

Supplementary information, Data S1 Materials and Methods

Mouse cortical astrocyte culturing *in vitro*. Primary astrocytes were isolated from P1 (one day post birth) mouse brain as previously described. Briefly, mouse pup was sprayed with 70% ethanol then sacrificed by decapitation. After taking out the brain into dissecting dish filled with D-HANKS on ice, following dissection procedures were performed under a stereomicroscope. Olfactory bulbs, cerebellum and meninges were all carefully removed using the fine dissecting forceps. The remaining cortex pieces were transferred into Falcon tube and digested with 1.25% trypsin in water bath at 37 °C for 30 min. Mixed by occasional shaking every 10 min. Pelleted cortex tissue pieces by centrifuge at 300 × g for 5 min and decanted supernatant. Added 10 ml astrocyte culturing medium and vigorously pipetted to dissociate tissue pieces into single cells proofing under a hemacytometer¹⁶. Dissociated single cell suspension was plated on poly-D-lysine (Sigma-Aldrich, catalogue # P0899) coated dish and incubated at 37 °C in the CO₂ incubator for one week. After reaching confluence, dishes were rigorously shaken for about 2 hours to remove microglia or oligodendrocyte precursor cells. Astrocytes were further passaged three times for further experiments. Astrocyte culture purity was characterized by microscopy morphological studies and cell marker expression. Astrocyte culture medium was DMEM/F12 (Gibco, catalogue # 11330-032) supplemented with 10% fetal bovine serum, B27 (Gibco, catalogue # 17504), 10 ng/ml EGF (Gibco, catalogue # PHG0311), 10 ng/ml FGF2 (Gibco, catalogue # PHG0021) and penicillin/streptomycin. As control, mouse neural progenitor cells were derived from

E12.5 mouse embryos and expanded in neural expansion medium (Millipore, catalogue # SCM003) supplemented with 30 ng/ml heparin, 20 ng/ml EGF and 20 ng/ml bFGF as described. Isolation and culture of primary neurons was same as that of primary astrocytes except that dissociated single cells were not cultured in astrocyte medium, but cultured in DMEM with 10% FBS and 10% F12 firstly then switched into neuron medium (Neurobasal with 1xB27 and 1% Glutamax) 4 hours later.

Retrovirus production. The CAG promoter of pCAG-GFP-IRES-GFP (gift of Prof. Fred Gage) was replaced by the human GFAP promoter cloned from genome to generate pGFAP-GFP-IRES-GFP retroviral vector according to previous report. Retrovirus was produced by transfection of plat-E cells with retroviral vectors, using EugeneHD transfection reagent (Roche) as previously described. Retroviral solution was collected and concentrated by Retro-Concentin virus precipitation solution (System Biosciences, catalogue # EMB100A-1) following protocol provided by the manufacturer.

Immunohistochemistry and quantification. Cells cultured on glass coverslips were fixed in 4% PFA solution for 10 min then incubated in blocking buffer (1% bovine serum albumin in PBS) with or without 0.5% Triton X-100 for 30 min at room temperature (RT). Afterwards, samples were incubated with primary antibodies at 4 °C overnight and then with appropriate fluorescent probe-conjugated secondary

antibodies for one hour at RT. Nuclei were counterstained with DAPI. Images were taken by fluorescence microscope (Zeiss Observer. Z1).

Electrophysiological analysis. Electrophysiological analysis was performed as our previous report. Whole-cell patch clamp recordings were carried out on astrocytes-derived neurons. Recordings were made using Multiclamp 700B amplifier (Molecular Devices). The bath was constantly perfused with fresh artificial cerebrospinal fluid (ACSF) at RT. The ACSF contained (in mM) 126 NaCl, 3 KCl, 1.25 KH₂PO₄, 1.3 MgSO₄, 3.2 CaCl₂, 26 NaHCO₃, and 10 glucose, bubbled with 95% O₂ / 5% CO₂. Signals were sampled at 10 kHz with a 2 kHz low-pass filter. The whole-cell capacitance was fully compensated. Recordings with Ra > 50M or fluctuation > 20 % were excluded. The intracellular solution contained (in mM): 93 K-gluconate, 16 KCl, 2 MgCl₂, 10 HEPES, 4 ATP-Mg, 0.3 GTP-Na₂, 10 creatine phosphate, 0.5% Alexa Fluor 568 hydrazide (Invitrogen), and 0.4% neurobiotin (Invitrogen) (pH 7.25, 290/300 mOsm). Membrane potentials were hold around -70 mV, and step currents with an increment of 5 pA were injected to elicit action potentials. Step voltages with an increment of 10 mV were injected to elicit inward sodium current. To block sodium current, TTX were added into the chamber in a final concentration of 1 μM. EPSCs were blocked by NBQX (10 μM) and IPSCs were inhibited by bicuculline (10 μM). Data were analyzed using pClamp 10 (Clampfit).

Quantitative RT-PCR. Total RNAs were extracted from cells using Trizol reagent

according to the manufacturer's instructions (Sigma-Aldrich, catalogue # T9424). RNA was reverse-transcribed to cDNA using random hexamers and M-MLV Reverse Transcriptase (Promega, catalogue # M5301). cDNA samples were then mixed with 2 × PCR Mix (Qiagen) and Eva Green (Biotium) and subjected to real-time quantitative PCR (Q-PCR) analysis with an MX3000P Stratagene PCR machine. The relative expression values were normalized against the internal controls (*HPRT*).

Statistical analysis. All quantified data were statistically analyzed and presented as mean ± s.e.m. Unless otherwise stated, one-way ANOVA and Turkey's Multiple comparison Test were used to calculate statistical significance with *P* values detailed in the text and figure legends.