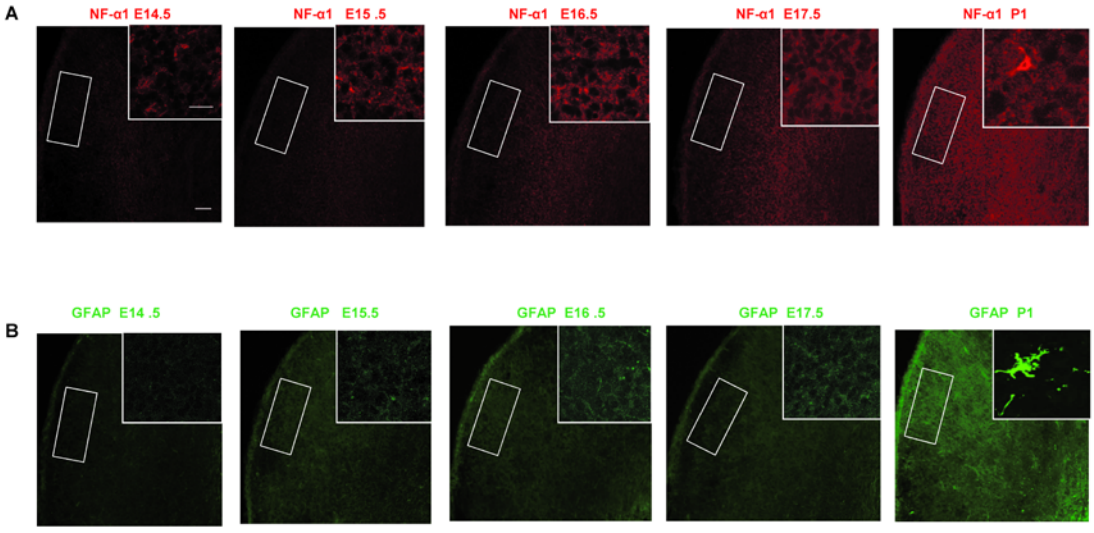

Supplementary Figure Legends

Figure S1: Immunostaining of NF-α1 and GFAP in the neocortex of mice at E14.5, E15.5, E16.5, E17.5 and P1. Coronal sections (16um) from E14.5, E15.5, E16.5, E17.5 and P1 mouse brains (8 random areas/section, 4 sections/embryo, N=3 embryo/phenotype) were immunostained for NF-α1(red) (A) and GFAP (green) (B). The rectangular box in the immunostained sections represents the area of the neocortex used to measure and quantify the intensity of NF-α1 and GFAP immunostaining. Low magnification (×10) and high magnification (×60, see insert) with oil immersion lens (Zeiss LSM 510 Inverted Meta) images were used to show immunostained cell pictures. Magnification (×20) images were used for quantification of intensity. Scale bar=100um for (×10) and scale bar= 20 um for (×60). Expression of NF-α1 from immunostained sections showed a gradual increase in intensity from E14.5 to P1(A), while GFAP immunoreactivity showed low intensity from E14.5 -E17.5 and a surge at P1(B). See Fig 7D and E for bar graphs showing intensity quantification.*$p<0.05$ and **$p<0.01$, N=3.

Supplementary Information:

Supplementary Materials and Methods

Western Blot

Cell lysates were prepared from E14.5 ,E15.5, E16.5, E17.5, P1 cortex as described previously [34]. Twenty μg of protein from the supernatants were analyzed by standard western blotting procedures using nitrocellulose. Protein bands were visualized and quantified by the Odyssey infrared imaging system and software v2.1 (LI-COR Inc.). The protein expression level for each sample was normalized to β-actin.

Immunohistochemistry (IHC)

For immunohistochemistry of E14.5, E15.5, E16.5, E17.5 and P1 mouse brains, pups were sacrificed by decapitation and processed as described previously [74]. The sections were processed also as described previously [74] and incubated with primary antibodies. Slides were examined using a fluorescence microscope (Nikon Eclipse 80i, Tokyo, Japan). The cortical layer was examined for astrocyte (GFAP) and NF-a1 immunostaining. The intensity of the GFAP and NF-α1 immunostaining within rectangular boxed fields (8 random areas/section, 4 sections/embryo, N=3 embryo/phenotype) in each section representing the whole neocortex was analyzed and quantified using imageJ software. Low magnification (×10) and high magnification (×60) with oil immersion lens (Zeiss LSM 510 Inverted Meta) images were used to show immunostained cell pictures and 20x magnification images were used for quantification of intensity.

<table>
<tr><td colspan="2">Immunoblotting</td></tr>
<tr><td>Primary antibodies</td><td>Secondary antibodies</td></tr>
<tr><td>Goat anti-hCPE antibodies (1:5000)
(R&D systems)</td><td rowspan="3">Anti-mouse, anti-goat and anti-rabbit secondary antibodies conjugated to Fluor®700 or 800 (Donkey IgG,1:10000)
(Bio-rad)</td></tr>
<tr><td>Rabbit anti-GFAP antibodies (1:5000)
(abcam)</td></tr>
<tr><td>Mouse Anti-β-actin antibodies (1:10000)
(BD Bio-science)</td></tr>
<tr><td colspan="2">Immunohistochemistry (IHC)</td></tr>
<tr><td>Primary antibodies</td><td>Secondary antibodies</td></tr>
<tr><td>Rabbit anti-GFAP antibodies (1:2000)
(abcam)</td><td>Alexa Fluor®488 anti-rabbit
(Donkey IgG, 1:2000)
(Invitrogen)</td></tr>
<tr><td>Goat anti-hCPE antibodies (1:2000)
(R&D systems)</td><td>Alexa Fluor®555 anti-goat
(Donkey IgG, 1:2000)
(Invitrogen)</td></tr>
</table>

Supplementary Figure Legend

Figure S1: Immunostaining of NF-α1 and GFAP in the neocortex of mice at E14.5, E15.5, E16.5, E17.5 and P1. Coronal sections (16um) from E14.5, E15.5, E16.5, E17.5 and P1 mouse brains (8 random areas/section, 4 sections/embryo, N=3 embryo/phenotype) were immunostained for NF-α1(red) (A) and GFAP (green) (B). The rectangular box in the immunostained sections represents the area of the neocortex used to measure and quantify the intensity of NF-α1 and GFAP immunostaining. Low magnification (×10) and high magnification (×60, see insert) with oil immersion lens (Zeiss LSM 510 Inverted Meta) images were used to show immunostained cell pictures. Magnification (x20) images were used for quantification of intensity. Scale bar=100um for (×10) and scale bar= 20 um for (×60). Expression of NF-α1 from immunostained sections showed a gradual increase in intensity from E14.5 to P1(A), while GFAP immunoreactivity showed low intensity from E14.5 -E17.5 and a surge at P1(B). See Fig 7D and E for bar graphs showing intensity quantification. *$p<0.05$ and **$p<0.01$, N=3.

Supplemental Table S1

The sequences of primers for quantitative real-time PCR

mouse CPE/NF-α1 forward	5’-CTCATCAGCTACCTGGAGCA-3’
mouse CPE/NF-α1 reverse	5’- AGCAAGCAATCGCCAGTAAT-3’
mouse 18S forward	5’-CTCTTAGCTGAGTGTCCCGC-3’
mouse 18S reverse	5’-CTGATCGTCTTCGAACCTCC-3’
mouse Sox9 forward	5’-CATCACCCGCTCGCAATAC-3’
mouse Sox9 reverse	5’-CCGGCTGCGTGACTGTAGTA-3’
mouse Gfap forward	5’- ACATCGAGATCGCCACCTAC-3’
mouse Gfap reverse	5’-TGCTTCGACTCCTTAATGACC-3’

Supplemental Table S2

Primary Antibodies

Immunoblotting

Antibodies	Conditions
anti-ERK(Cell Signaling)	mouse (1:1000)
anti-p-ERK(Cell Signaling)	rabbit (1:5000)
anti-β-Catenin (Santa Cruz)	mouse (1:500)
anti-alpha-tubulin(Cell Signaling)	rabbit IgG1, (1:5000)
anti-Sox9 (Abcam)	rabbit IgG, (1:5000)
anti-CPE antibodies (BD Bio-science)	mouse (1:2500)
anti-β-actin (BD Bio-science)	mouse (1:10000)
anti-CPE/NF-α1 (generated in our laboratory)	rabbit (1:250)
anti-hCPE antibodies(R&D systems)	Goat(1:5000)
anti-GFAP antibodies(abcam)	Rabbit(1:5000)

Immunocytochemistry/Immunohistochemistry

Antibodies	Conditions
anti-Nestin (Millipore)	mouse IgG1, (1:3000)
anti-GFAP (Abcam)	rabbit IgG, (1:2000)
anti-CNPase (Abcam)	mouse IgG1, (1:3000)
anti- β III tubulin (Millipore)	mouse IgG1, (1:3000)
anti-MAP2 antibodies (Millipore)	mouse (1:2000)
anti-hCPE antibodies(R&D systems)	Goat(1:2000)

Secondary antibodies

Immunoblotting

Antibodies (LI-COR – Odyssey)	Conditions
anti-mouse,anti-goat and anti-rabbit secondary antibodies conjugated to Fluor® 700 or 800 (Bio-rad)	donkey IgG, 1:10,000

Immunocytochemistry/Immunohistochemistry

Antibodies (Life technologies)	Conditions
Alexa Fluor® 488 anti-mouse IgG	donkey IgG, 1:2500
Alexa Fluor® 594 anti-rabbit IgG	donkey IgG, 1:2500
Alexa Fluor® 488 anti-mouse IgG	donkey IgG, 1:50
Alexa Fluor® 594 anti-rabbit IgG	donkey IgG, 1:50
Alexa Fluor®488 anti-rabbit IgG (Invitrogen)	donkey IgG, 1:2000
Alexa Fluor®555 anti-goat IgG (Invitrogen)	donkey IgG, 1:2000