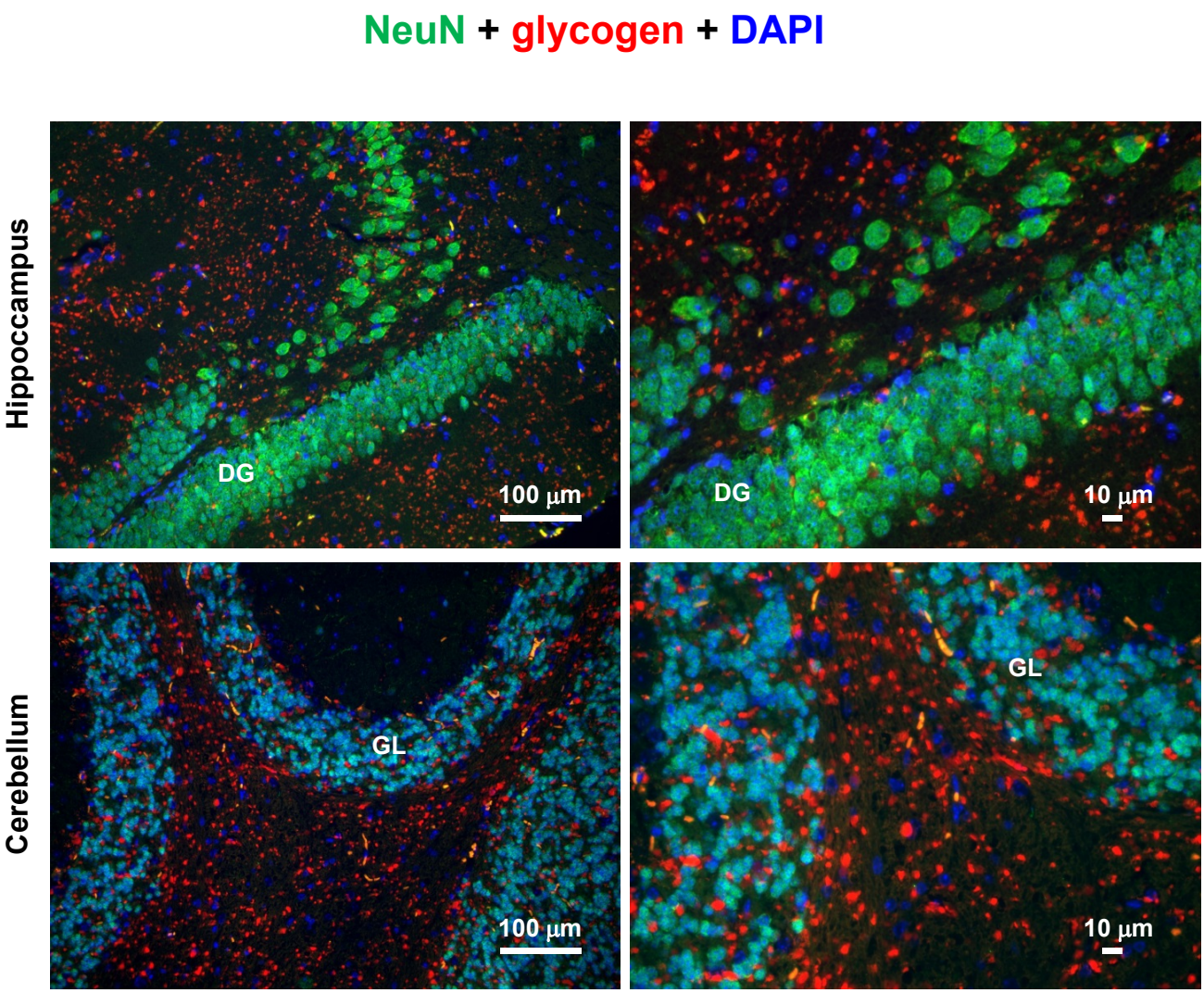
Supplementary Fig. S5: Immunohistofluorescence analyses of glycogen inclusions in brain of control and *Epm2b-/-* mice of 3 months of age. Lateral sections of hippocampus and cerebellum were incubated with anti-glycogen antibody and a secondary anti-IgM AlexaFluor (red) 633 conjugated. Sections were then stained with DAPI to visualize nuclei. Images were acquired with a Leica DM RXA2 microscope. Representative images are presented. Scale bars: 100 μm. Bottom panels: sections from *Epm2b-/-* mice were pretreated with α-amylase as described in Materials and Methods, and then analyzed by immunohistofluorescence as above. DG: hippocampus dentate gyrus; GL: cerebellar granular layer.

Supplementary Fig. S6: Immuno co-localization experiments using anti-Tuj1 or anti-NeuN and anti-glycogen antibodies. Lateral sections of hippocampus and cerebellum from *Epm2b-/-* mice of 3 months of age were immunodetected using a combination of anti-Tuj1 (A) or anti-NeuN (B) (neuronal markers) and anti-glycogen antibodies, and secondary anti-rabbit AlexaFluor (green) 488 and anti-IgM AlexaFluor (red) 633 conjugated antibodies respectively. Sections were then stained with DAPI to visualize nuclei. Images were

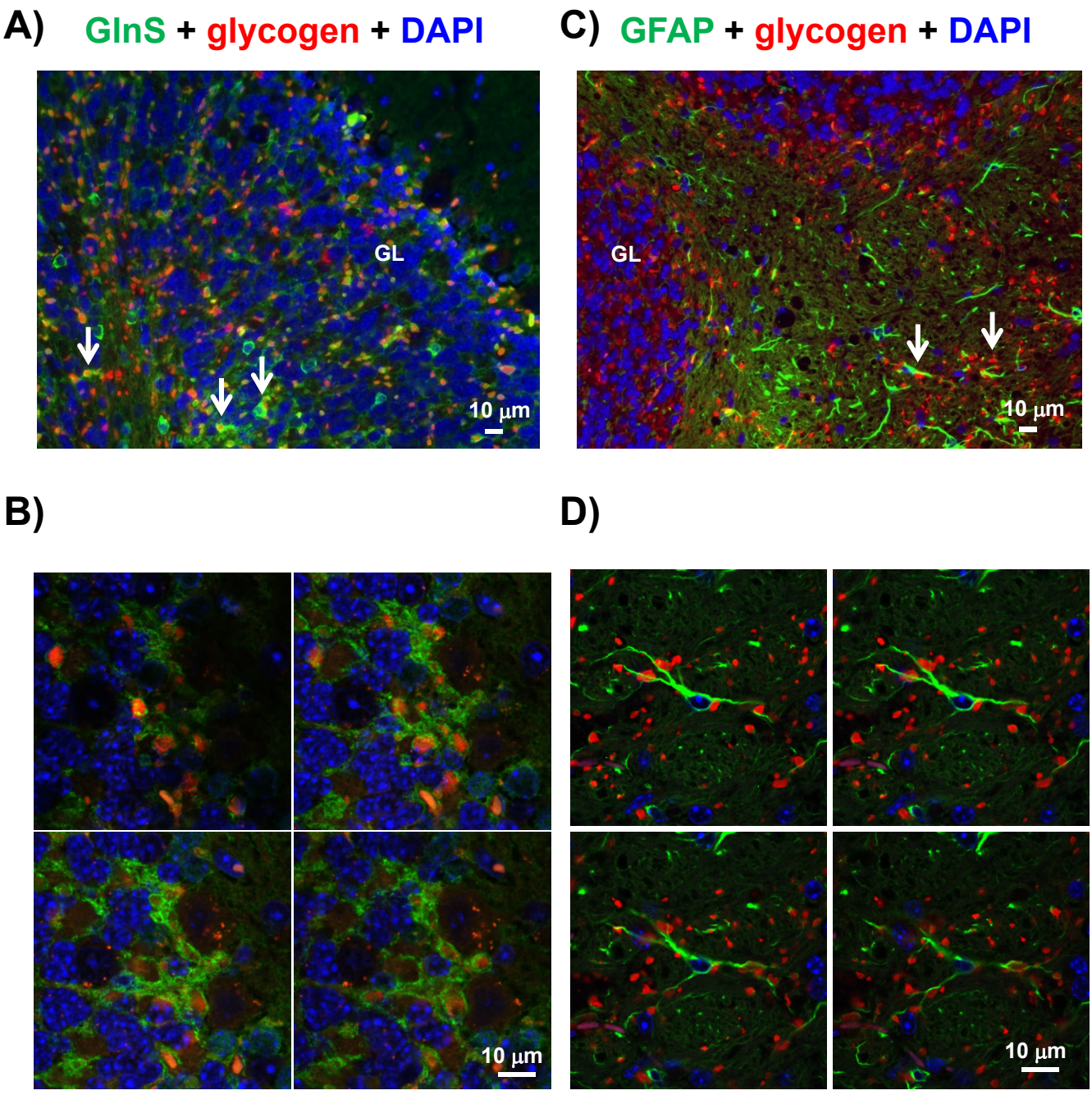
acquired with a Leica DM RXA2 microscope. Representative images are presented. Scale bars: 100 μm. CA1: hippocampus cornus ammonis 1, GL: cerebellar granular layer, PC: Purkinje cells.

Supplementary Fig. S7: Immuno co-localization experiments using anti-GlnS, anti-GFAP and anti-glycogen antibodies. Lateral sections of hippocampus and cerebellum from *Epm2b-/-* mice of 3 months of age were immunodetected using a combination of anti-GlnS (A) or anti-GFAP (C) and anti-glycogen antibodies, and secondary anti-rabbit AlexaFluor (green) 488 and anti-IgM AlexaFluor (red) 633 conjugated antibodies respectively. Sections were then stained with DAPI to visualize nuclei. Images were acquired with a Leica DM RXA2 microscope. Representative images are presented. Scale bars: 10 μm. DG: hippocampus dentate gyrus, GL: cerebellar granular layer. Bottom panels, images were also acquired from similar preparations using a Leica TCS SP8 confocal microscope (B, anti-GlnS; D, anti-GFAP). Four consecutives images from the Z-stacks are shown.

Supplementary Fig. S8: Primary cerebellar astrocytes from mouse models of LD accumulate higher levels of glycogen. A) Primary cultures of cerebellar astrocytes from seven days old control, *Epm2a-/-* and *Epm2b-/-* mice were grown in high glucose containing medium (25 mM glucose) for 24 hours. Then, glycogen content was measured as described in Materials and Methods. B) Primary cultures of astrocytes as above were grown in high glucose conditions for 24 hours. Then, they were shifted to a medium without glucose for 6 and 24 hours and glycogen content was measured as described in Materials and Methods. Results are expressed as means of at least three independent batches of astrocytes in each genotype. In B) the results are normalized to the levels of glycogen present in the respective basal conditions (high glucose; dotted line). Bars indicate standard deviation. In A), significant differences between the LD astrocytes and control are indicated (*$p < 0.05$). In B), significant differences between the levels of glycogen respect to basal conditions (high glucose) are indicated (**$p < 0.01$, ***$p < 0.001$).

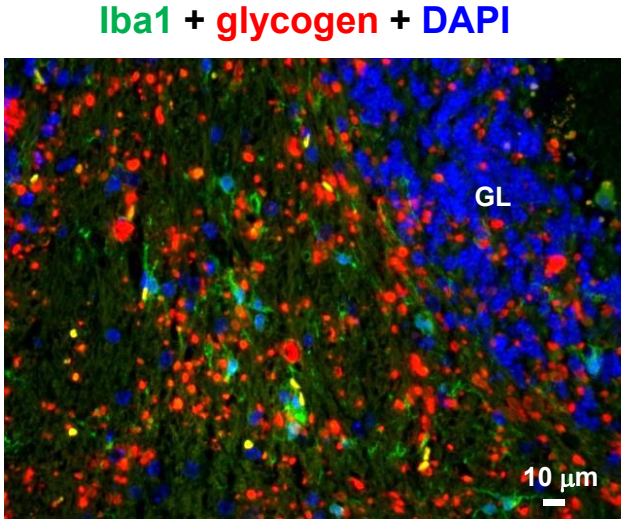


Supplementary Fig. S1

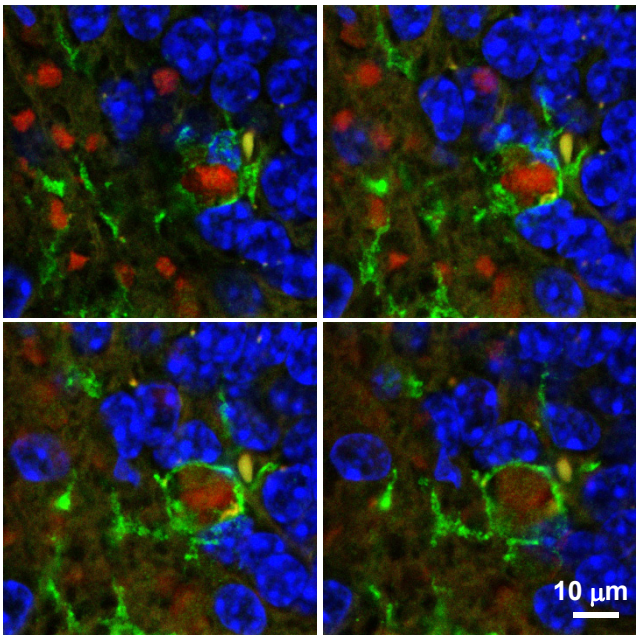


Supplementary Fig. S2

A)

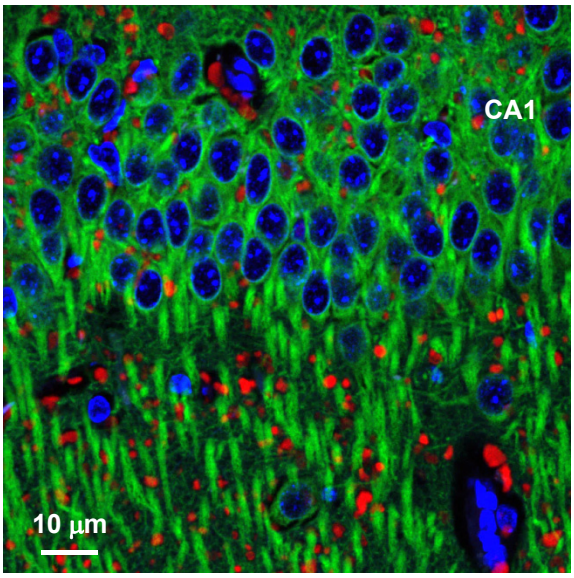


B)

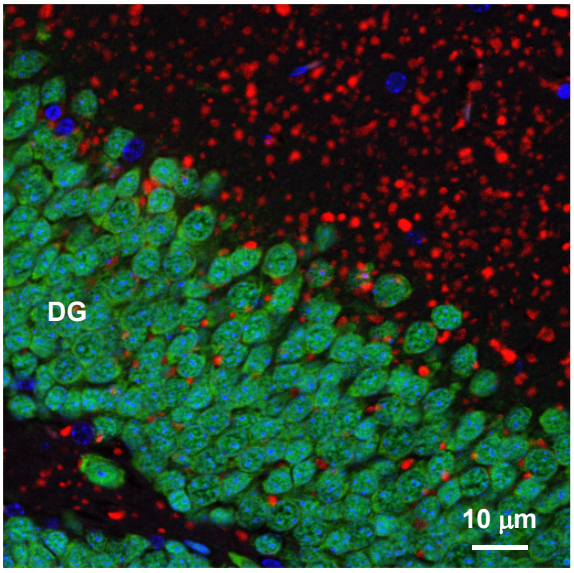


Supplementary Fig. S3

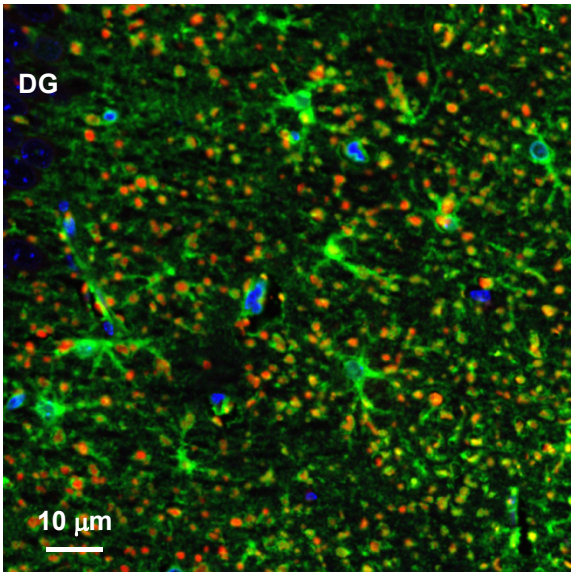
A) Tuj1 + glycogen + DAPI



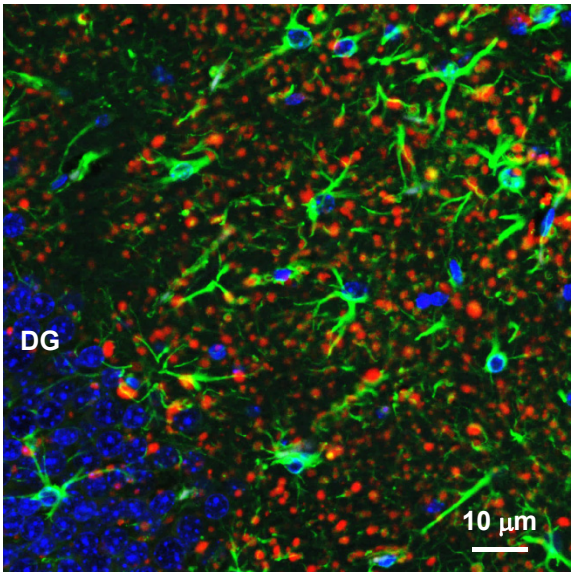
B) NeuN + glycogen + DAPI



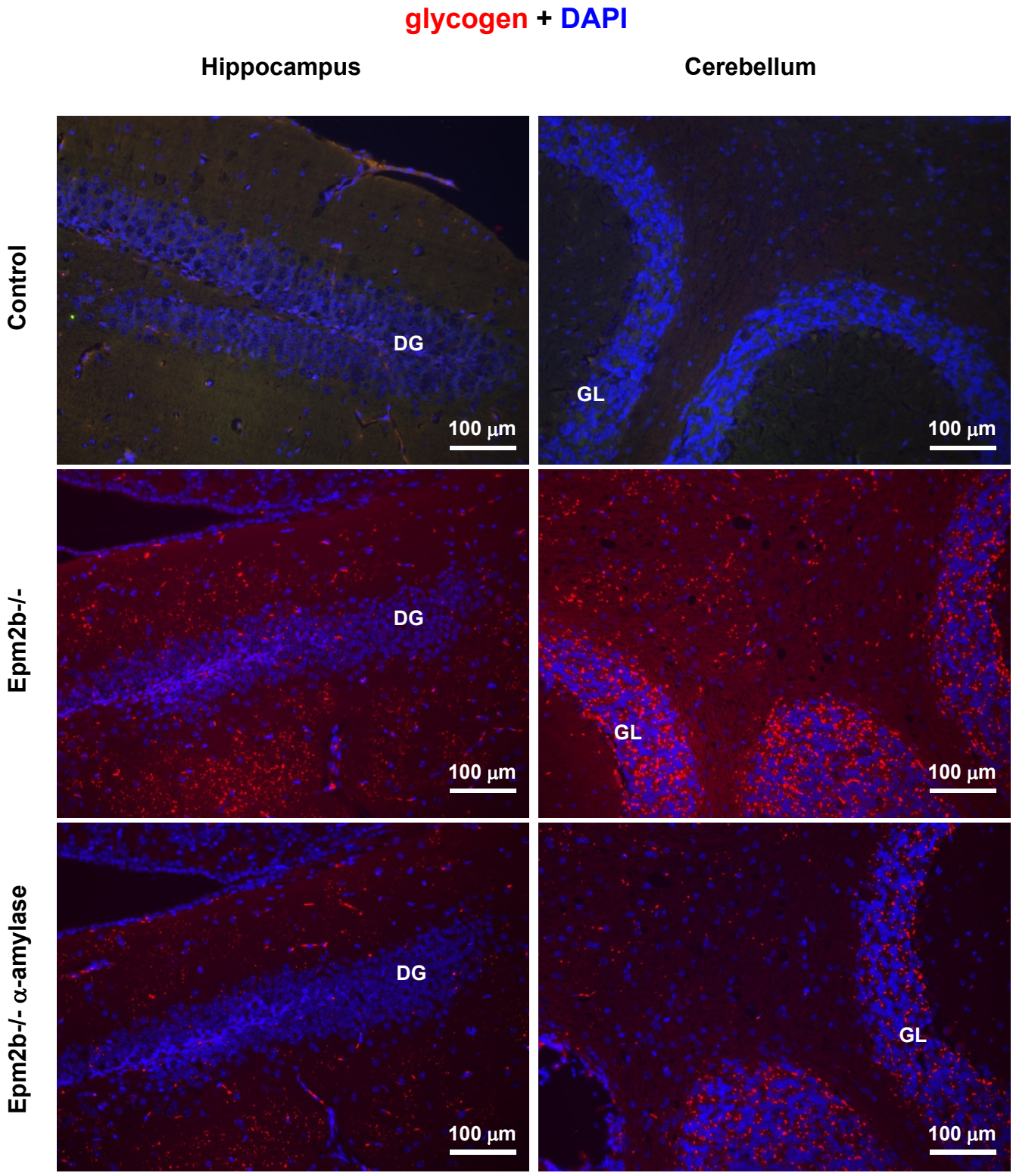
C) GlnS + glycogen + DAPI



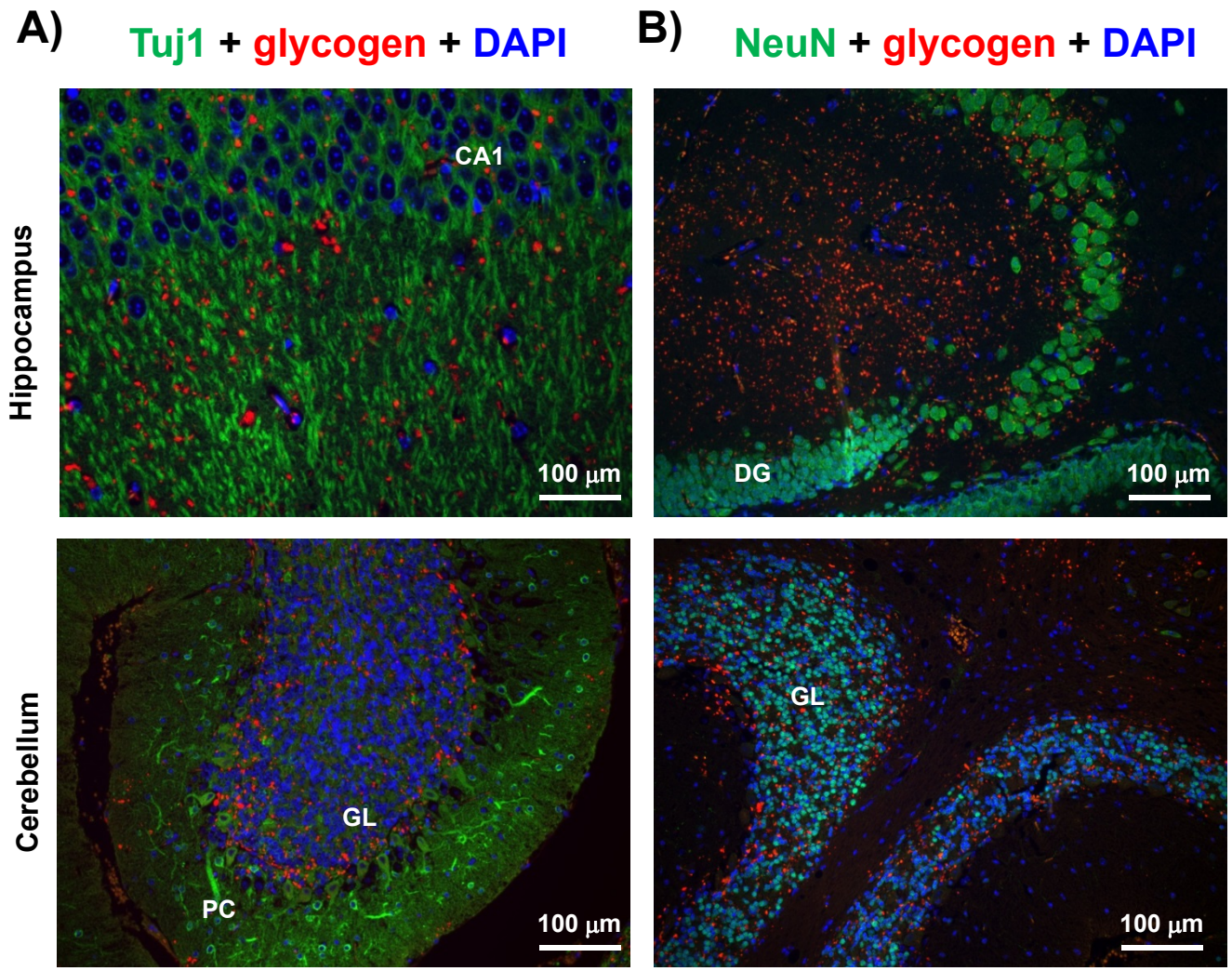
D) GFAP + glycogen + DAPI



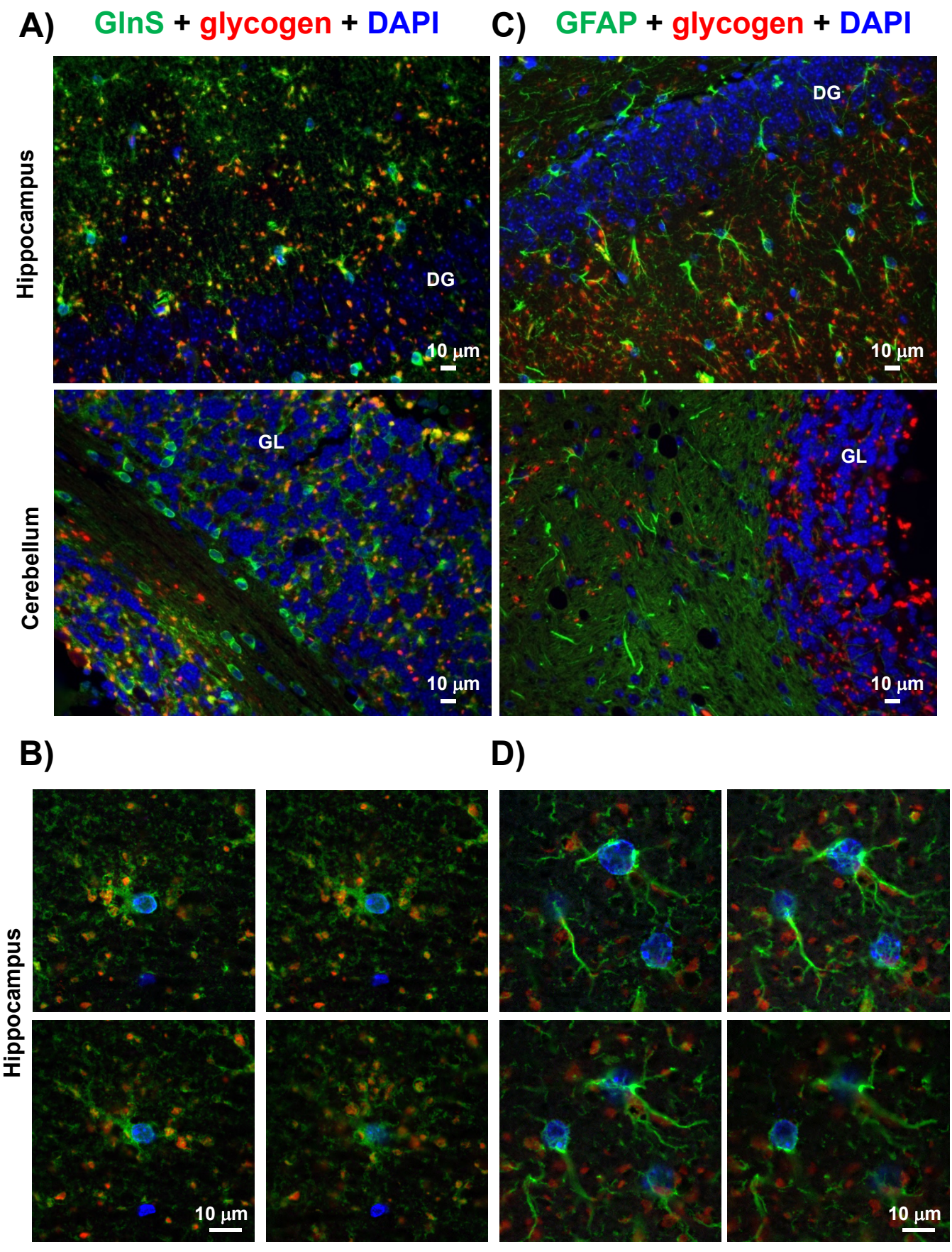
Supplementary Fig. S4



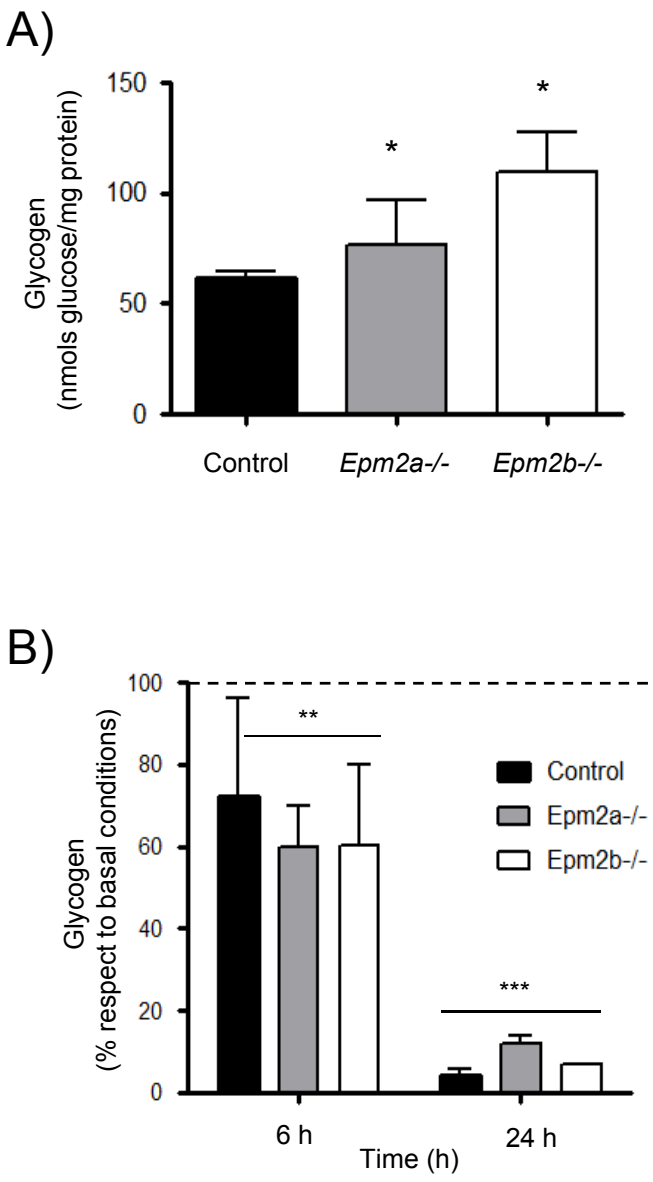
Supplementary Fig. S5



Supplementary Fig. S6



Supplementary Fig. S7



Supplementary Fig. S8