Fig S1

A. Atg5 and GFAP normalized density across E12, E15, E19, P2, and P7.

B. LC3 images at E12, E15, E19, and P2.

C. GFAP images at E12, E15, E19, and P2.
Fig S2
Fig S3
Fig S4
SUPPLEMENTARY INFORMATION

SUPPLEMENTARY METHODS

Mice

ICR mice were used in utero and postnatal electroporation experiments. For timed breedings, if vaginal plug is detected, the next day is considered to be E1. The \( \text{Atg}^5(\text{floxed}) \) mice were provided from Riken animal center. Mice are maintained and bred in the Experiment Animal Center of Institute of Zoology, Chinese Academy of Sciences.

Plasmids

The full length cDNAs encoding mouse \( \text{Atg}^5 \), human \( \text{Atg}^5 \), \( \text{Cre} \), mouse \( \text{STAT}^3 \), \( \text{STAT}^3-C \) (constitutively activated STAT3, changing alanine 662 to cysteine and asparagine 664 to cysteine) (Kitamura T, 2003) were amplified by PCR and subcloned into the pCDH lentiviral vector (System Biosciences, CD511B-1) to generate destination constructs. To generate \( \text{Atg}^5 \) shRNA and \( \text{SOCS}^2 \) shRNA expressing plasmids, the oligonucleotides were subcloned into the lentiviral vector pSicoR-GFP (Addgene, 12093). The shRNAs sequences targeting to \( \text{Atg}^5 \) and \( \text{SOCS}^2 \) were provided in the supplementary table S1.

Cell culture and virus packaging

Lentivirus was produced by 293FT cells using plasmids mentioned above. CNS neuronal progenitor cells used in immunofluorescence (IF) and western blot (WB) were isolated from E12 mouse embryonic brains and cultured in Neural Stem Cell
Basal Medium (Millipore, SM008) supplemented with bFGF (5ng/ml, Invitrogen, PHG0026), EGF (5ng/ml, Invitrogen, PHG0311) and 1% penicillin/streptomycin. Prior to infection, precursor cells were seeded in 6 well plates (WB, 1.5x10^6 cells per well) or 48 well plates (IF, 2x10^4 cells per well), which were pre-coated with Poly-D-ornithine (10μg/ml, Sigma, P3655) and Laminin (10μg/ml, Invitrogen, 23017015). Lentivirus solution (MOI=10) was added to each well and medium was changed after 8h infection. The next day, cells were grown in glial cell differentiation medium with DMEM medium supplemented with 1%FBS and 1 x B27. LIF (50ng/ml, Millipore, ESG1107) was added in some experiments. Medium was changed every other day. Four days later, cells were harvested for western blot assay or used for immunocytochemistry. To detect phosphorylation form of JAK2 and STAT3, LIF was added into differentiation medium before harvest for a 30 min short-term treatment. Then cells were collected to analyze the expression of phosphorylation JAK2 and STAT3.

**Western blot**

Mouse cortices or cell samples were lysed in RIPA lysis buffer, ultrasonicated on ice, and centrifuged at 12,000 rpm for 10 min at 4 °C. Then protein concentration was measured by the Pierce BCA Protein Assay Kit (Thermo Scientific). Equal amounts of protein samples were size-separated by 12% SDS-PAGE and blotted onto nitrocellulose membrane by the semi-dry electrophoretic transfer according to the standard protocols (BioRad). The primary antibodies used for WB were: GFAP (Dako, Z033429, 1/3000); Atg5 (MBL International Corporation, M153-3, 1/400); LC3
(MBL, PM036, 1/1000); β-actin (Proteintech, 60008-1, 1/2000); STAT3 and phosho-STAT3 (Tyr705) (Cell Signaling, 4904P, 9145S, 1/2000); JAK2 and phosho-JAK2 (Bioworld, BS1194, BS4109, 1/500); Flag (Sigma, F7425, 1/2000) and SOCS-2 (E67) (Bioworld, BS2914, 1/500). The secondary antibodies used were: 800CW Donkey-Anti-Rabbit IgG, 800CW Donkey-Anti-Mouse IgG, and 680LT Donkey-Anti-Rabbit IgG (Odyssey, 926-32213, 926-32212, 926-68072). Membranes were scanned with the Odyssey Infrared Imaging Systems.

**In utero and postnatal electroporation**

Timed pregnant mice were deeply anesthetized by pentobarbital sodium (70mg/kg) and embryos were carefully exposed. Neonatal mice were cryo anesthetized. Overexpression and knockdown plasmids (1.5μg/μl) were mixed with a EGFP-encoding plasmid by a molar ratio of 3:1. Fast green (0.05%), as a tracer, was added into DNA solution and mixed. Then, the mixture was microinjected into the lateral ventricle of every embryo or newborn pup (1.5ul, about 2ug per ventricle). Sterile physiological saline preheated to 37°C was poured onto the exposed embryo position. Using 5 mm paddle electrodes, embryos were electroporated with five 50 ms square pulses of 40 V with 950 intervals (BTX electroporator) and gently returned to the abdominal cavity. For neonatal pups, they were electroporated with four 50 ms pulses at 90V with a 950 ms interval.

**Sections preparation**

The heads of embryos and newborn pups were harvested at defined developmental stages after electroporation. The brains were peeled off, fixed with 4%
paraformaldehyde in PBS for 24h at 4°C, dehydrated in 30% sucrose solution for 48h at 4 °C, embedded in OCT at -20°C, and cryosectioned to 15 µm coronal sections on a Leica cryostat. Then the sections reserved for immunohistochemistry were stored at -80°C.

**Immunofluorescence staining**

Brain sections were rinsed with PBS once and fixed in 4% PFA/PBS for 40min, washed with PBS twice and blocked for 1h in 5% BSA in PBS containing 0.1% Triton-X (PBST). The primary antibodies were used for incubation overnight at 4°C. The next day, sections were washed three times with PBS, and incubated with the appropriate secondary antibody for 2h at room temperature. After washed 3 times in PBS, counterstained with Dapi (2ug/ml) and washed twice in PBS, the number of positive cells could be observed. The steps for immunocytochemistry were similar to the above, but incubation time was adjusted. The information of primary antibodies:

- GFAP (Dako, Z033429, 1/2000); Atg5 (Abcam, ab78073, 1/100); GLAST (MBL, BMP009, 1/500); BLBP (Millipore, abn14, 1/300); CD44 (Abcam, ab51037, 1/1000); FGFR3 (W153) (Bioworld, BS1125, 1/100); ALDH1L1 (BIOSS, bs-5709R, 1/100); S100B (Proteintech, 15146, 1/100); GFP (Invitrogen, A11122, 1/1000); Flag (CST, #8146S, 1/1000); HA (CST, #5017, 1/800). The secondary antibodies used were: Cy3 Donkey-Anti-Mouse IgG, Cy3 Donkey-Anti-Rabbit IgG, Cy5 Donkey-Anti-Mouse IgG, Cy5 Donkey-Anti-Rabbit IgG (Jackson ImmunoResearch, 715-165-150, 711-165-152, 715-175-150, 711-175-152); Alexa488 Donkey-Anti-Rat IgG (Life Technologies, A21208). All images were acquired with confocal Zeiss LSM 780
microscope.

**RT-PCR analysis**

Total RNA was prepared with TRIzol (Invitrogen, 15596) and cDNA was synthesized from 0.6ug of RNA using Fast Quant RT Kit (TIANGEN). RT-PCR was performed with the SYBR Green PCR Kit (TaKaRa). Primers used were listed in the supplementary table S1.

**Co-immunoprecipitation**

Samples were incubated overnight at 4 °C in the tube containing with 50ul Dynabeads Protein A (Life Technologies, 10001D), which was incubated with antibody (1μg) diluted in 300ul PBS with Tween-20 (Buffer A). The mixture in the tube was placed on the magnet and the supernatant was removed afterwards. Beads-Antibody-Sample complex was washed by gentle pipetting using Buffer A. The supernatant was removed and resuspended with 20ul loading buffer. The sample was heated for 10 min at 70°C and harvested to run for western blot with detection antibody.

**TUNEL staining**

Neural precursor cells were fixed with 4% PFA/PBS and stained for TUNEL analysis using In situ cell death Detection Kit, TMR red (Roche, 1215679291) as the operating instruction. The results were shown as a ratio of TUNEL-positive cell number to total cell number.

**Statistical analysis**

Images were further analyzed by Adobe Photoshop and the “Ipwin 32” was used for
cell counting. Quantification of bands in WB was performed using the software (Odyssey V3.0). Statistical analysis was performed with the student’s T-test. All bar graphs are plotted as mean±SD. Probabilities of P < 0.05 were considered as significant (not significant, n.s., p>0.05; * p<0.05; ** p<0.01; *** p<0.001).

SUPPLEMENTARY FIGURE LEGENDS

Fig S1. Atg5 expression in the developing mouse brain.

(A) Quantification of Atg5, LC3, and GFAP protein expression levels (related to Fig 1A) in the brain cortex from E12 to P7 during embryonic cortex development.

(B,C) Immunostaining showing the LC3 and GFAP expression pattern in the developing brain from E12 to P2.

Fig S2. Atg5 knockdown results in astrocyte differentiation defects.

(A) Left and middle, quantification of Atg5 and LC3 protein expression levels (related to Fig 1C) in neuronal progenitor cells (NPCs) infected with control or Atg5 shRNA lentiviruses. Right, quantification of GFAP expression (related to Fig 1E) in NPCs that infected with lentiviruses and treated with LIF for 4 days.

(B) Immunostaining with Atg5 in coronal sections displaying the effects of Atg5 shRNA in vivo. The embryos were electroporated in utero with Atg5 shRNA or control plasmids at E14 and developed until E18 (Scale bar: 50 μm).

(C) LC3 immunostaining showing the autophagy defects in embryonic brains after electroporation with Atg5 shRNA plasmids (Scale bar: 10μm).
(D) E12 wild-type NPCs were cultured for 2 (2D), 4 (4D) and 6 days (6D) in the absence (-) or presence (+) of LIF (50 ng/ml). The results of the western blot analysis show the expression of GFAP protein. β-actin served as a loading control.

(E) Atg5 knockdown resulting in a decrease of GFAP-positive cells in P8 mouse brains electroporated control and Atg5 shRNA plasmids at E16. The right graphs show the percentages of double-labeled GFAP/GFP cells relative to the numbers of GFP-positive cells (Scale bar: 50μm).

(F) Atg5 knockdown leading to reduced astrocyte-restricted precursor cells, as demonstrated through CD44 staining. The percentages of double-positive CD44-GFP cells relative to GFP positive cells are shown on the right (Scale bar: 50μm).

(G) Tunnel staining of cultured NPCs showing that Atg5 knockdown does not lead to cell death. Cells incubated with DNase I served as positive controls (Scale bar: 50μm).

(H) Tunnel staining of brain cortices showing that there is no significant difference in cell death between Atg5 knockdown and control cells. The graph shows the quantification of the percentage of apoptotic cells (Scale bar: 50μm).

The data are represented as the mean ± SD, n=3, NS, not significant, * p≤0.05, ** p≤0.01, *** p≤0.001, student’s T-test.

Fig S3. Astrocyte differentiation defects through mAtg5 knockdown are rescued
by hAtg5.

(A) Abnormal astrocyte differentiation induced through mAtg5 shRNA was rescued by hAtg5 overexpression in culture. NPCs were infected with different combinations of lentivirus and cultured for 4 days. GFAP immunostaining displays an increase of GFAP+ cells due to human Atg5 overexpression. The percentage of GFAP+ cells relative to total cells is calculated in the graph.

(B) The GFAP protein expression was analyzed through western blotting after hAtg5 overexpression with mAtg5 knockdown. The quantification of GFAP protein expression was calculated.

(C) Decrease of astrocyte-restricted precursor cells through mAtg5 shRNA was rescued by hAtg5, as shown with CD44 staining. Left, confocal images of sections stained for CD44 and GFP. Right, the percentages of CD44+ GFP+ cells relative to GFP+ cell numbers. The data are represented as the mean ± SD. n=3, * p≤0.05, ** p≤0.01. Scale bar: 50 μm.

**Fig S4. Atg5 affects astrocyte differentiation through SOCS2-JAK2-STAT3 signaling pathway.**

(A) E12 NPCs infected with lentiviruses of Atg5 overexpression and control were cultured for 4 days. Western blot analysis shows total JAK2, pJAK2, total STAT3, and pSTAT3 proteins in those groups with or without short 30 minutes LIF treatment. In the right panel, the percentage of pJAK2 /JAK2, pSTAT3/ STAT3
was calculated.

(B) Defective astrocyte differentiation induced through Atg5 shRNA was rescued by STAT3 overexpression in culture. The percentage of GFAP+GFP+ cells relative to the total cells was calculated in the right panel. Scale bar: 50 μm.

(C) Disruption of astrocyte differentiation induced by Atg5 shRNA was rescued through STAT3-C (constitutively activated STAT3, A661C/N663C) overexpression in vivo. Left, confocal images of sections stained for GFAP and GFP. The right graph shows the percentages of double-labeled GFAP+GFP+ cells relative to GFP+ cells. Scale bar: 50 μm.

(D) Real-time PCR analysis showing the change of SOCS1, SOCS2, SOCS3, and SHP-2 when Atg5 was overexpressed.

(E) Western blot revealing the knockdown efficiency of SOCS2 shRNA in vitro.

(F) The expression of SOCS2 at different periods were reduced after Atg5 knockdown. Western blot analysis showing the expression of SOCS2 in cells transfected with Atg5 overexpression plasmid 36h or 96h.

(G) Disruption of astrocyte differentiation induced through Atg5 shRNA was rescued with SOCS2 shRNA in vivo. Left, confocal images of the sections stained for GFAP and GFP. Right, quantification of double-labeled GFAP-GFP cells. Scalebar: 50 μm.

(H) The co-location of LC3 and SOCS2 in 293FT cells cotransfected with Flag-tagged LC3 and HA-tagged SOCS2. Scale bar: 10 μm.

Data are represented as mean ± SD, n=3, * p≤0.05, ** p≤0.01.
Fig S5. Sections from Atg5 knockdown brain were stained with Nestin, Pax6, and TuJ1.

(A) Nestin were used to stain sections from brains electroporated at E16 and analyzed at E19.

(B) Pax6 staining of sections from brains electroporated at E16 and harvested at P2.

(C) Immunostaining with TuJ1 in coronal sections from brains electroporated at E16 and analyzed at E19.
## Supplementary Table S1

Primer sequences used in this study.

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