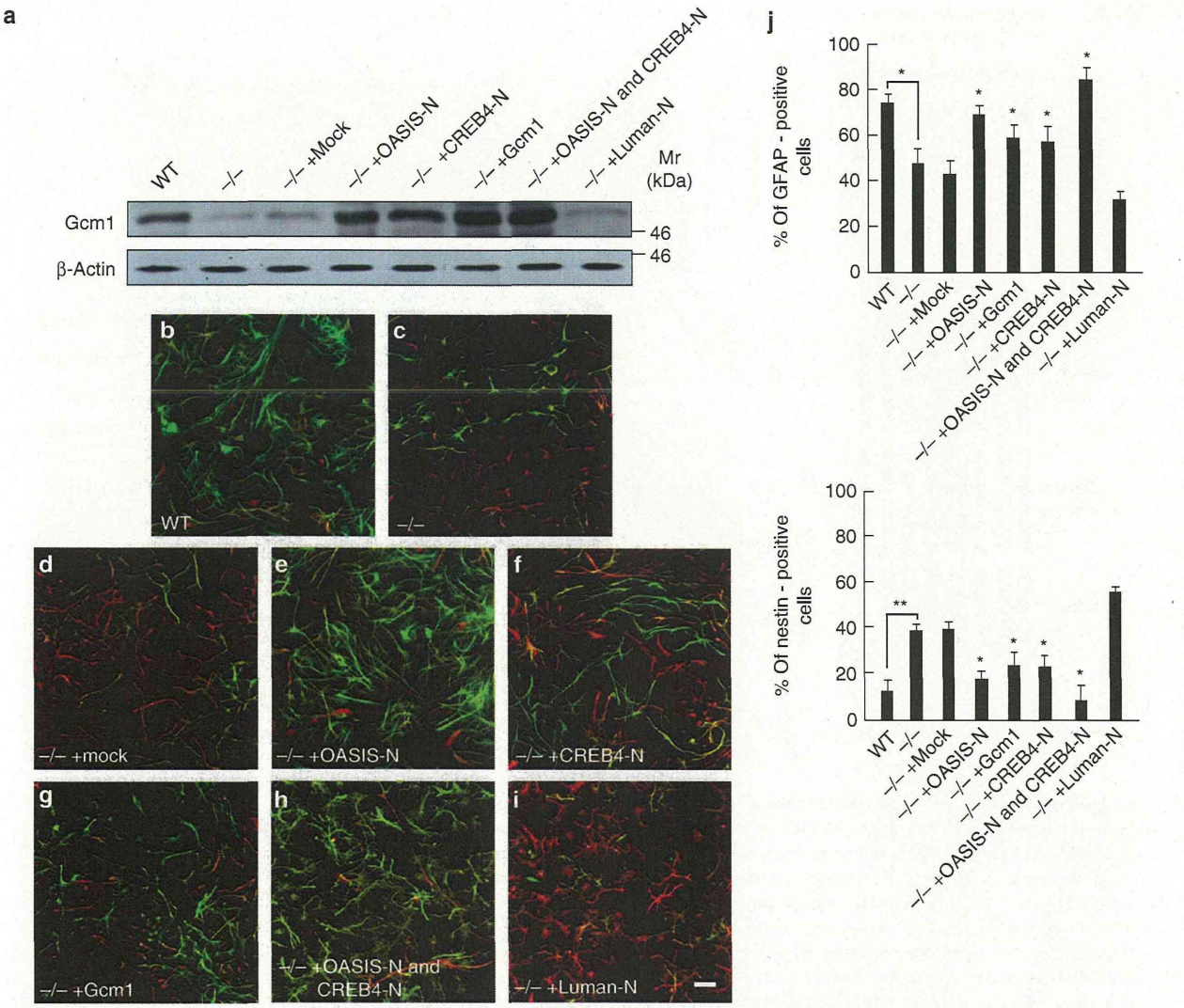


Figure 6 | OASIS family members and Gcm1 rescue the delay in astrocyte differentiation in *Oasis*$^{-/-}$ NPCs. (**a**) Western blotting of Gcm1 in NPCs treated with LIF and BMP2 for 4 days. In *Oasis*$^{-/-}$ NPCs, Gcm1 was upregulated by the introduction of the OASIS N-terminus, the CREB4 N-terminus, or co-expression of both. However, expression of the Luman N-terminus did not affect the expression of Gcm1. Mock indicates empty vector. (**b–i**) Immunostaining of GFAP (green) and nestin (red) in primary cultured NPCs treated with LIF and BMP2 for four days. Mock indicates empty vector. Scale bar, 50 μm. (**j**) The percentages of GFAP- (left panel) and nestin-positive cells (right panel) in (**b–i**). The number of positive cells was measured in five fields per well. All bars represent the mean values ±s.d. of ten experiments. Significant difference between two samples was determined by unpaired Student's *t*-test. $^{*}P<0.05$, $^{**}P<0.01$, between indicated pairs. Multiple comparisons were made using One-way ANOVA followed by Tukey's *post hoc* test. $^{*}P<0.05$, among all indicated samples except for WT.

CREB/ATF transcription factors on the *Gcm1* promoter should be precisely analysed to elucidate the detailed mechanisms underlying cell differentiation regulated by Gcm1.

The mammalian homologues of *gcm* are *Gcm1* and *Gcm2*. These molecules show little homology and have no common functional domains except for the GCM-motif[28]. Previous data have shown that both have the potential to accelerate demethylation of the *Hes5* promoter by direct binding to the GCM-binding site in the promoter followed by acquiring the stem cell properties, but *Gcm2* is a more crucial factor than *Gcm1* for the demethylation of the *Hes5* promoter because of the lower expression of *Hes5* in *Gcm2*$^{-/-}$ mice than in *Gcm1*$^{-/-}$ mice[31]. In our data, expression of *Gcm2* was not changed in *Oasis*$^{-/-}$ mice (data not shown), indicating that *Gcm2* is not associated with the impaired differentiation of astrocytes observed in *Oasis*$^{-/-}$ mice. The expression of *Gcm1* was significantly upregulated in the late stage of mouse embryonic development (Supplementary Fig. S3), whereas that of *Gcm2* was transiently upregulated in the early stage[31]. Therefore, we presume that *Gcm2* is mainly involved in the demethylation of *Hes5* promoter in the early stage of mouse embryonic development, and conversely, that *Gcm1* mainly has a role in the demethylation of the *Gfap* promoter in the late stage. The distinct expression patterns and target promoters for demethylation between *Gcm1* and *Gcm2* may determine glial and neuronal cell lineages, respectively.

We showed that OASIS, CREB4 and Luman activate the UPR signalling in response to mild ER stress, during astrocyte differentiation. Previous studies have shown that UPR signalling activated by mild ER stress is crucial for the differentiation of secretory cells including

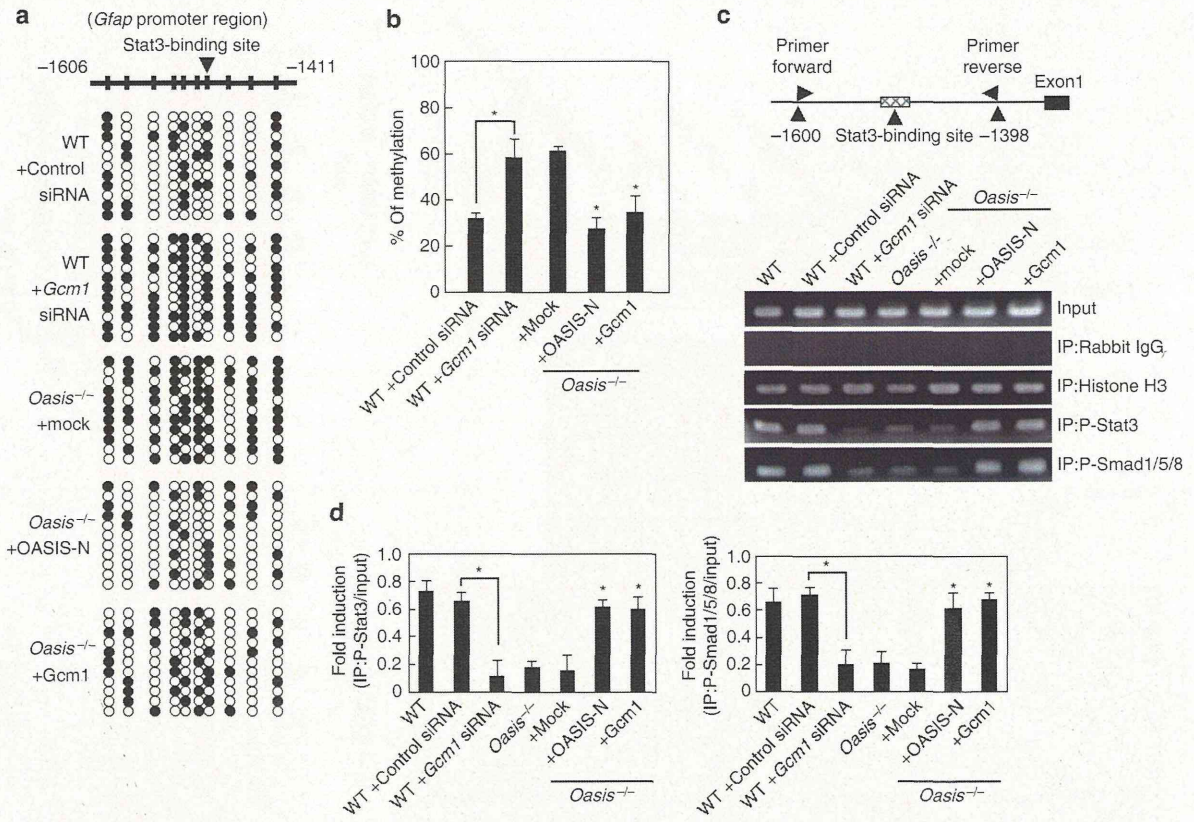


Figure 7 | Gcm1 is involved in demethylation of the *Gfap* promoter. (**a**) Bisulfite sequencing results for the *Gfap* promoter in primary cultured NPCs treated with LIF and BMP2 for four days. (•) and (○) indicate methylated and unmethylated CpG sites, respectively. Experiments were performed using 11 independent samples. Mock indicates empty vector. (**b**) The percentages of methylated sites in the *Gfap* promoter. (**c**) The top panel shows a schematic representation of the *Gfap* promoter and the annealing sites of the primer set used in the ChIP assays. The bottom panel shows the results of PCR amplification of the *Gfap* promoter region containing the Stat3-binding site (−1,398 to −1,600) after immunoprecipitation with the indicated antibodies. Primary cultured NPCs treated with LIF and BMP2 for 4 days were used for ChIP assays. (**d**) Quantitative analysis of PCR amplification after immunoprecipitation by anti-P-Stat3 (top panel) and anti-P-Smad1/5/8 (bottom panel) antibodies in (**c**). All bars represent the mean values ±s.d. of 11 (**b**) and 4 (**d**) experiments. Significant difference between two samples was determined by unpaired Student's *t*-test. $^*P<0.05$, between indicated pairs. Multiple comparisons were made using One-way ANOVA followed by Tukey's *post hoc* test. $^*P<0.05$, among the samples named +mock, +OASIS-N and +Gcm1 (**b**) and *Oasis*−/−, +mock, +OASIS-N and +Gcm1 (**d**).

osteoblasts[21,46–48], chondrocytes[14] and plasma cells[49]. During the differentiation of progenitor cells into these mature secretory cells, secretory materials are gradually produced, and abundant nascent proteins are delivered to the ER. Such an event may serve as a trigger for mild ER stress. Astrocytes also synthesize and secrete various neurotrophic factors and cytokines. During differentiation of NPCs into mature astrocytes, UPR signalling in response to the mild ER stress caused by production of abundant secretory proteins could be activated. Indeed, we found that ER stress-related genes were transiently upregulated during astrocyte differentiation. However, it remains unclear what extent of ER stress is needed for cell differentiation or how such mild ER stress activates only UPR signalling, but does not cause ER stress-induced apoptosis.

The cerebral cortices of *Oasis*$^{-/-}$ mice contained few astrocytes in the embryonic stages, but interestingly, the numbers of astrocytes completely recovered to those in WT mice in adulthood (data not shown). We showed that transfection of NPCs with the CREB4 N-terminus activated the transcription of *Gcm1*. Although the detailed underlying mechanisms are unknown, it is possible that CREB4 has the functional complement to promote astrocyte differentiation in *Oasis*$^{-/-}$ mice in adulthood. It has been reported that one of the aetiologies of psychiatric diseases such as schizophrenia and Rett syndrome is disturbance of the neuronal network caused by a decrease in the number of astrocytes in embryonic and infant stages[50,51]. Although the numbers of astrocytes in the cerebral cortices of *Oasis*$^{-/-}$ mice are normal in adulthood at first glance, a functional disturbance of the CNS may occur in adult *Oasis*$^{-/-}$ mice like in the CNS of such human diseases. It is necessary to perform advanced studies such as precise histological analysis and behavioural tests to clarify the association between OASIS and neuronal diseases.

Methods

Mice. C57BL/6 mice or *Oasis*$^{-/-}$ mice were used in this study. The *Oasis*$^{-/-}$ mice were previously established in our laboratory[21]. In all studies comparing WT and *Oasis*$^{-/-}$ mice, sex-matched littermates derived from the mating of *Oasis*$^{+/-}$ mice were used. The experimental procedures and housing conditions for animals were approved by the Committee of Animal Experimentation, Hiroshima University.

Cell culture, plasmids, transfection and adenovirus. Primary cultured NPCs were cultured as previously described[52]. Briefly, the telencephalons of E14.5 WT and *Oasis*$^{-/-}$ mice were triturated in Hank's balanced salt solution (Invitrogen) by mild pipetting with 1 ml pipette tips (Gilson, Greiner Bio-one). Dissociated cells were cultured in 1.27 g l^{-1} NaHCO3, 25 mg l^{-1} insulin (Sigma), 100 mg l^{-1} apo-transferrin (Sigma), 16 mg l^{-1} putrescin (Sigma), 30 nM sodium selenite (Sigma), 20 nM progesterone (Sigma) in D-MEM-F12 (Gibco), containing

10 ng ml^{-1} basic fibroblast growth factor (Pepro Tech) on culture dishes precoated with poly-ornithine (Sigma) and fibronectin (Sigma) for 4 days. The following day, the cells were stimulated with 50 ng ml^{-1} LIF (Sigma) and 50 ng ml^{-1} BMP2 (Sigma) to initiate astrocyte differentiation. For knockdown of *Gcm1*, NPCs were transfected with a Gcm1-targeting siRNA (Ambion; 4390771) or a non-targeting siRNA (Thermo Scientific; D-001206-13-05) as a control using Lipofectamine 2000 reagent (Invitrogen). Complementary DNAs encoding the N-termini of mouse OASIS, BBF2H7, ATF6, Luman, CREBH and CREB4, the spliced form of XBP1, and full-length ATF4 were inserted into pCDNA3.1 + (Invitrogen). The complementary DNA sequences are summarized in Supplementary Table S1. Cells were transfected with each expression plasmid using Lipofectamine 2000 reagent. The recombinant adenovirus carrying mouse OASIS was generated previously[21]. Adenovirus vectors expressing the mouse Gcm1 were constructed using the AdenoX Expression system (Clontech), according to the manufacturer's protocol.

Immunohistochemistry. Cerebral cortices of WT and *Oasis*$^{-/-}$ mice at the indicated stages were fixed in 4% paraformaldehyde (PFA). Samples were then dehydrated with ethanol, embedded in paraffin and sectioned (5 μm). Paraffin sections were stained with antibodies and visualized under a fluorescence microscope (BX51, Olympus) or a confocal microscope (FV1000D, Olympus). The following antibodies and dilutions were used: anti-GFAP (Sigma; 1:200), and anti-nestin (Abcam; 1:200). The anti-OASIS monoclonal antibody (1:500) was generated previously[21].

***In situ* hybridization**. *In situ* hybridization was performed using digoxigenin-labelled *Oasis* antisense RNA (cRNA) probes[21]. *Oasis* cRNA probes were made by *in vitro* transcription in the presence of digoxigenin-labelled dUTP, using *Oasis* cRNA subcloned into the pGEM-Teasy vector (Promega) as templates. Cerebral cortices were frozen immediately and sectioned (5 μm). Frozen sections were fixed for 20 min with 4% formalin. Then the sections were treated with 0.1% proteinase K for 5 min. After washing with PBS, the sections were re-fixed for 20 min with 4% formalin, and treated with 0.1 M triethanolamine, 2.5% anhydrous acetic acid for 10 min. Sections were prehybridized for 1 h at 37 °C in hybridization buffer (0.01% dextran sulfate, 0.01 M Tris–HCl pH 8.0, 0.05 M NaCl, 50% formamide, 0.2% sarcosyl, 1× Denhardt's solution, 0.5 mg ml^{-1} yeast tRNA, 0.2 mg ml^{-1} salmon testis DNA), and hybridized overnight at 55 °C. After washing with 4×saline sodium citrate buffer for 20 min at 60 °C followed by 2×saline sodium citrate buffer, 50% formamide for 30 min at 60 °C, sections were treated with RNaseA in RNase buffer (10 mM Tris–HCl pH 7.4, 1 mM 0.5 M EDTA (pH 8.0), 0.5 M NaCl) for 30 min at 37 °C to remove un-hybridized probe. After RNase treatment, sections were washed with 2×saline sodium citrate buffer, 50% formamide for 30 min at 60 °C, and then blocked with 1.5% blocking reagent in 100 mM Tris–HCl pH 7.5, 150 mM NaCl for 60 min at room temperature. For detection of digoxigenin-labelled cRNA probes, anti-digoxigenin antibody conjugated to alkaline phosphatase (Roche) was used at a dilution of 1:500, and colour was developed by incubation with 4-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate solution.

Quantitative real-time PCR and RT–PCR. Total RNA was isolated using an RNeasy kit (Qiagen) according to the manufacturer's protocol. First-strand cDNA was synthesized in a 20-μl reaction volume using random primers (Takara) and Moloney murine leukemia virus reverse transcriptase (Invitrogen). Quantitative real-time PCR reactions were performed using a Light Cycler 480 system II (Roche) and Light Cycler SYBR Green I Master (Roche). RT–PCR was performed using each specific primer set in a total volume of 30 μl containing 0.8 μM of each primer, 0.2 mM dNTPs, 3 units of Taq polymerase, and 10×PCR buffer (Agilent). The density of each band on RT–PCR was quantified using Photoshop Elements 2.0 (Adobe Systems). Primer sequences are summarized in Supplementary Table S2.

Western blotting. Proteins were extracted from cells or mice cerebral cortices using cell extraction buffer containing 2.5 mM methionine, 33.3 mM Tris-acetate pH 8.5, 5 mM EDTA, 0.3% SDS, 1.5% Triton, and protease inhibitor cocktail (MBL). Lysates were incubated on ice for 45 min. After centrifugation at 15,000 *g* for 15 min, the soluble protein concentration was measured using BCA protein assay reagents (Pierce). The following antibodies and dilutions were used: anti-β-actin (Sigma; 1:3,000), anti-GFAP (Sigma; 1:1,000), anti-S100β (Abcam; 1:1,000), anti-nestin (Abcam; 1:500), anti-brain lipid-binding protein (Abcam; 1:500), anti-NeuN (CHEMICON; 1:1,000), anti-GSTπ (BD Transduction Laboratories; 1:1,000), anti-phosphorylated-Stat3 (Santa Cruz Biotechnology; 1:500), anti-phosphorylated-Smad1/5/8 (Cell Signaling; 1:1,000), anti-Gcm1 (Santa Cruz Biotechnology; 1:500), anti-Luman (Santa Cruz Biotechnology; 1:250), anti-ATF4 (Santa Cruz Biotechnology; 1:500), anti-ATF6 (Santa Cruz Biotechnology; 1:250), anti-JNK (Cell Signaling; 1:1,000), anti-phosphorylated-JNK (Cell Signaling; 1:1,000), anti-Caspase 3 (Cell Signaling; 1:1,000), anti-BiP (MBL; 1:1,000), and anti-XBP1 (Santa Cruz Biotechnology; 1:250). The anti-OASIS monoclonal antibody (1:1,000) was generated previously[21]. The anti-CREB4 polyclonal antibody (1:1,000) was generated by immunizing mice against mouse recombinant CREB4 (amino acids 1–162). The density of each band was quantified using Photoshop Elements 2.0 (Adobe Systems).

Immunofluorescence. Cells were fixed in 4% PFA and then permeabilized in 0.5% Triton-X 100. The following antibodies and dilutions were used: anti-GFAP (Sigma; 1:500), and anti-nestin (Abcam; 1:250). Cells were visualized under a fluorescence microscope or a confocal microscope (FV1000D, Olympus). The number of positive cells was measured in five fields per well.

TUNEL assay. Primary cultured NPCs prepared from E14.5 WT and *Oasis*$^{-/-}$ mice were fixed in 4% PFA and then permeabilized in 0.1% sodium citrate and 0.1% Triton-X 100. TdT-mediated dUTP nick end labeling (TUNEL) staining was performed using the Cell Death Detection kit, Fluorescein (Roche), according to the manufacturer's protocol. Cells were visualized under a fluorescence microscope. The number of positive cells was measured in five fields per well.

Immunoprecipitation assay. Cells were washed with PBS and lysed with 1 ml of 1% CHAPSO (Sigma) buffer. Cell lysates were centrifuged at 15,000*g* at 4 °C for 15 min and supernatants collected and then incubated with anti-OASIS antibody overnight. Then, samples were incubated with protein G–Sepharose beads (GE Healthcare Life Sciences) for an additional 2 h at 4 °C. Beads were washed with TNE buffer, containing 10 mM Tris, 1 mM EDTA and 150 mM NaCl. Subsequently, samples were subjected to western blotting with anti-CREB4 or anti-Luman antibody.

Luciferase assay. Primary cultured NPCs prepared from E14.5 WT and *Oasis*$^{-/-}$ mice were cultured for 4 days and transfected with 0.2 μg of a pGL3 basic reporter plasmid carrying the firefly luciferase gene (Promega) and 0.02 μg of the reference plasmid pRL-SV40 carrying the Renilla luciferase gene under the control of the SV40 enhancer and promoter (Promega), together with 0.2 μg of an effector protein expression plasmid, using Lipofectamine 2000 reagent. UPRE-LUC reporter was kind gift from Ron Prywes (Columbia University)[53]. After 24 h, luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega) and a luminometer (Berthold Technologies), according to the manufacturer's protocol. Relative activity was defined as the ratio of firefly luciferase activity to that of Renilla luciferase.

Chromatin immunoprecipitation assay. The chromatin immunoprecipitation assay was performed as previously described[10]. The primers used for the mouse *Gcm1* promoter were: 5′-GAAAAATTATTAACATGTGTGAATGCAT-3′ (forward) and 5′-CTCAAAAGAGGGTGGTGGGGGGCTTA-3′ (reverse) yielding a 127-bp product. Those used for the mouse *Gfap* promoter were: 5′-CCTTCCC TATGGTGGGACTCATTAGGAG-3′ (forward) and 5′-CATGCTTGGGCTTC TGGTGTCTACTCCAG-3′ (reverse) yielding a 209-bp product. The following antibodies were used: anti-OASIS[21], anti-CREB4 (generated by immunizing mice against mouse recombinant CREB4: amino acids 1–162), anti-histone H3 (Santa Cruz Biotechnology), and rabbit IgG (Sigma).

Bisulfite sequencing. Procedures were performed as previously described[52]. Sodium bisulfite treatment of genomic DNA was performed using a Methylamp DNA Modification kit (Epigentek), according to the manufacturer's protocol. The region in the *Gfap* promoter containing the Stat-binding site of the bisulfite-treated genomic DNA was amplified by PCR using the following primers: 5′-GGGATTT ATTAGGAGAATTTTAGAAGTAG-3′ (forward) and 5′-TCTACCCATACTTAAA CTTCTAATATCTAC-3′ (reverse). The PCR products were cloned into pCR–Blunt II-TOPO vector (Invitrogen) and at least 11 randomly selected clones were sequenced.

Statistical analysis. Statistical comparisons were made using the unpaired Student's-*t*-test (between two samples) and One-way ANOVA followed by Tukey's *post hoc* test (among multiple samples). The statistical significance of a difference between each sample was determined on the basis of a *P*-value <0.05. *P*-values of <0.05, 0.01 or 0.001 are described as $^{*}P<0.05$, $^{**}P<0.01$, or $^{***}P<0.001$, respectively.

References

1. Ron, D. Translational control in the endoplasmic reticulum stress response. *J. Clin. Invest.* **110,** 1383–1388 (2002).
2. Kaufman, R. J. Orchestrating the unfolded protein response in health and disease. *J. Clin. Invest.* **101,** 1389–1398 (2002).
3. Harding, H. P., Zhang, Y. & Ron, D. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature* **397,** 271–274 (1999).
4. Tirasophon, W., Welihinda, A. A. & Kaufman, R. J. A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells. *Genes Dev.* **12,** 1812–1824 (1998).
5. Calfon, M. *et al.* IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* **415,** 92–96 (2002).
6. Yoshida, H. *et al.* ATF6 activated by proteolysis binds in the presence of NF-Y (CBF) directly to the cis-acting element responsible for the mammalian unfolded protein response. *Mol. Cell. Biol.* **20,** 6755–6767 (2000).

7. Shen, J., Chen, X., Hendershot, L. & Prywes, R. ER stress regulation of ATF6 localization by dissociation of BiP/GRP78 binding and unmasking of Golgi localization signals. *Dev. Cell* **3,** 99–111 (2002).
8. Kondo, S., Saito, A., Asada, R., Kanemoto, S. & Imaizumi, K. Physiological unfolded protein response regulated by CREB/ATF family members, transmembrane bZIP transcription factors. *IUBMB Life* **63,** 233–239 (2011).
9. Asada, R., Kanemoto, S., Kondo, S., Saito, A. & Imaizumi, K. The signaling from endoplasmic reticulum-resident bZIP transcription factors involved in diverse cellullar physiology. *J. Biochem.* **149,** 507–518 (2011).
10. Kondo, S. *et al.* OASIS, a CREB/ATF-family member, modulates UPR signalling in astrocytes. *Nat. Cell Biol.* **7,** 186–194 (2005).
11. Murakami, T. *et al.* Cleavage of the membrane-bound transcription factor OASIS in response to endoplasmic reticulum stress. *J. Neurochem.* **96,** 1090–1100 (2006).
12. Saito, A., Hino, S- I., Murakami, T., Kondo, S. & Imaizumi, K. A novel ER stress transducer, OASIS, expressed in astrocytes. *Antioxid. Redox Signal.* **9,** 563–571 (2007).
13. Kondo, S. *et al.* BBF2H7, a novel transmembrane bZIP transcription factor, is a new type of endoplasmic reticulum stress transducer. *Mol. Cell. Biol.* **27,** 1716–1729 (2007).
14. Saito, A. *et al.* Regulation of endoplasmic reticulum stress response by a BBF2H7-mediated Sec23a pathway is essential for chondrogenesis. *Nat. Cell Biol.* **11,** 1197–1204 (2009).
15. Omori, Y. *et al.* CREB-H: a novel mammalian transcription factor belonging to the CREB/ATF family and functioning via the box-B element with a liver-specific expression. *Nucleic Acids Res.* **29,** 2154–2162 (2001).
16. Zhang, K. *et al.* Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. *Cell* **124,** 587–599 (2006).
17. Adham, I. M. *et al.* Reduction of spermatogenesis but not fertility in Creb3l4-deficient mice. *Mol. Cell. Biol.* **25,** 7657–7664 (2005).
18. Stelzer, G. & Don, J. Atce1: a novel mouse cyclic adenosine 3′,5′-monophosphate-responsive element-binding protein- like gene exclusively expressed in postmeiotic spermatids. *Endocrinology* **143,** 1578–1588 (2002).
19. Nagamori, I. *et al.* The testes-specific bZip type transcription factor Tisp40 plays a role in ER stress responses and chromatin packaging during spermiogenesis. *Genes Cells* **11,** 1161–1171 (2006).
20. Lu, R., Yang, P., O'Hare, P. & Misra, V. Luman, a new member of the CREB/ATF family, binds to herpes simplex virus VP16-associated host cellular factor. *Mol. Cell. Biol.* **17,** 5117–5126 (1997).
21. Murakami, T. *et al.* Signalling mediated by the endoplasmic reticulum stress transducer OASIS is involved in bone formation. *Nat. Cell Biol.* **11,** 1205–1211 (2009).
22. Chihara, K. *et al.* Increased vulnerability of hippocampal pyramidal neurons to the toxicity of kainic acid in OASIS-deficient mice. *J. Neurochem.* **110,** 956–965 (2009).
23. Hosoya, T., Takizawa, K., Nitta, K. & Hotta, Y. Glial cells missing: a binary switch between neuronal and glial determination in Drosophila. *Cell* **82,** 1025–1036 (1995).
24. Jones, B. W., Fetter, R. D., Tear, G. & Goodman, C. S. Glial cells missing: a genetic switch that controls glial versus neuronal fate. *Cell* **82,** 1013–1023 (1995).
25. Vincent, S., Vonesch, J. L. & Giangrande, A. Glide directs glial fate commitment and cell fate switch between neurones and glia. *Development* **122,** 131–139 (1996).
26. Takizawa, K. & Hotta, Y. Pathfinding analysis in a glia-less gcm mutant in Drosophila. *Dev. Genes Evol.* **211,** 30–36 (2001).
27. Kim, J. *et al.* Isolation and characterization of mammalian homologs of the Drosophila gene glial cells missing. *Development* **95,** 12364–12369 (1998).
28. Akiyama, Y., Hosoya, T., Poole, A. M. & Hotta, Y. The gcm-motif: a novel DNA-binding motif conserved in *Drosophila* and mammals. *Proc. Natl Acad. Sci. USA* **93,** 14912–14916 (1996).
29. Egger, B. *et al.* Gliogenesis in Drosophila: genome-wide analysis of downstream genes of glial cells missing in the embryonic nervous system. *Development* **129,** 3295–3309 (2002).
30. Iwasaki, Y. *et al.* The potential to induce glial differentiation is conserved between Drosophila and mammalian glial cells missing genes. *Development* **130,** 6027–6035 (2003).
31. Hitoshi, S. *et al.* Mammalian *Gcm* genes induce *Hes5* expression by active DNA demethylation and induce neural stem cells. *Nat. Neurosci.* **14,** 957–964 (2011).
32. Nakashima, K. *et al.* Synergistic signaling in fetal brain by STAT3-Smad1 complex bridged by p300. *Science* **284,** 479–482 (1999).
33. Bonni, A. *et al.* Regulation of gliagenesis in the central nervous system by JAK-STAT signaling pathway. *Science* **278,** 477–483 (1997).
34. Koblar, S. A. *et al.* Neural precursor differentiation into astrocytes requires signaling through the leukemia inhibitory factor receptor. *Proc. Natl Acad. Sci. USA* **95,** 3178–3181 (1998).
35. Nakashima, K. *et al.* Developmental requirement of gp130 signaling in neuronal survival and astrocyte differentiation. *J. Neurosci.* **19,** 5429–5434 (1999).
36. He, F. *et al.* A positive autoregulatory loop of Jak-STAT signaling controls the onset of astrogliagenesis. *Nat. Neurosci.* **8,** 616–625 (2005).
37. Rajan, P. & Mckay, R. D. Multiple routes to astrocytic differentiation in the CNS. *J. Neurosci.* **18,** 3620–3629 (1998).
38. Takizawa, T. *et al.* DNA methylation is a critical cell-intrinsic determinant of astrocyte differentiation in the fetal brain. *Dev. Cell* **1,** 749–758 (2001).
39. Schubert, S. W. *et al.* bZIP-Type transcription factors CREB and OASIS bind and stimulate the promoter of the mammalian transcription factor GCMa/Gcm1 in trophoblast cells. *Nucleic Acids Res.* **36,** 3834–3846 (2008).
40. Clauss, I. M., Chu, M., Zhao, J. L. & Glimcher, L. H. The basic domain/leucine zipper protein hXBP-1 preferentially binds to and transactivates CRE-like sequences containing an ACGT core. *Nucleic Acids Res.* **24,** 1855–1864 (1996).
41. Harris, M. *et al.* Transcriptional regulation of the thyrotropin-releasing hormone gene by leptin and melanocortin signaling. *J. Clin. Invest.* **107,** 111–120 (2001).
42. Berhane, K. & Boggaram, V. Identification of a novel DNA regulatory element in the rabbit surfactant protein B (SP-B) promoter that is a target for ATF/CREB and AP-1 transcription factors. *Gene* **268,** 141–151 (2001).
43. Cote-Vélez, A. *et al.* Dexamethasone represses cAMP rapid upregulation of TRH gene transcription: identification of a composite glucocorticoid response element and a cAMP response element in TRH promoter. *J. Mol. Endocrinol.* **34,** 177–197 (2005).
44. Sheng, Z. *et al.* A genome-wide RNA interference screen reveals an essential CREB3L2-ATF5-MCL1 survival pathway in malignant glioma with therapeutic implications. *Nat. Med.* **16,** 671–677 (2010).
45. Hai, T. & Hartman, M. G. The molecular biology and nomenclature of the activating transcription factor/cAMP responsive element binding family of transcription factors: activating transcription factor proteins and homeostasis. *Gene* **273,** 1–11 (2001).
46. Wei, J., Sheng, X., Feng, D., McGrath, B. & Cavener, D. R. PERK is essential for neonatal skeletal development to regulate osteoblast proliferation and differentiation. *J. Cell Physiol.* **217,** 693–707 (2008).
47. Saito, A. *et al.* Endoplasmic reticulum stress response mediated by the PERK-eIF2á-ATF4 pathway is involved in osteoblasts differentiation induced by BMP2. *J. Biol. Chem.* **286,** 4809–4818 (2011).
48. Tohmonda, T. *et al.* The IRE1á-XBP1 pathway is essential for osteoblasts differentiation through promoting transcription of *Osterix*. *EMBO Rep.* **12,** 451–457 (2011).
49. Gass, J. N., Gifford, N. M. & Brewer, J. W. Activation of an unfolded protein response during differentiation of antibody-secreting B cells. *J. Biol. Chem.* **277,** 49047–49054 (2002).
50. Cotter, D. R., Pariante, C. M. & Everall, I. P. Glial cell abnormalities in major psychiatric disorders: the evidence and implications. *Brain Res. Bull.* **55,** 585–595 (2001).
51. Nagai, K., Miyake, K. & Kubota, T. A transcriptional repressor MeCP2 causing Rett syndrome is expressed in embryonic non-neuronal cells and controls their growth. *Dev. Brain Res.* **157,** 103–106 (2005).
52. Sanosaka, T. *et al.* Identification of genes that restrict astrocyte differentiation of midgestational neural precursor cells. *Neuroscience* **155,** 780–788 (2008).
53. Wang, Y. *et al.* Activation of ATF6 and an ATF6 DNA binding site by the endoplasmic reticulum stress response. *J. Biol. Chem.* **275,** 27013–27020 (2000).

Acknowledgements

We thank M. Tohyama and S. Shiosaka for helpful discussions and critical reading of the manuscript. We also thank S. Nakagawa, K. Takedachi, and M. Harada for technical support. This work was partly supported by grants from the Japan Society for the Promotion of Science KAKENHI (#22020030, #22800049), Sumitomo Foundation, Mochida Memorial Foundation for Medical and Pharmaceutical Research, Astellas Foundation for Research on Metabolic Disorders, Takeda Science Foundation, The Pharmacological Research Foundation Tokyo, Daiichi-Sankyo Foundation of Life Science, The Naito Foundation, Senri Life Science Foundation, Hokuto Foundation for Bioscience, and The Japan Prize Foundation.

Author contributions

A.S. and K.I. designed the experiments. A.S., S.K., N.K., R.A., H.I., M.O., H.M. and S.I. performed the experiments. T.S. and K.N guided the experiments of astrocyte differentiation. T.S. and K.N. provided substantial input into the writing of the manuscript. A.S. and K.I. wrote the manuscript. K.I. supervised the project.

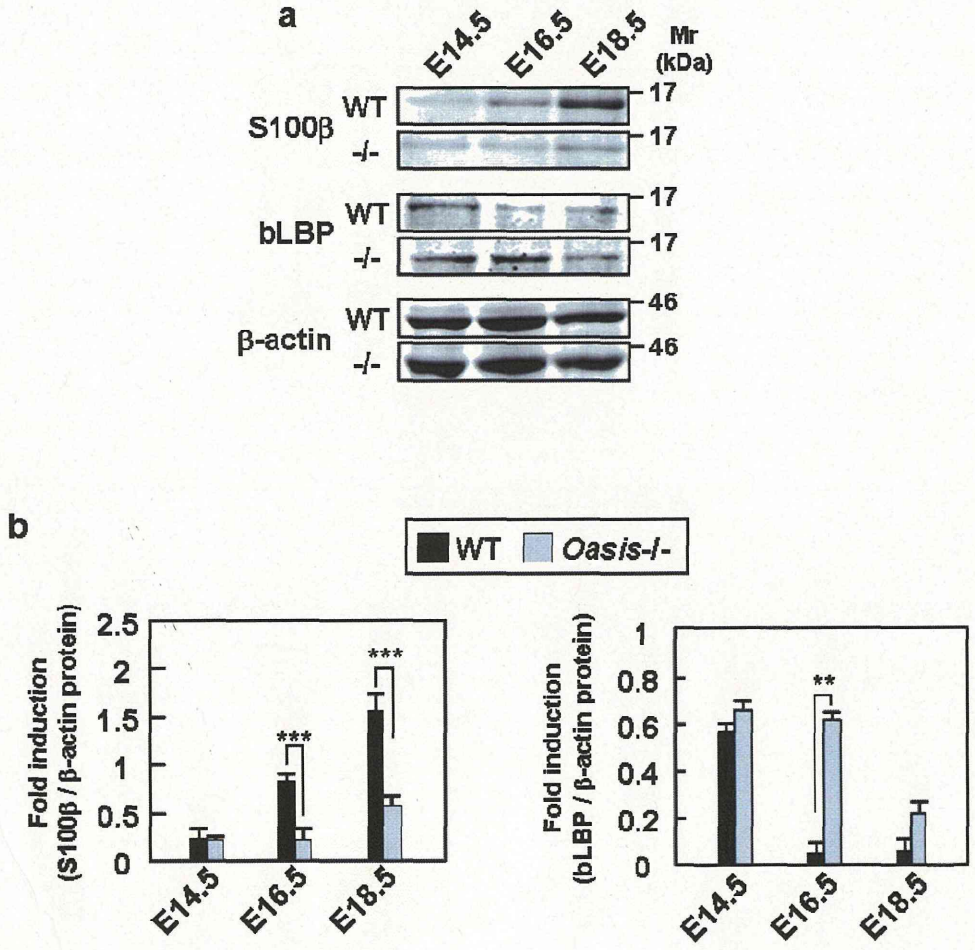
Additional information

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

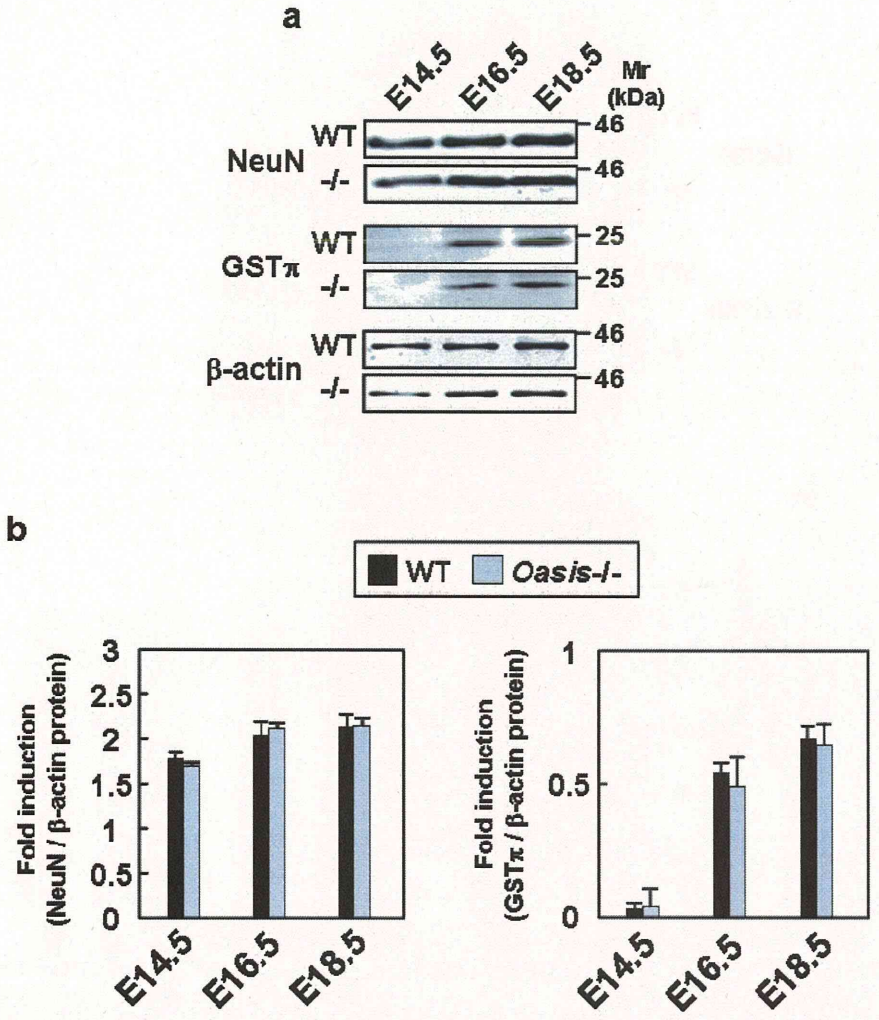
Competing financial interests: The authors declare no competing financial interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

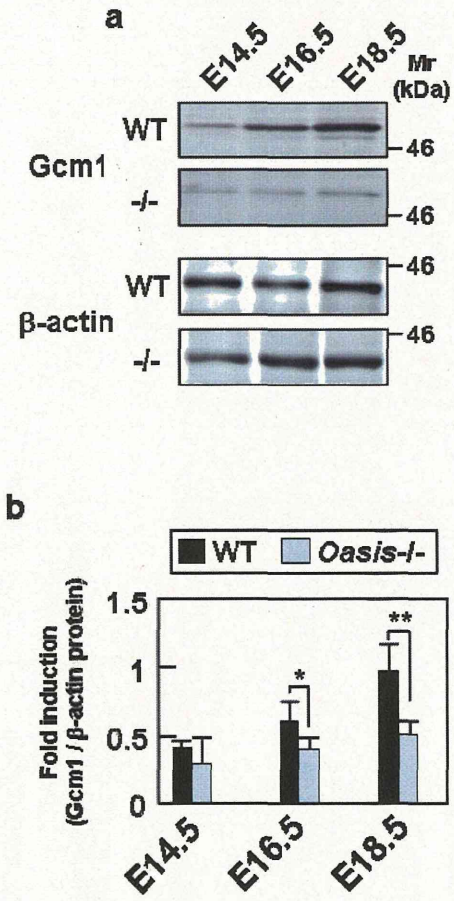
How to cite this article: Saito, A. *et al.* Unfolded protein response, activated by OASIS family transcription factors, promotes astrocyte differentiation. *Nat. Commun.* 3:967 doi: 10.1038/ncomms1971 (2012).



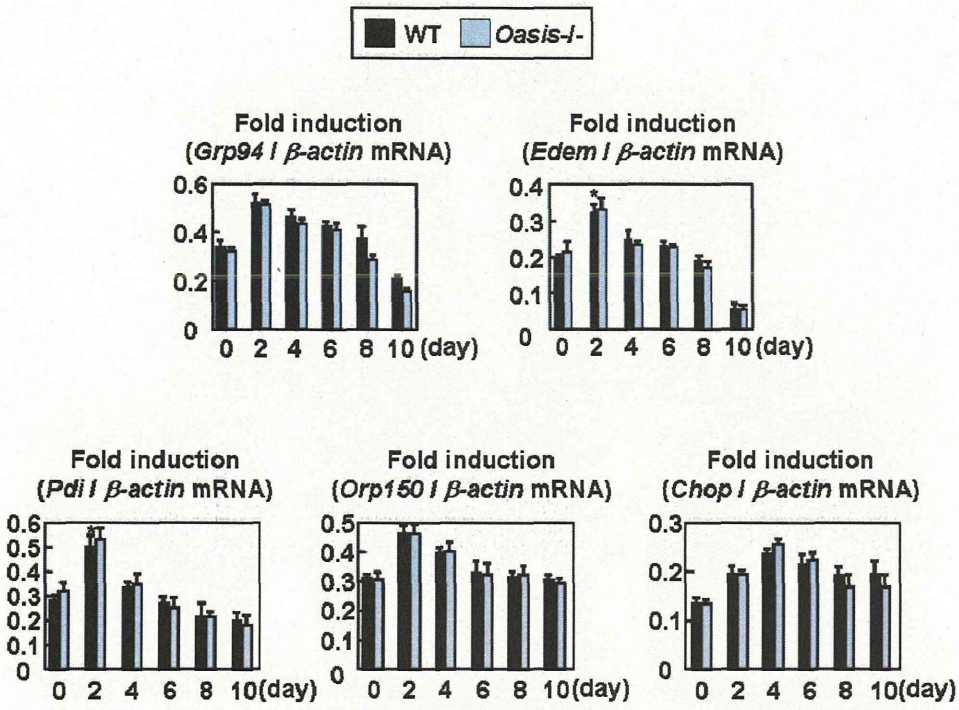
Supplementary Figure S1. Cerebral cortices of *Oasis*$^{-/-}$ in the embryonic stage. (**a**) Western blotting of S100β and bLBP in the cerebral cortices of wild-type (WT) and *Oasis*$^{-/-}$ mice. The expression levels of S100β were markedly lower, and those of bLBP were higher, in the cerebral cortices of *Oasis*$^{-/-}$ mice. (**b**) Quantitative analysis of protein expression levels in (**a**). All bars represent the mean values ±s.d. of 3 experiments. Significant difference was determined by unpaired Student's-*t*-test. $^{**}P < 0.01$, $^{***}P < 0.001$, between indicated pairs.



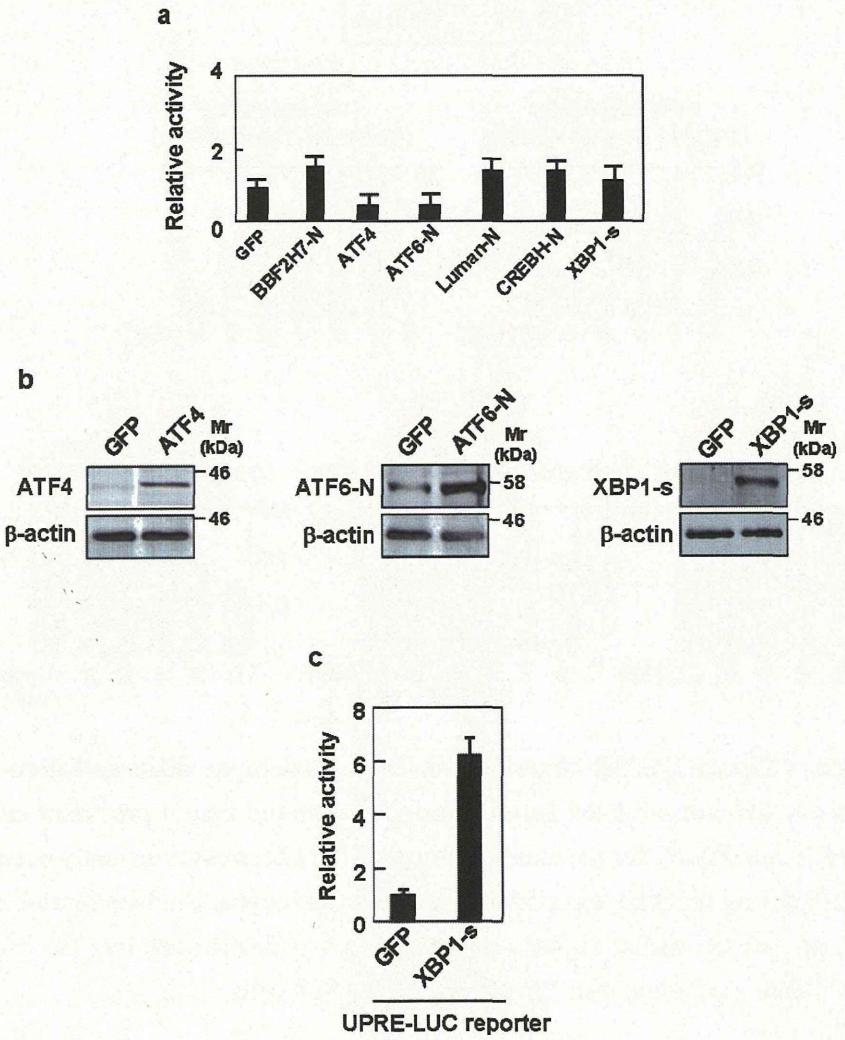
Supplementary Figure S2. *Oasis* deficiency does not affect the differentiation of NPCs. (**a**) Western blotting of NeuN and GSTπ in the cerebral cortices of WT and *Oasis*$^{-/-}$ mice. NeuN, a neuronal marker, and GSTπ, an oligodendrocyte marker, expression levels were not affected by *Oasis* deficiency. (**b**) Quantitative analysis of protein expression levels in (**a**). All bars represent the mean values ±s.d. of 3 experiments.



Supplementary Figure S3. Expression of Gcm1 is down-regulated in *Oasis*$^{-/-}$ mice. (**a**) Expression of Gcm1 was induced in the cerebral cortices of mouse embryos after embryonic day (E) 16.5. In *Oasis*$^{-/-}$ mice, it was inhibited compared with that in WT mice. (**b**) Quantitative analysis of Gcm1 expression in (**a**). All bars represent the mean values ±s.d. of 3 experiments. Significant difference was determined by unpaired Student's-*t*-test. $*P < 0.05$, $**P < 0.01$, between indicated pairs.



Supplementary Figure S4. ER stress occurs during astrocyte differentiation. Real-time PCR analysis of ER stress-related genes in primary cultured neural precursor cells (NPCs) treated with LIF and BMP2 for the indicated times. Mild ER stress transiently occurred (from day 2 to day 6) during the differentiation of NPCs into astrocytes. All bars represent the mean values ±s.d. of 4 experiments. Significant difference was determined by One way ANOVA followed by Tukey's post hoc test. $*P < 0.05$, among WT cells.



Supplementary Figure S5. Other CREB/ATF family members don't affect *Gcm1* promoter activation. (**a**) Reporter assays using NPCs. Vectors expressing the N-termini of mouse BBF2H7, ATF6, Luman and CREBH and the spliced form of XBP1 (XBP1-s), ATF4, and green fluorescent protein (GFP) were co-transfected with each *Gcm1*-Luc construct. GFP was used as a control. N: N-terminus. (**b**) Western blotting analysis in NPCs transfected with each expression plasmid. (**c**) Reporter assays using NPCs. Vectors expressing the XBP1-s was co-transfected with UPRE-LUC reporter. All bars represent the mean values ±s.d. of 3 (**a**) and 2 (**c**) experiments.