

LETTER

Glutamine synthetase sustains cortical circuit development via mTOR-mediated astrocyte maturation

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Dear Editor,

The developing cerebral cortex requires precise metabolic regulation to support neurogenesis and circuit formation (Belanger et al., 2011; Namba et al., 2021). Glutamine synthetase (GS), which catalyzes glutamate-to-glutamine conversion, sustains neurotransmitter recycling and nitrogen homeostasis (Tani et al., 2014). Human *GLUL* mutations cause lethal neurodevelopmental disorders (Häberle et al., 2005), and GS deletion in mice leads to postnatal death (He et al., 2010), underscoring its essential role. While adult cortical GS deficiency triggers neurodegeneration (Zhou et al., 2019), its function in early cortical development remains elusive, despite evidence linking astrocytic metabolism to circuit maturation (Chung et al., 2015; Pekny et al., 2016).

Spatiotemporal analysis revealed high GS expression in embryonic neural stem cells (NSCs) within the ventricular/subventricular zone, transitioning to S100β⁺ astrocytes postnatally (Figs. 1A–D and S1A–C). GS enzymatic activity followed a biphasic pattern, peaking during early postnatal stages coincident with astrocyte maturation (Fig. S1D), suggesting stage-specific roles in cortical development.

To dissect the *in vivo* function of GS, we generated cortex-specific GS knockout (GS-cKO) mice by crossing *Glu1^{flox/flox}* mice with *Emx1-Cre* mice (Fig. 1E). This strategy induces gene ablation in radial glial progenitors from embryonic day 10.5 onward while preserving postnatal

viability, thereby enabling examination of GS function across developmental stages (Gorski et al., 2002). Efficient GS deletion was confirmed in both embryonic and postnatal forebrains (Figs. 1F and S1E–G). By postnatal day 14 (P14), GS-cKO mice exhibited reduced brain size and cortical thickness (Fig. S1H–K), accompanied by notable astrocytic morphological abnormalities, as revealed by AAV2/9-GFAP-mCherry labeling (Fig. 1G–I). In contrast, embryonic neurogenesis and neuronal migration remained largely intact (Figs. 1J, 1K, and S2A–I). Although early GS loss mainly impaired astrocyte maturation, by P28, the cortex of GS-cKO mice displayed pronounced astrocyte activation, characterized by robust GFAP upregulation, cellular hypertrophy, and thickened astrocytic processes, indicative of reactive gliosis (Fig. S3A–C). Notably, dietary glutamine supplementation partially restored astrocyte maturation, as evidenced by improved morphological complexity and enhanced process elaboration (Fig. S4A–C), highlighting the essential role of GS-derived glutamine in supporting astrocytic structural development.

GS deficiency severely impaired dendritic growth, spine density, and synaptogenesis, as shown by EGFP electroporation, Golgi staining, and synaptic marker analysis (Figs. 1M, 1N, 1Y, 1Z and S4D). Electrophysiological recordings confirmed reduced miniature excitatory and inhibitory synaptic activity (Fig. 1O–T). Remarkably, glutamine supplementation restored synaptic density, indicating

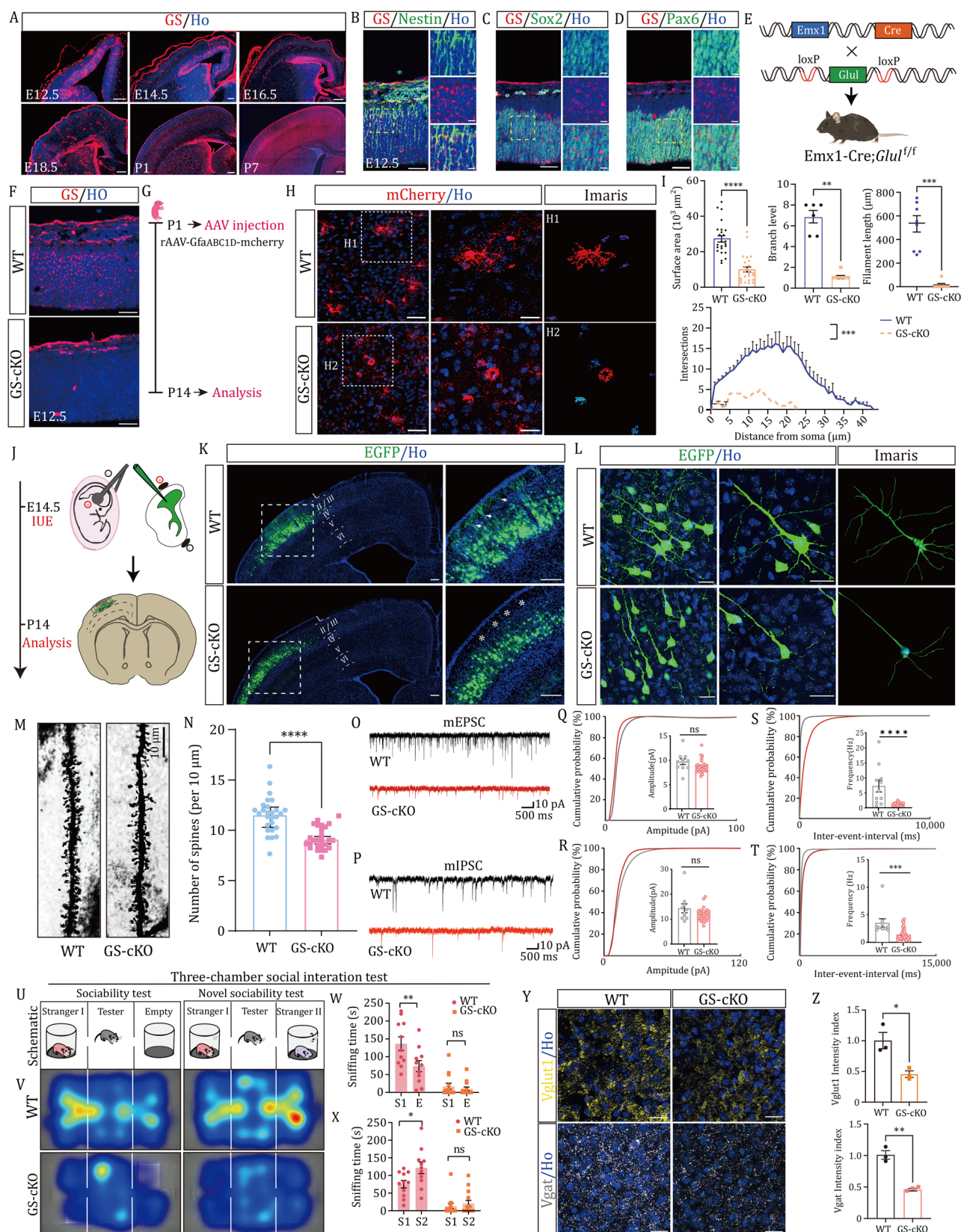


Figure 1. Glutamine synthetase regulates cortical astrocyte maturation and synaptic development. (A) Representative images of cortical sections stained for glutamine synthetase (GS) at E12.5, E14.5, E16.5, E18.5, and postnatal days P1 and P7. Scale bars: 200 μ m. (B-D) Representative images of GS co-stained with antibodies against Nestin (B), Sox2 (C), or Pax6 (D) in the cortex at E12.5. Yellow dashed outlines indicate regions enlarged on the right. Scale bars: 50 μ m (left); 20 μ m (right). (E) Breeding strategy for generating GS conditional knockout (cKO) mice. (F) Immunofluorescent staining of GS in wild-type (WT) and GS-cKO mice at E12.5. Scale bars: 50 μ m. (G) Schematic and timeline for selective astrocyte labeling in the postnatal cortex. (H) Representative mCherry⁺ astrocytes in P14 WT and GS-cKO mice. (I) Quantification of astrocyte morphology. Surface area ($10^3 \mu\text{m}^2$), Branch level, and Filament length (μ m) are shown. Intersections vs. Distance from soma (μ m) is also plotted. (J) Schematic and timeline for selective astrocyte labeling. (K) Representative EGFP/HO staining in WT and GS-cKO mice. (L) Representative EGFP/HO staining in WT and GS-cKO mice. (M) Micrographs of dendrites. (N) Quantification of dendritic spines (per 10 μ m). (O-P) Electrophysiological recordings of mEPSCs and mIPSCs. (Q-T) Cumulative probability plots of mEPSCs and mIPSCs. (U) Three-chamber social interaction test. (V) Heatmaps of social interaction. (W) Quantification of sniffing time. (X) Quantification of sniffing time. (Y) Vgat1/HO staining. (Z) Quantification of Vgat1 intensity index.

metabolite dependence of GS-mediated circuit maturation (Fig. S4E–H). Consistently, GS-cKO mice displayed behavioral abnormalities in motor coordination (Figs. S5A–E, S5G, and S5H) and social interaction (Figs. 1U–X and S5F), paralleling behavioral deficits in autism and epilepsy models (Varghese et al., 2017; Sharma et al., 2018).

Astrocyte development was prominently disrupted in GS-cKO mice. GS deficiency reduced S100 β ⁺, Aldh1L1⁺, Sox2⁺, and Sox9⁺ cell numbers (Fig. 2A–H) without increasing apoptosis (Fig. S6A and S6B). Single-nucleus RNA sequencing (snRNA-seq) of P7 cortex identified major cell types but showed no change in excitatory neuron or astrocyte proportions between genotypes (Fig. 2I–K). However, analysis of astrocyte-specific gene expression revealed distinct alterations, with a downregulation of *Blbp* (a key regulator of astrocyte maturation) and an upregulation of *ApoE* (a lipid transporter implicated in neuroinjury response), respectively; in contrast, the glutamate transporter *Slc1a3* (GLAST) remained unchanged in GS-cKO astrocytes (Figs. 2L, S6H, S6J, and S6M). These changes emerged by P1 (Fig. S6C–F) and were confirmed by reduced *Blbp* protein and increased *ApoE* mRNA via immunostaining and *in situ* hybridization (Fig. S6H–O).

Metabolically, GS loss reduced glutamine, glutamate, aspartate, and asparagine, while elevating glycine and alanine (Fig. 2M and 2N). snRNA-seq revealed selective suppression of the mTOR pathway in astrocytes (Fig. 2O and 2P). Western blotting confirmed decreased phosphorylation of S6 (mTORC1) and AKT (mTORC2), with key mTOR pathway genes (*Atp6v1c1*, *Atp6v1g2*, *Eif4b*) downregulated (Fig. 2P–X). Thus, GS deficiency disrupts cortical amino acid homeostasis and suppresses astrocytic mTOR signaling, likely underlying the observed astrocyte dysfunction and neuronal deficits.

Our findings establish GS as a key regulator of cortical circuit maturation through astrocyte metabolic programming. GS expression shifts from NSCs to astrocytes during

cortical expansion, indicating stage-specific roles. Neurogenesis remains unaffected, likely due to maternal or placental glutamine supply (Wu et al., 2015), but postnatal GS deficiency profoundly impairs astrocyte maturation and suppresses mTOR signaling, suggesting that mTOR inactivation is central to the observed developmental deficits.

As a metabolic sensor, mTOR critically depends on intracellular amino acid availability. Glutamine and its metabolites (e.g., α -ketoglutarate and arginine) not only directly activate mTORC1 but also fuel mTOR-dependent anabolic processes (Jewell et al., 2015). Thus, GS loss removes both signaling input and metabolic support for mTOR, leading to selective pathway impairment. In contrast, the Hippo/YAP pathway, although sensitive to energy stress (Sheng et al., 2024), lacks a glutamine-sensing mechanism and is therefore less affected. This specificity explains why mTOR signaling is preferentially disrupted in GS-deficient astrocytes.

mTOR suppression likely compromises astrocytic metabolic support, reducing protein synthesis and precursor provision for nucleotide and neurotransmitter production. Such deficits during critical developmental windows disrupt synaptogenesis and circuit refinement, offering a mechanistic link between GS–mTOR dysregulation and neurodevelopmental disorders, such as specific autism spectrum disorder subtypes. Meanwhile, upregulation of *ApoE* may initially serve as a compensatory response to metabolic stress but, when prolonged, can lead to lipid dysregulation, toxic protein accumulation, and increased susceptibility to neurodegenerative conditions, consistent with APOE ϵ 4-associated Alzheimer's disease (Zheng and Wang, 2025).

Our observations align with the previously reported *Glul* conditional knockout model (Zhou et al., 2019), which also exhibited seizures, motor deficits, and reactive astrogliosis. Although our study documents cortical thinning earlier and more quantitatively, careful review of their data suggests similar trends. Differences in phenotypic

cortices. Enlarged insets show reconstructed astrocytes (H1, H2) (Scale bars: 50 μ m; 25 μ m for H1–H2) (≥ 20 astrocytes from 3–4 mice per genotype, $n \geq 3$ mice). (I) Quantification of astrocyte morphology, including surface area, branch level, total process length, and Sholl analysis (≥ 20 astrocytes from 3 to 4 mice per genotype, $n \geq 3$ mice). (J) Schematic of the experimental approach. Embryos were electroporated at E14.5, and brain sections were collected at P14. (K) Representative images of P14 brain sections following in utero electroporation (IUE) with a pCAG-EGFP plasmid at E14.5. White rectangles indicate regions enlarged on the right. Arrowheads highlight complex dendritic branching in the wild-type cortex, while asterisks denote sparse dendritic branching in GS-cKO mice. Scale bars: 200 μ m. (L) Representative images of EGFP⁺ pyramidal neurons reconstructed using Imaris software. (M) Representative images of dendritic spines in WT and GS-cKO mice at P28. Scale bars: 10 μ m. (N) Quantification of spine density per 10 μ m on secondary dendrites (10 neurons per animal, 3 animals per genotype). (O and P) Representative traces of whole-cell mEPSC (O) and mIPSC (P) recordings from layer II/III pyramidal neurons in acute M1 slices of P28 WT and GS-cKO mice. (Q and R) Cumulative distributions of the amplitude of mEPSC (Q) and mIPSC (R) in P28 WT and GS-cKO pyramidal neurons. (S and T) Cumulative distributions of the inter-event interval of mEPSC (S) and mIPSC (T) in P28 WT and GS-cKO pyramidal neurons. (U) Schematic of the three-chamber sociability test apparatus. (V) Representative heatmaps of movement traces from WT and GS-cKO mice during the three-chamber sociability and novel sociability tests. (W and X) Quantification of sniffing time during the sociability test (W) and novel sociability test (X) (WT: $n = 18$ mice; GS-cKO: $n = 15$ mice). (Y) Immunofluorescent images of excitatory (VGLUT1) and inhibitory (VGAT) synapses in layer II/III neurons of P28 WT and GS-cKO mice. Scale bars: 25 μ m. (Z) Quantification of VGLUT1 and VGAT intensity indices in P28 WT and GS-cKO mice ($n = 3$ mice). Statistical significance was determined using an unpaired two-tailed Student's *t*-test (N, P, Q, S, T, W, X, Z) or an unpaired two-tailed Mann-Whitney test (I). $P < 0.05$, $P < 0.01$, $^*P < 0.001$, $^{**}P < 0.0001$; ns, not significant. Nuclei were counterstained with Hoechst 33342 (Ho; blue).

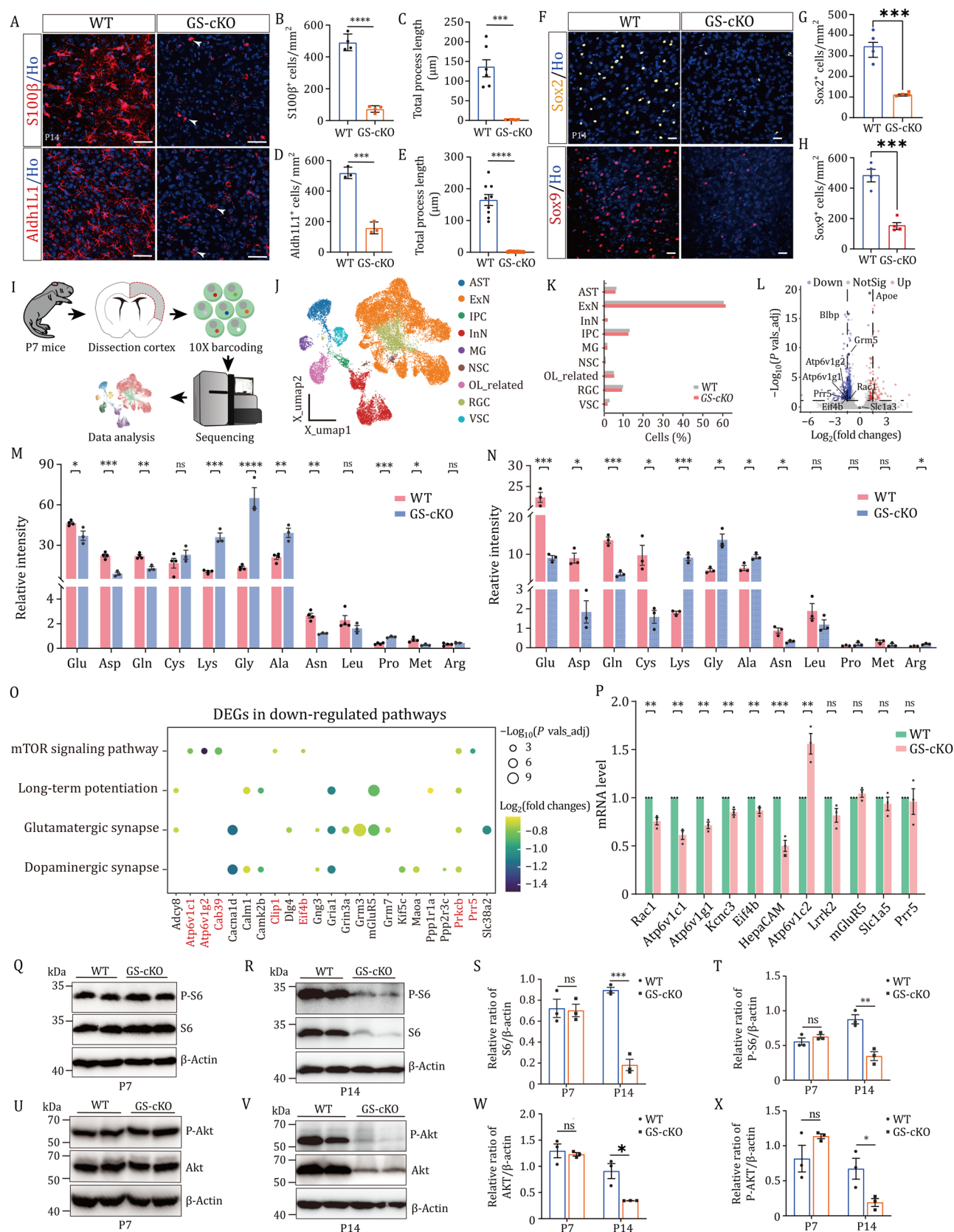


Figure 2. GS deficiency suppresses mTOR signaling and disrupts amino acid homeostasis in cortical astrocytes. (A) Immunofluorescent staining of S100 β and Aldh1L1 in P14 WT and GS-cKO mice. Arrowheads indicate unbranched astrocytes in the GS-cKO cortex (Scale bars: 50 μ m). (B–E) Quantification of S100 β ⁺ or Aldh1L1⁺ astrocytes (≥ 20 cells per mouse) and astrocyte complexity (based on total processes) from 3 to 4 mice per genotype ($n \geq 3$ mice). (F) Representative images of P14 WT and GS-cKO brain sections stained for Sox2 (orange) or Sox9 (red) (Scale bars: 25 μ m). (G and H) Quantification of Sox2⁺ or Sox9⁺ cells in the cortex at P14 ($n \geq 3$ mice). (I) Schematic of snRNA-seq using cortex samples from WT and GS-cKO mice at P7. (J) t-SNE plot showing broad cell-type clustering

presentation likely reflect variations in timing and targeting strategies; nevertheless, both models converge on severe astrogliosis by the fourth postnatal week, highlighting GS loss as a potent driver of neuroinflammatory pathology.

In conclusion, this study uncovers an essential role for GS in postnatal brain development and reveals how a metabolic enzyme can regulate cell-specific gene expression, protein translation, and morphology. These insights offer new therapeutic perspectives for treating neurological disorders involving astrocytic dysfunction and impaired cortical connectivity, such as epilepsy and autism.

Footnotes

We thank the members of the Qin laboratory for their valuable suggestions and comments. This work was supported by grants from the National Natural Science Foundation of China (Nos. 31871477, 32170971), the Natural Science Foundation of Shanghai (No. 18ZR1403800) awarded to S.Q.; as well as grants from the CAMS Innovation Fund for Medical Sciences (CIFMS, No. 2024-I2M-ZD-012), the STI2030-Major Projects (No. 2022ZD0204700), and the National Natural Science Foundation of China (No. 32170964) awarded to W.-P.G.

The authors declare that they have no competing interests, financial or non-financial, that could be perceived as influencing the work reported in this manuscript.

All animal procedures were approved by the Fudan University Institutional Animal Care and Use Committee and performed in accordance with institutional and national guidelines.

All authors have provided informed consent for participation and confirmed their agreement to publish this work.

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

S.Q., W.P., J.G., and S.D. conceived the project. S.Q., W.P., and P.G. designed experiments. P.G. performed most of the experiments. X.C. performed single-cell analysis. W.C. performed electrophysiological experiments. W.H., Y.L., G.Q., X.S., Z.W., and Z.L. assisted with the experiments and helped to analyze the data. P.G. and S.Q. wrote the article.

References

- Belanger M, Allaman I, Magistretti PJ. Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. *Cell Metab* 2011;**14**:724–738.
- Chung WS, Allen NJ, Eroglu C. Astrocytes control synapse formation, function, and elimination. *Cold Spring Harb Perspect Biol* 2015;**7**:a020370.
- Gorski JA, Talley T, Qiu M et al. Cortical excitatory neurons and glia, but not GABAergic neurons, are produced in the *Emx1*-expressing lineage. *J Neurosci* 2002;**22**:6309–6314.
- Häberle J, Görg B, Rutsch F et al. Congenital glutamine deficiency with glutamine synthetase mutations. *N Engl J Med* 2005;**353**:1926–1933.
- He Y, Hakvoort TB, Vermeulen JL et al. Glutamine synthetase deficiency in murine astrocytes results in neonatal death. *Glia* 2010;**58**:741–754.
- Jewell JL, Kim YC, Russell RC et al. Metabolism. Differential regulation of mTORC1 by leucine and glutamine. *Science* 2015;**347**:194–198.
- Namba T, Nardelli J, Gressens P et al. Metabolic regulation of neocortical expansion in development and evolution. *Neuron* 2021;**109**:408–419.
- Pekny M, Pekna M, Messing A et al. Astrocytes: a Central element in neurological diseases. *Acta Neuropathol* 2016;**131**:323–345.
- Sharma SR, Gonda X, Tarazi FI. Autism spectrum disorder: classification, diagnosis and therapy. *Pharmacol Ther* 2018;**190**:91–104.
- Sheng X, Xia Z, Yang H et al. The ubiquitin codes in cellular stress responses. *Protein Cell* 2024;**15**:157–190.
- Tani H, Dulla CG, Farzampour Z et al. A local glutamate-glutamine cycle sustains synaptic excitatory transmitter release. *Neuron* 2014;**81**:888–900.
- Varghese M, Keshav N, Jacot-Descombes S et al. Autism spectrum disorder: neuropathology and animal models. *Acta Neuropathol* 2017;**134**:537–566.
- Wu X, Xie C, Zhang Y et al. Glutamate-glutamine cycle and exchange in the placenta-fetus unit during late pregnancy. *Amino Acids* 2015;**47**:45–53.
- Zheng Q, Wang X. Alzheimer's disease: insights into pathology, molecular mechanisms, and therapy. *Protein Cell* 2025;**16**:83–120.
- Zhou Y, Dhaher R, Parent M et al. Selective deletion of glutamine synthetase in the mouse cerebral cortex induces glial dysfunction and vascular impairment that precede epilepsy and neurodegeneration. *Neurochem Int* 2019;**123**:22–33.

based on transcriptomic profiles. (K) Histogram of the percentage of different cell types. AST, astrocytes; ExN, excitatory neurons; IPC, intermediate precursor cells; InN, inhibitory neurons; MG, microglia; NSC, neural stem cells; OL_related, oligodendrocytes and OPCs; RGC, radial glial cells; VAC, vascular endothelial cells. (L) Differentially expressed genes in the astrocyte cluster, highlighting astrocyte markers and mTOR pathway-related genes. (M and N) Relative amino acid levels in cortical tissues from WT and GS-cKO mice at P14 (M) or P28 (N) ($n \geq 3$ mice). (O) Dot plot of differentially expressed genes in astrocytes from GS-cKO mice compared to WT, highlighting KEGG pathway enrichment. (P) qRT-PCR analysis of candidate genes based on RNA-seq data from WT and GS-cKO mice at P14 ($n=3$ mice). (Q and R) Western blot analysis of S6 and phosphorylated S6 (p-S6) in the cortex of WT and GS-cKO mice at P7 (Q) and P14 (R) ($n=3$ mice). (S and T) Quantification of S6 (S) and p-S6 (T) protein levels normalized to β -actin ($n=3$ mice). (U and V) Western blot analysis of AKT and phosphorylated AKT (p-AKT) in the cortex of WT and GS-cKO mice at P7 (U) and P14 (V) ($n=3$ mice). (W and X) Quantification of AKT (W) and p-AKT (X) protein levels normalized to β -actin ($n=3$ mice). Statistical significance was determined using an unpaired two-tailed Student's *t*-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; ns, not significant.