manner (5, 17). Therefore, p29 is thought to play a key role in ciliary movements in response to cAMP. The deduced amino acid sequence of *ODAL1* includes several phosphorylation sites (Fig. 1), and the ciliary orientation on cortical sheets from *ODAL1*-RNAi cells lost cAMP-dependent control (Fig. 5B). This may be due to the absence of the cAMP-dependent phosphorylation of some axonemal proteins induced by *ODAL1* silencing. To test this possibility, we examined the effects of *ODAL1* silencing on the cAMP-dependent phosphorylation of axonemal proteins. As a result, the cAMP-dependent phosphorylation of p29 was not detected in the axonemes from *ODAL1*-RNAi cells (Fig. 7A and B). This result indicates that *ODAL1* silencing impairs the cAMP-dependent phosphorylation of p29. The SDS-PAGE band pattern and the observation of cross-sectional images of the *ODAL1*-RNAi axonemes showed that the outer dynein arms were decreased to some extent (Fig. 6 and 8). These results suggest that the defect in the ciliary response to cAMP caused by *ODAL1* silencing may be due to a reduction in the levels of p29 in the outer dynein arm. Our results also suggest that the structural integrity of the outer dynein arm may be essential to produce a high ciliary beat frequency. Further studies, e.g., determining ciliary movements using gene silencing for the other outer dynein arm components, will be required to clarify the mechanisms regulating the ciliary beat frequency.

In conclusion, we demonstrated that the *ODAL1* gene is essential for controlling the ciliary response by cAMP-dependent phosphorylation. The *ODAL1* product may be the p29-phosphorylatable LC of the *Paramecium* 22S dynein. The use of gene silencing by RNAi using the feeding method and the analysis of ciliary movements using cortical sheets could provide further information for an understanding of the molecular mechanism of ciliary movements.

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Unfolded protein response, activated by OASIS family transcription factors, promotes astrocyte differentiation

Atsushi Saito[1], Soshi Kanemoto[1], Noritaka Kawasaki[1], Rie Asada[1], Hideo Iwamoto[1], Mami Oki[1], Hidetaka Miyagi[1], Soutarou Izumi[1], Tsukasa Sanosaka[2], Kinichi Nakashima[2] & Kazunori Imaizumi[1]

OASIS is a member of the CREB/ATF family of transcription factors and modulates cell- or tissue-specific unfolded protein response signalling. Here we show that this modulation has a critical role in the differentiation of neural precursor cells into astrocytes. Cerebral cortices of mice specifically deficient in OASIS (*Oasis*$^{-/-}$) contain fewer astrocytes and more neural precursor cells than those of wild-type mice during embryonic development. Furthermore, astrocyte differentiation is delayed in primary cultured *Oasis*$^{-/-}$ neural precursor cells. The transcription factor Gcm1, which is necessary for astrocyte differentiation in *Drosophila*, is revealed to be a target of OASIS. Introduction of Gcm1 into *Oasis*$^{-/-}$ neural precursor cells improves the delayed differentiation of neural precursor cells into astrocytes by accelerating demethylation of the *Gfap* promoter. Gcm1 expression is temporally controlled by the unfolded protein response through interactions between OASIS family members during astrocyte differentiation. Taken together, our findings demonstrate a novel mechanism by which OASIS and its associated family members are modulated by the unfolded protein response to finely control astrocyte differentiation.

[1] Department of Biochemistry, Institute of Biomedical & Health Sciences, University of Hiroshima, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan. [2] Laboratory of Molecular Neuroscience, Graduate School of Biological Sciences, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0101, Japan. Correspondence and requests for materials should be addressed to K.I. (email: imaizumi@hiroshima-u.ac.jp).

To manage the burden of protein synthesis, cells augment the folding capacity of the endoplasmic reticulum (ER) in response to the accumulation of unfolded proteins. This is called the unfolded protein response (UPR)[1,2]. In mammalian cells, the three major transducers of the UPR are PKR-like endoplasmic reticulum kinase[3], inositol-requiring 1 (refs 4,5), and activating transcription factor 6 (ATF6)[6,7]. Additionally, novel types of ER stress transducers that are structurally included in the CREB/ATF family and share a region of high sequence similarity with ATF6 have been identified[8,9]. These proteins have a transmembrane domain, which allows them to associate with the ER, a transcription-activation domain and a basic leucine zipper (bZIP) domain. These ER stress transducers include OASIS[10–12], BBF2H7[13,14], CREBH[15,16], CREB4 (refs 17–19), and Luman[20], and are collectively referred to as OASIS family members. These molecules have unique cell- or tissue-specific expression patterns, indicating that OASIS family members may be associated with a distinct physiological response that is dependent on the cell or tissue.

Oasis was originally identified as a gene that is specifically induced in long-term cultured astrocytes. Under normal conditions, OASIS is expressed at the ER membrane. In response to ER stress, OASIS is cleaved at the transmembrane region, and processed fragments of OASIS (the OASIS amino-terminus) containing the bZIP domain are translocated into the nucleus to promote transcription of target genes[10–12]. *In vivo*, OASIS is preferentially expressed in osteoblasts of bone tissues and astrocytes in the central nervous system (CNS). *Oasis*-deficient (*Oasis*$^{-/-}$) mice exhibit severe osteopaenia caused by a decrease in the levels of type I collagen, a major component of the bone matrix and an OASIS target gene in osteoblasts[21]. In the CNS, OASIS is significantly induced in astrocytes after brain injury[10]. OASIS is also upregulated in reactive astrocytes after neuronal degeneration induced by kainic acid. The number of glial fibrillary acidic protein (GFAP)-positive reactive astrocytes was decreased in the hippocampi of *Oasis*$^{-/-}$ mice compared with those of wild-type (WT) mice[22], indicating that the number of mature reactive astrocytes is decreased in *Oasis*$^{-/-}$ mice after brain injury. Although these findings suggest that OASIS is involved in the proliferation and differentiation of astrocytes, the functional details of OASIS in astrocytes remain unclear.

The *glial cell missing* (*gcm*) gene was identified in *Drosophila* through the study of loss-of-function mutants[23–25]. Mutation in the *gcm* gene causes presumptive glial cells to differentiate into neurons, whereas ectopic expression of *gcm* makes all CNS cells differentiate into glial cells. Thus, *gcm* functions as a binary switch between glial and neuronal fates[26]. Two *gcm* homologues (*Gcm1* and *Gcm2*) have been identified in mice[27]. *Gcm1* and *Gcm2* encode transcription factors with a unique DNA-binding domain called the GCM-motif within their N-terminal regions allowing these factors to bind to GCM-binding sites[28] and regulate the expression of several genes[29]. The introduction of Gcm1 into cultured brain cells derived from mice resulted in the induction of an astrocyte lineage[30]. Recent work showed that mammalian *Gcm1* and *Gcm2* are involved in the genetic regulation of *Hes5* transcription by accelerating DNA demethylation followed by induction of neural stem cells in the early embryonic stage[31]. However, the roles of *Gcm* in the differentiation of neural precursor cells (NPCs) into glial cell lineage in mammals are still unknown. Here we show that the UPR signalling mediated by OASIS and its associated family members, regulate the expression of Gcm1 and subsequently, differentiation of NPCs into astrocytes, via the demethylation of the *Gfap* promoter.

Results

Oasis$^{-/-}$ mice exhibit decrease in the number of astrocytes. OASIS is structurally similar to an ER stress sensor ATF6, and contains a transmembrane domain, a transcription-activation domain and a bZIP domain at its N-terminus (Fig. 1a). To examine the expression of OASIS in the embryonic CNS, we performed *in situ* hybridization using mouse cerebral cortices. Although signals for *Oasis* messenger RNA were hardly observed in the cerebral cortices of embryonic day (E) 14.5 mice, strong signals were detected at E16.5 and E18.5 (Fig. 1b). Western blotting showed that both full-length and N-terminal OASIS (an active form) were expressed at E16.5 and the amounts of these proteins were increased at E18.5 (Fig. 1c), the findings of which are consistent with the findings regarding expression of *Oasis* mRNA. These data indicate that OASIS is expressed in the cerebral cortices of mouse embryos in the late stages of embryonic development. It is well known that astrocytes are largely differentiated from NPCs, around birth[32]. We noticed that upregulation of OASIS was synchronized with the initiation of differentiation of NPCs into astrocytes, and we hypothesized that OASIS may be involved in the regulation of astrocyte differentiation. To verify this hypothesis, we first investigated the expression of astrocyte and NPC markers in the embryonic stages by immunohistochemistry using cerebral cortices of WT and *Oasis*$^{-/-}$ mice. The numbers of cells positive for GFAP, an astrocyte marker, were significantly higher in the cerebral cortices of WT mice than in those of *Oasis*$^{-/-}$ mice from E16.5 (Fig. 1d). By contrast, those of nestin, an NPC marker, were higher in the cerebral cortices of *Oasis*$^{-/-}$ mice than in those of WT mice at E16.5 and E18.5 (Fig. 1e). The double staining of GFAP and OASIS (Fig. 1f), or GFAP and nestin (Fig. 1g), showed that GFAP and OASIS were co-expressed in the same cells, but not GFAP and nestin (Fig. 1f,g). To examine the expression levels of each marker, we carried out western blotting (Fig. 1h). In the embryonic stages, the expression levels of GFAP and S100β were markedly lower, and those of nestin and brain lipid-binding protein, NPC and radial glial cell markers, respectively, were higher in the cerebral cortices of *Oasis*$^{-/-}$ mice (Fig. 1h,i; Supplementary Fig. 1). These results suggest that differentiation of astrocytes from NPCs may be disturbed in the cerebral cortices of *Oasis*$^{-/-}$ mice in the embryonic stages.

Differentiation of NPCs is inhibited in _Oasis_$^{-/-}$ NPCs. Next, we investigated the expression of astrocyte and NPC markers using primary cultured NPCs prepared from E14.5 mice telencephalons. To promote differentiation of NPCs into astrocytes, the activation of Stat3 mediated by the stimulation of cytokines such as leukaemia inhibitory factor (LIF) is needed[32–36]. The bone morphogenetic protein (BMP) family is another group of astrocyte-inducing cytokines, members of which activate Smads[32,35,37]. Therefore, we treated NPCs with LIF and BMP2 (on day 0). During the differentiation of WT NPCs into astrocytes, the expression level of *Gfap* was increased, and conversely, that of *Nestin* was decreased (Fig. 2a). In *Oasis*$^{-/-}$ cells, the induction of *Gfap* expression and the reduction of *Nestin* expression were significantly impaired (Fig. 2a). The expression levels of these proteins coincided with those of their mRNAs (Fig. 2b). These results are consistent with *in vivo* findings that astrocyte differentiation is disturbed in *Oasis*$^{-/-}$ mice. Interestingly, when the culture was continued for a long time, the expression levels of these genes were not different between WT and *Oasis*$^{-/-}$ cells (data not shown). Thus, we concluded that the differentiation of NPCs into astrocytes was delayed in *Oasis*$^{-/-}$ cells, but was not completely inhibited. The expression of neuronal nuclear antigen (NeuN), a neuronal marker, and glutathione *S*-transferase-pi (GSTπ), an oligodendrocyte marker, were not affected in *Oasis*$^{-/-}$ mice (Supplementary Fig. S2), indicating that differentiation of NPCs into neurons or oligodendrocytes was intact in *Oasis*$^{-/-}$ mice.

Demethylation of *Gfap* promoter is inhibited in _Oasis_$^{-/-}$ NPCs. It is well known that the activation of Stat3 and Smads, and epigenetic regulation of the methylation status of the *Gfap* promoter are essential for differentiation of NPCs into astrocytes. Phosphorylated-Stat3 and Smads bind to demethylated sites in the

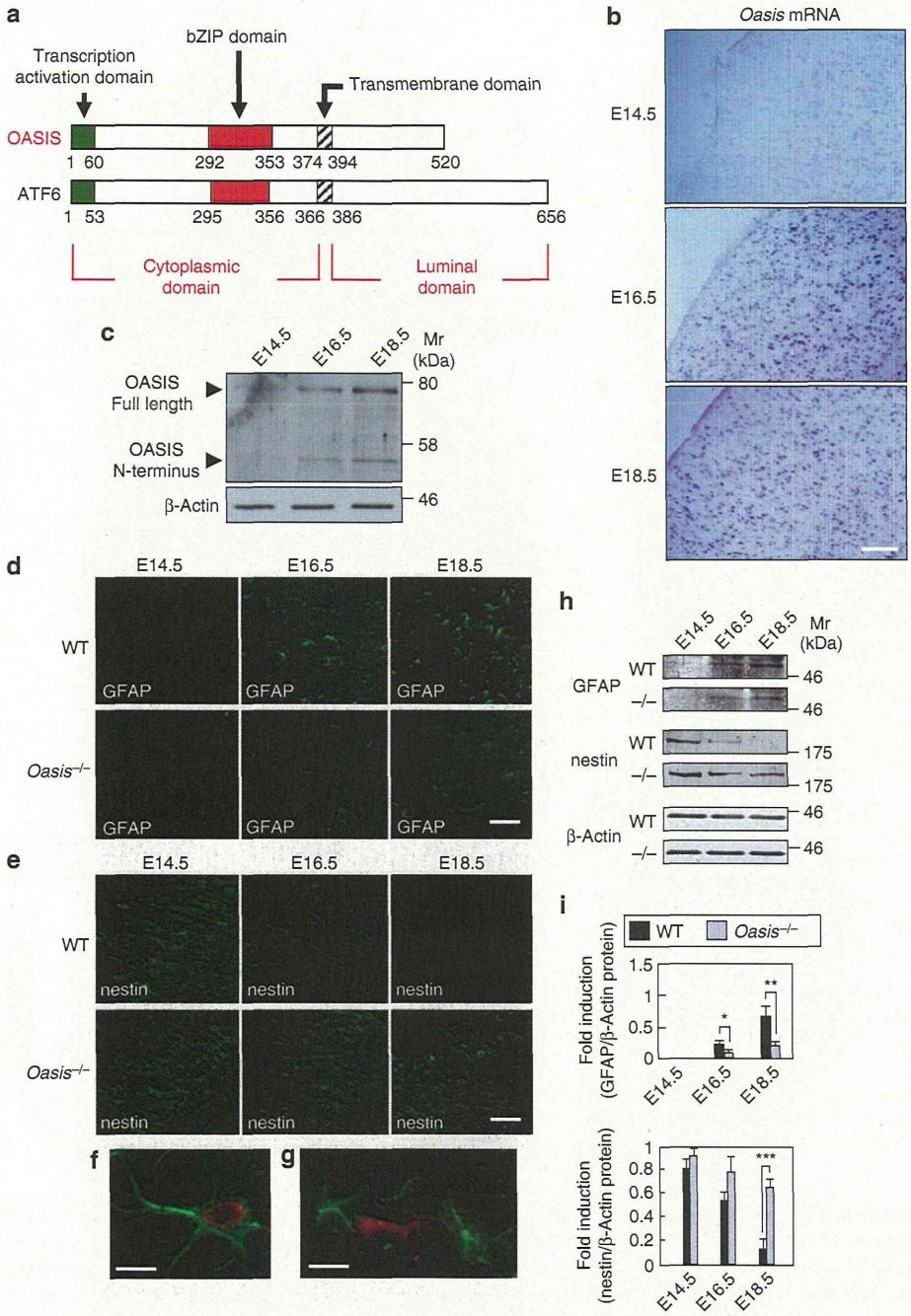

Figure 1 | Cerebral cortices of *Oasis*$^{-/-}$ mice. (**a**) Predicted peptide features of mouse OASIS and ATF6. (**b**) *In situ* hybridization of *Oasis* mRNA in the cerebral cortices of embryonic day (E) 14.5, E16.5 and E18.5 mice. *Oasis* expression was induced in the cerebral cortices of E16.5 and E18.5 mice. Scale bar, 150 μm. (**c**) Western blotting of OASIS in the cerebral cortices of E14.5, E16.5 and E18.5 mice. Both full-length OASIS and its N-terminus were detected at E16.5 and were increased at E18.5. (**d**,**e**) Immunohistochemical analysis of (**d**) GFAP and (**e**) nestin in the cerebral cortices of E14.5, E16.5 and E18.5 WT and *Oasis*$^{-/-}$ mice. In *Oasis*$^{-/-}$ mice, the number of GFAP-positive cells was lower and that of nestin-positive cells was higher at E16.5 and E18.5 mice. Scale bars, 100 μm. (**f**,**g**) Double staining of GFAP (green) and OASIS (red) (**f**), GFAP (green) and nestin (red) (**g**) in the cerebral cortices of E18.5 WT mice. Scale bars, 50 μm. (**h**) Western blotting of GFAP and nestin in the cerebral cortices of WT and *Oasis*$^{-/-}$ mice. (**i**) Quantitative analysis of protein expression levels in (**h**). All bars represent the mean values ±s.d. of three experiments. Significant difference was determined by unpaired Student's-*t*-test. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, between indicated pairs.

Gfap promoter to form a Stat3-Smads-p300 complex and promote transcription of *Gfap*[32,35,37,38]. We examined levels of phosphorylated Stat3 and Smads and the methylation status of the *Gfap* promoter in *Oasis*$^{-/-}$ mice. Although the levels of phosphorylated Stat3 and Smads were not changed (Fig. 2c,d), the amount of demethylation of the *Gfap* promoter was significantly decreased in *Oasis*$^{-/-}$ NPCs (about 45% demethylated) compared with WT NPCs (about 65% demethylated) (Fig. 2e,f). Furthermore, the binding of Stat3

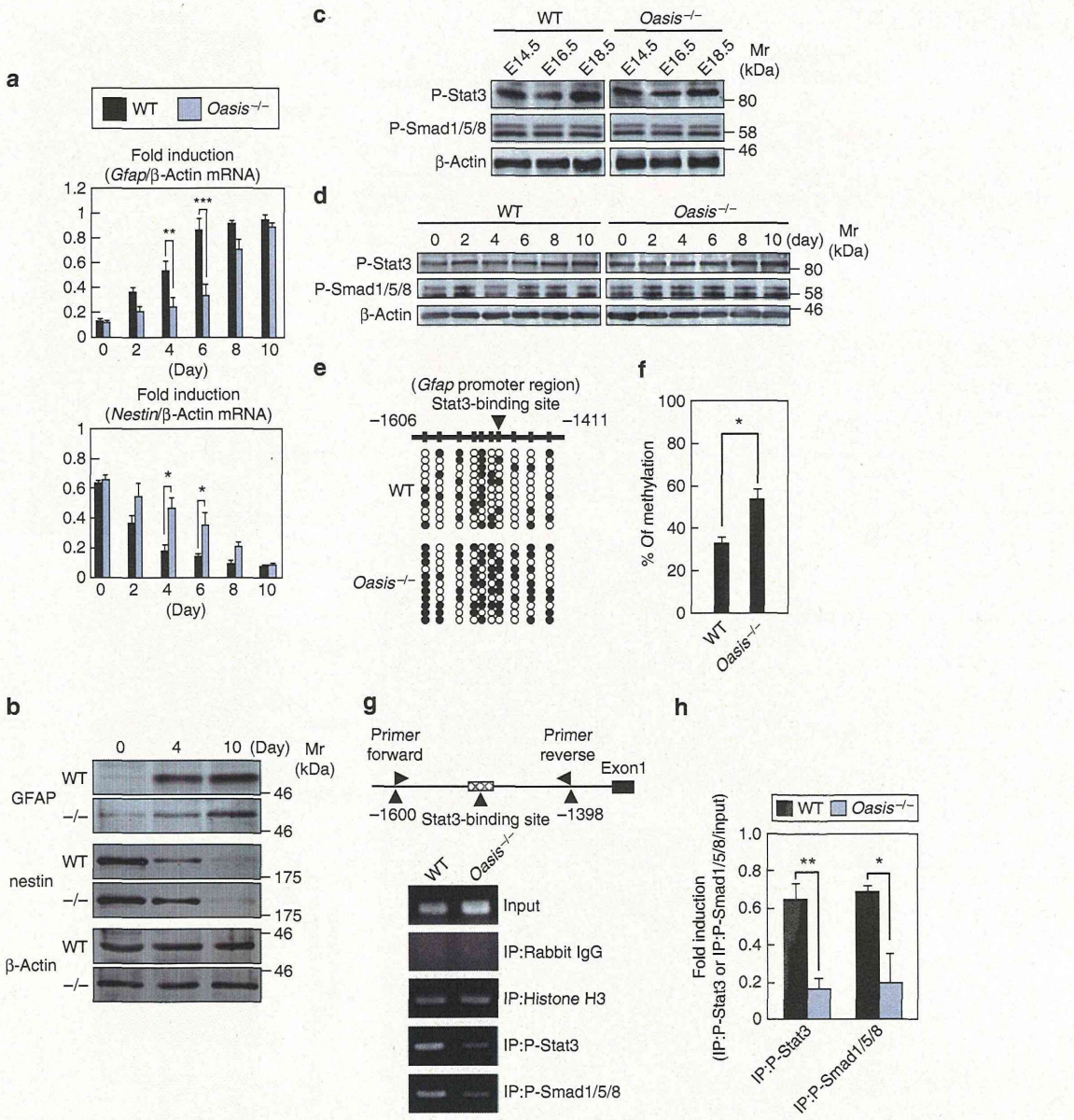

Figure 2 | Demethylation of *Gfap* promoter is inhibited in primary cultured *Oasis*$^{-/-}$ NPCs. (**a**) RT-PCR analysis of *Gfap* and *Nestin* in primary cultured NPCs treated with LIF and BMP2 for the indicated times. The upregulation of *Gfap* and the downregulation of *Nestin* were inhibited in *Oasis*$^{-/-}$ cells. (**b**) Western blotting of GFAP and nestin in primary cultured WT and *Oasis*$^{-/-}$ NPCs treated with LIF and BMP2 for the indicated times. (**c**,**d**) Western blotting of phosphorylated (P)-Stat3 and P-Smad1/5/8 in the cerebral cortices of WT and *Oasis*$^{-/-}$ mice (**c**) and primary cultured NPCs treated with LIF and BMP2 for the indicated times (**d**). The phosphorylation of these proteins was not affected by *Oasis* deficiency. (**e**) Bisulfite sequencing results for the CpG site within the Stat3 recognition sequence and other CpG sites around this sequence of the *Gfap* promoter in NPCs treated with LIF and BMP2 for 4 days. (■) and (○) indicate methylated and demethylated CpG sites, respectively. Experiments were performed using 11 independent samples. (**f**) The percentages of methylated sites in the *Gfap* promoter. (**g**) 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 by indicated antibodies. Primary cultured NPCs treated with LIF and BMP2 for 4 days were used for ChIP assays. (**h**) Quantitative analysis of PCR amplification after immunoprecipitation by anti-P-Stat3 and anti-P-Smad1/5/8 antibodies in (**g**). All bars represent the mean values ±s.d. of 3 (**a**), 11 (**f**) and 4 (**h**) experiments. Significant difference was determined by unpaired Student's-*t*-test. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, between indicated pairs.

and Smads to the *Gfap* promoter was significantly inhibited in *Oasis*$^{-/-}$ NPCs (Fig. 2g,h). These findings suggest that the delayed astrocyte differentiation in *Oasis*$^{-/-}$ cells is caused by inhibition of demethylation of the *Gfap* promoter.

The target of OASIS in NPCs is Gcm1. Previously, it was reported that OASIS could activate the transcription of *Gcm1* in trophoblasts[39]. The *Gcm1 Drosophila* orthologue, *gcm*, has been reported to control neuronal and glial fates[23–25]. Further, a recent study showed

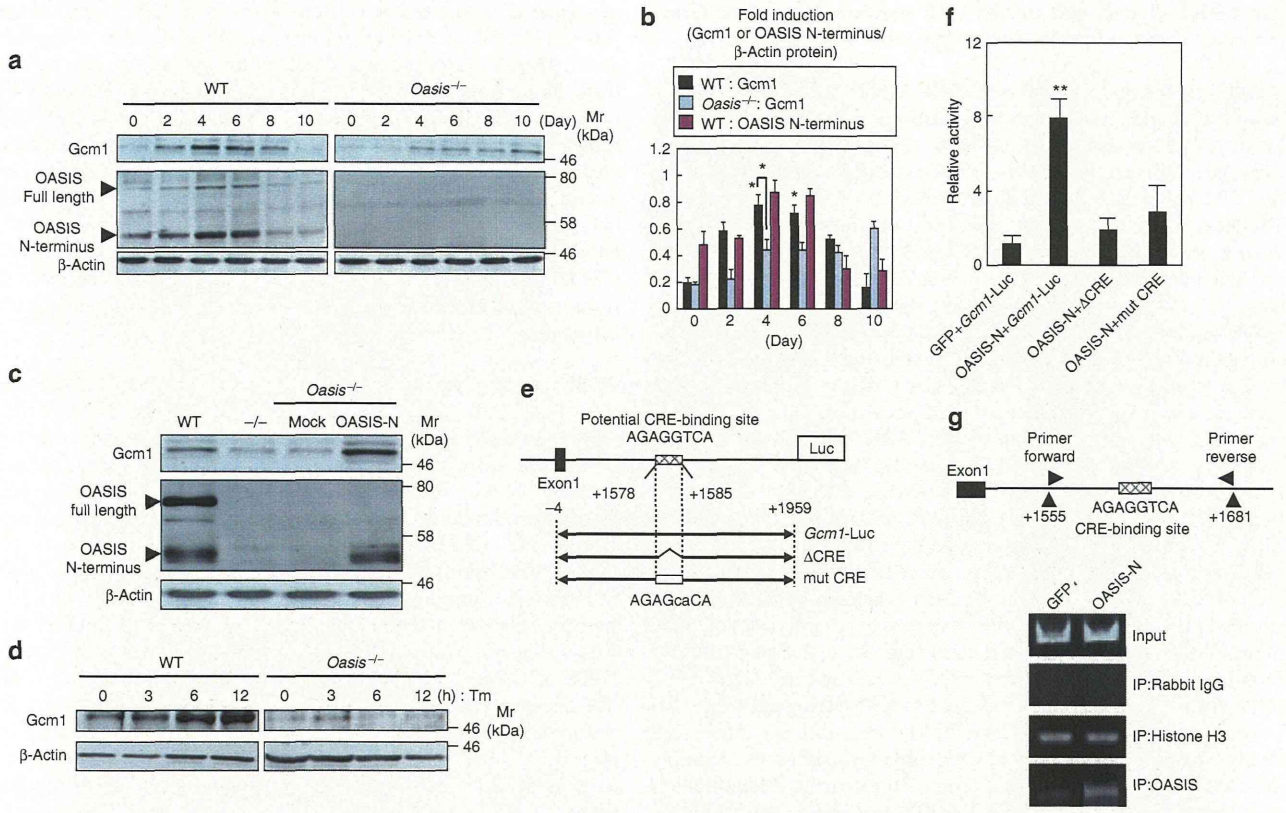

Figure 3 | Gcm1 is a direct target of OASIS in NPCs. (**a**) Western blotting of Gcm1 in NPCs treated with LIF and BMP2 for the indicated times. In WT cells, Gcm1 is transiently upregulated at the same time as OASIS. By contrast, it was significantly downregulated in *Oasis*$^{-/-}$ NPCs. (**b**) Quantitative analysis of expression of Gcm1 and OASIS N-terminus in (**a**). (**c**) Western blotting of Gcm1 using NPCs cultured for 4 days. Adenoviruses expressing OASIS N-terminus were infected with *Oasis*$^{-/-}$ NPCs. Mock indicates empty vector. (**d**) Western blotting of Gcm1 using NPCs treated with LIF and BMP2 for 4 days. Cells were exposed to 3 μg ml^{-1} tunicamycin (Tm; an ER stressor) for the indicated times. (**e**) Scheme of the mouse *Gcm1* promoter region and reporter constructs. The *Gcm1*-Luc construct consists of 2.0 kb from the *Gcm1* promoter region. ΔCRE lacks the CRE-binding site (AGAGGTCA), and mut CRE is mutated (small letters of CRE sequence) in the CRE-binding site. Luc, luciferase gene. (**f**) Reporter assays using NPCs. Vectors expressing the mouse OASIS N-terminus or green fluorescent protein (GFP) were co-transfected with each reporter construct. GFP was used as a control. (**g**) The top panel shows a schematic representation of the *Gcm1* 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 *Gcm1* promoter region containing the CRE-binding site (+1,555 to +1,681). NPCs were transfected with the vector expressing the mouse OASIS N-terminus. GFP was used as a control. All bars represent the mean values ±s.d. of three experiments. Significant difference between two samples was determined by unpaired Student's *t*-test (**b**). $^{*}P<0.05$, between indicated pairs. Multiple comparisons were made using One way ANOVA followed by Tukey's *post hoc* test. $^{*}P<0.05$, $^{**}P<0.01$, among WT cells (**b**) and all indicated samples (**f**).

that Gcm1 is involved in the demethylation of promoter regions in specific genes[31]. *Gcm1* might be one of the direct target genes of OASIS in NPCs, and it is possible that OASIS is involved in the regulation of astrocyte differentiation through the expression of Gcm1 followed by the demethylation of the *Gfap* promoter. Therefore, we examined the expression levels of Gcm1 during astrocyte differentiation using primary cultured NPCs. Gcm1 was transiently upregulated during the differentiation of NPCs into astrocytes, and the expression pattern was similar to that of OASIS (Fig. 3a,b). However, Gcm1 was dramatically downregulated to about 50% of the WT level on day 4 in *Oasis*$^{-/-}$ cells. We infected primary cultured *Oasis*$^{-/-}$ NPCs with adenovirus expressing the OASIS N-terminus. Gcm1 expression was markedly upregulated by overexpression of the OASIS N-terminus (Fig. 3c). As OASIS is cleaved in response to ER stress, Gcm1 could be induced in NPCs exposed to ER stress, if Gcm1 is a direct target of OASIS in NPCs. Indeed, the expression level of Gcm1 was upregulated in WT NPCs under the ER stress condition. By contrast, the upregulation of Gcm1 was significantly inhibited in *Oasis*$^{-/-}$ NPCs (Fig. 3d). In the cerebral cortices of *Oasis*$^{-/-}$ mice, Gcm1 expression was downregulated (Supplementary Fig. S3); the findings were consistent with the findings in primary cultured NPCs.

To determine whether OASIS acts on the *Gcm1* promoter and activates transcription of *Gcm1* in NPCs, we performed promoter assays using a reporter gene carrying a 2.0-kb promoter region of *Gcm1* (*Gcm1*-Luc) (Fig. 3e). In NPCs transfected with a *Gcm1*-Luc construct, reporter activities were dramatically induced by expression of the OASIS N-terminus (Fig. 3f). The *Gcm1* promoter includes a cAMP-responsive element (CRE) (+1,585 to +1,592 bp) that OASIS can bind to[10]. We next performed a promoter assay using the reporter constructs ΔCRE, which lacks the CRE-binding site, and mut CRE, which has a mutated CRE-binding site (Fig. 3e). In NPCs transfected with these constructs, reporter activities were markedly reduced, despite expression of the OASIS N-terminus (Fig. 3f). Furthermore, we performed chromatin immunoprecipitation (ChIP) assays and detected a high level of OASIS binding to the endogenous *Gcm1* promoter in primary cultured NPCs transfected with the OASIS N-terminus (Fig. 3g). These results indicate

that OASIS directly acts on the CRE sequence within the *Gcm1* promoter and facilitates its transcription in NPCs.

Mild ER stress and activation of UPR. As shown in Fig. 3a, the levels of full-length OASIS and its N-terminus were increased during astrocyte differentiation. The OASIS N-terminus is produced by the cleavage of full-length OASIS in response to ER stress. It is possible that ER stress occurs during differentiation of NPCs into astrocytes. Therefore, we examined the expression of ER stress-related genes during astrocyte differentiation. *Bip*, *p58^{IPK}* and *Erdj4* were slightly upregulated from day 2 to 6 during differentiation of WT and *Oasis*$^{-/-}$ NPCs into astrocytes (Fig. 4a; Supplementary Fig. S4), indicating that all of signalling pathways from the major three sensors including PKR-like endoplasmic reticulum kinase, ATF6 and inositol-requiring 1, are activated during astrocyte differentiation. Furthermore, the transient upregulation of these genes was synchronized with the expression of the OASIS N-terminus (Fig. 5d), indicating that mild ER stress is transiently induced and that OASIS is activated by this mild ER stress during astrocyte differentiation. Although it is unknown, the roles of each pathway of major ER stress sensors. Next, we examined the roles of mild ER stress in cell fates including differentiation and cell death. To determine the level of ER stress during astrocyte differentiation, we quantified the expression levels of *Erdj4* in NPCs exposed to several doses of dithiothreitol (DTT) or tunicamycin (Tm) (Fig. 4b,c). Consequently, we found that the level of ER stress in NPCs exposed to 125 μM DTT or 90 ng ml^{-1} Tm for 48 h was equal to that during astrocyte differentiation (Fig. 4b,c). This level of ER stress did not induce cell death (Fig. 4d–f), but activated UPR signalling pathways including the OASIS-Gcm1 pathway and promoted astrocyte differentiation after treatment with LIF and BMP2 (Fig. 4g). These results indicate that mild ER stress to a lesser extent than the levels that induce cell death is necessary for astrocyte differentiation.

Gcm1 expression modulation by OASIS family members. ER stress-related CREB/ATF family members have the potential to bind to a CRE-binding site in promoter region and promote its transcription[13,16,40]. Thus, we checked the effects of CREB/ATF family members, including OASIS family members, on a *Gcm1*-Luc reporter. Consequently, we found that CREB4 (Fig. 5a) promotes *Gcm1* reporter activities (Fig. 5b; Supplementary Fig. S5). Furthermore, we detected the binding of CREB4 to endogenous *Gcm1* promoter (Fig. 5c). Thus, not only OASIS but also CREB4 could activate the transcription of *Gcm1*. Two previous reports showed that CREB4 does not bind to the CRE sequence (TGACGTCA)[18,19]. Thus far, CREB/ATF family members are known to bind to the several sequences such as TGACCTCA, TGAGGTCA, TGCCGTCA and TGAAGTCA[41–44]. AGAGGTCA sequence in the *Gcm1* promoter region is not identical to TGACGTCA, which is the most common sequence as CREB/ATF family binding sequence. Therefore, it is possible that the manners of binding of OASIS and CREB4 are different from those of the other CREB/ATF family members. CREB/ATF family members are well known to form homodimers or heterodimers to promote transcription[16,45]. Thus, we examined the synergistic effects of CREB/ATF family members on *Gcm1* promoter activities. In NPCs transfected with a *Gcm1*-Luc construct, reporter activities were dramatically induced by co-expression of OASIS and CREB4 (Fig. 5b), and at the same time, the binding of OASIS to *Gcm1* promoter was also significantly increased (Fig. 5c). Conversely, co-expression of OASIS and Luman, which is also an OASIS family member, resulted in significant downregulation of reporter activities and decreased binding of OASIS to the *Gcm1* promoter (Fig. 5b,c).

We next investigated endogenous expression of and direct interactions among OASIS family members during astrocyte differentiation. Both full-length OASIS and CREB4 and their N-termini were upregulated from day 2 to 6 after treatment of NPCs with LIF and BMP2 (Fig. 5d,e). Further, robust binding of OASIS N-terminus and CREB4 N-terminus was detected on day 4 (Fig. 5f). By contrast, binding of OASIS N-terminus and Luman N-terminus was observed on day 10 (Fig. 5f), suggesting that OASIS and CREB4 form a heterodimer in the early stage of astrocyte differentiation, and that Luman binds to OASIS in the late stage of astrocyte differentiation. Taken together, we concluded that an OASIS-CREB4 heterodimer activates transcription of *Gcm1* during astrocyte differentiation. Conversely, Luman inhibits the formation of the OASIS-CREB4 heterodimer by binding to OASIS and downregulate the transcription of *Gcm1*, followed by terminating differentiation of astrocytes.

OASIS family members and Gcm1 promote astrocyte differentiation. To examine whether OASIS family members and Gcm1 could rescue the delay in astrocyte differentiation observed in *Oasis*$^{-/-}$ NPCs, we introduced these molecules into primary cultured *Oasis*$^{-/-}$ NPCs. The expression levels of Gcm1 were upregulated by the introduction of the OASIS N-terminus, or co-expression of OASIS and CREB4 N-termini. However, introduction of the Luman N-terminus did not affect the expression of Gcm1 (Fig. 6a). Immunocytochemical analysis showed that the number of GFAP-positive cells was increased in NPCs treated with LIF and BMP2 for 4 days (Fig. 6b). Conversely, these cells were decreased in *Oasis*$^{-/-}$ NPCs or *Oasis*$^{-/-}$ NPCs expressing the empty vector (Fig. 6c,d). The decrease in the number of GFAP-positive *Oasis*$^{-/-}$ cells was restored to those of WT cells by introduction of OASIS N-terminus (Fig. 6e,j), CREB4 N-terminus (Fig. 6f,j) or Gcm1 (Fig. 6g,j). The co-expression of OASIS N-terminus and CREB4 N-terminus in *Oasis*$^{-/-}$ NPCs resulted in a greater improvement of the delay in astrocyte differentiation than did expression of OASIS N-terminus or CREB4 N-terminus alone (Fig. 6 h,j). The overexpression of Luman N-terminus in *Oasis*$^{-/-}$ NPCs could not restore the delay (Fig. 6i,j). These data indicate that OASIS-CREB4 heterodimerization followed by the induction of Gcm1 is necessary for accelerating astrocyte differentiation, and conversely, that Luman does not accelerate the differentiation of NPCs into astrocytes.

Gcm1 is involved in demethylation of *Gfap* promoter. As shown in Fig. 2, demethylation of *Gfap* promoter was inhibited in *Oasis*$^{-/-}$ NPCs. To examine the mechanisms responsible for the impaired methylation status of the *Gfap* promoter in *Oasis*$^{-/-}$ NPCs, we performed bisulfite sequencing using the *Gfap* promoter (Fig. 7a,b). In NPCs cultured for 4 days, demethylation in the control was suppressed by transfection with a *Gcm1*-specific short interfering RNA (siRNA) (Fig. 7a,b). In *Oasis*$^{-/-}$ NPCs, demethylation of *Gfap* promoter was also inhibited. The introduction of OASIS N-terminus or Gcm1 into *Oasis*$^{-/-}$ NPCs resulted in restoration of the *Gfap* demethylation status (Fig. 7a,b).

We further examined whether demethylation of *Gfap* promoter by OASIS-Gcm1 signalling induces binding of the Stat3-Smads-p300 complex to the promoter by performing ChIP assays using primary cultured NPCs (Fig. 7c,d). The binding of Stat3 and Smads to the endogenous *Gfap* promoter was inhibited in NPCs transfected with the *Gcm1*-specific siRNA (Fig. 7c,d). In *Oasis*$^{-/-}$ NPCs, binding of Stat3 and Smads was also inhibited. The introduction of the OASIS N-terminus or Gcm1 into *Oasis*$^{-/-}$ NPCs restored the binding of Stat3 and Smads to the *Gfap* promoter to the levels seen in WT cells (Fig. 7c,d). These data suggest that the activation of OASIS-Gcm1 signalling accelerates demethylation of the *Gfap* promoter followed by binding of Stat3-Smads-p300 complex.

Discussion

It has been reported that the epigenetic regulation of the methylation status of the *Gfap* promoter is essential for accelerating differentiation

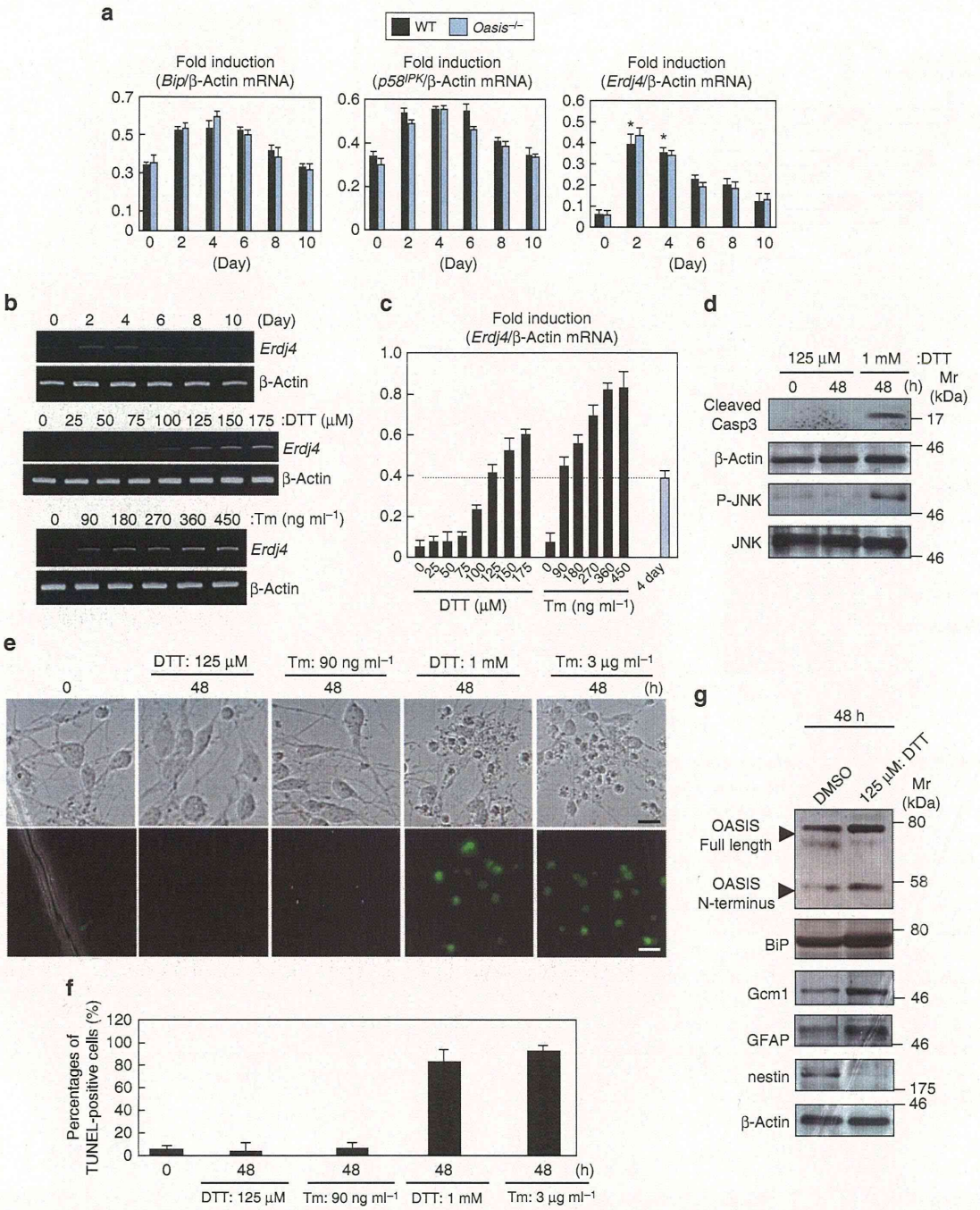

Figure 4 | Mild ER stress promotes astrocyte differentiation. (**a**) Real-time PCR analysis of ER stress-related genes in NPCs treated with LIF and BMP2 for the indicated times. Mild ER stress transiently occurred (from day 2 to day 6) during the differentiation of NPCs into astrocytes. (**b**) RT-PCR analysis of *Erdj4* in NPCs treated with LIF and BMP2 for the indicated times (top panel). Middle and bottom panels show the expression of *Erdj4* in NPCs exposed to DTT (an ER stressor) or Tm for 48 h. (**c**) Quantitative analysis of the expression of *Erdj4* in (**b**). The expression level of *Erdj4* in NPCs treated with LIF and BMP2 for 4 days was very similar to the expression levels in NPCs exposed to 125 μM DTT or 90 ng ml^{-1} Tm for 48 h. The dotted line indicates the expression level of *Erdj4* in NPCs treated with LIF and BMP2 for 4 days. (**d**) Western blotting of cleaved caspase 3 (Casp3) and phosphorylated (P)-JNK in NPCs treated with LIF and BMP2 for 4 days. Cleaved Casp3 and P-JNK were not induced by the same level of mild ER stress observed during astrocyte differentiation. (**e**) Phase contrast images (top panels) and TUNEL staining (bottom panels) in NPCs treated with LIF and BMP2 for 4 days. Cell death was not induced by mild ER stress (125 μM DTT, 90 ng ml^{-1} Tm). Scale bars, 25 μm. (**f**) The percentages of TUNEL-positive cells in (**e**). The number of TUNEL-positive cells was measured in five fields per well. (**g**) Western blotting of OASIS, BiP, Gcm1, GFAP and nestin in NPCs treated with LIF and BMP2 for 4 days. Mild ER stress activated UPR signalling and promoted astrocyte differentiation after treatment with LIF and BMP2. All bars represent the mean values ±s.d. of 4 (**a**,**c**) and 2 (**f**) experiments. Significant difference was determined by One-way ANOVA followed by Tukey's *post hoc* test. *P < 0.05, among WT cells.

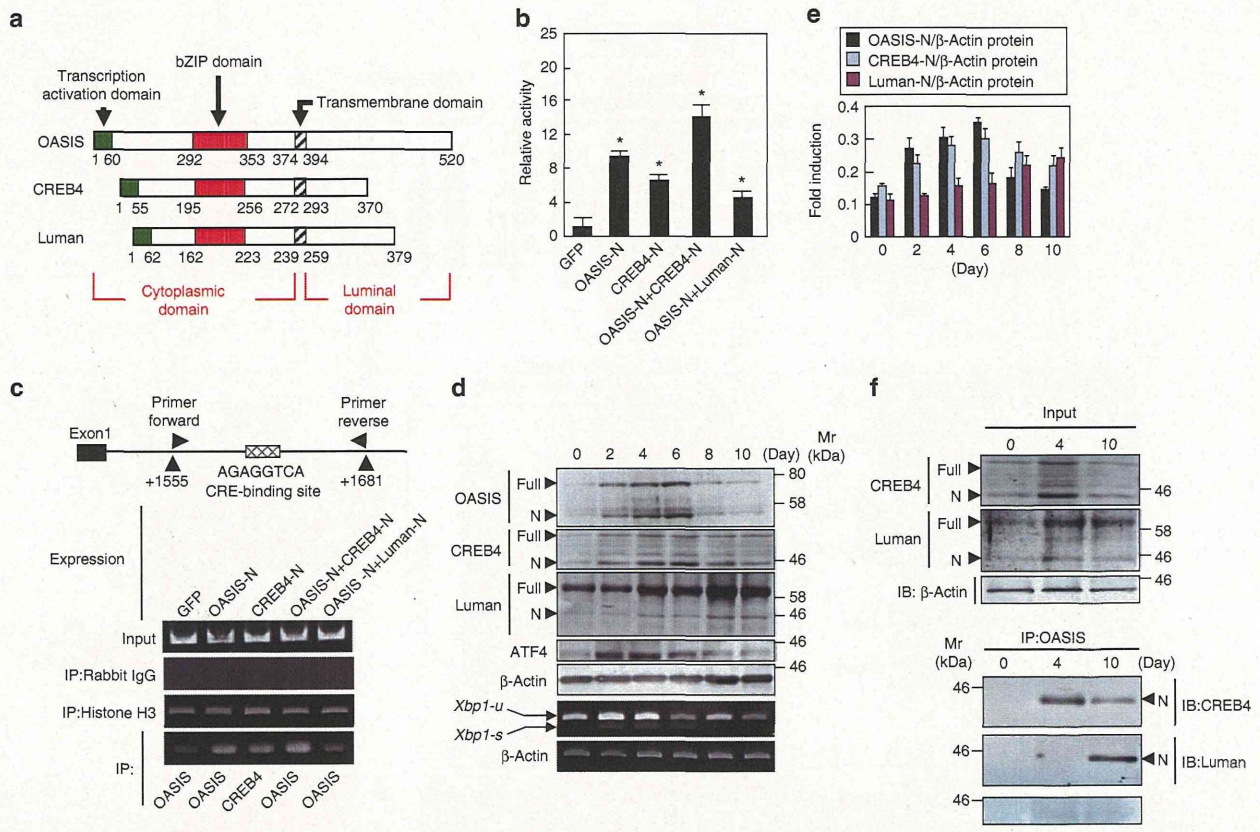

Figure 5 | OASIS and CREB4 form a heterodimer and promote Gcm1 expression. (**a**) Predicted peptide features of mouse OASIS, CREB4 and Luman. (**b**) Reporter assays in NPCs. Expression vectors for the N-terminus of mouse OASIS, CREB4, Luman and GFP were co-transfected with *Gcm1*-Luc. GFP was used as a control. (**c**) The top panel shows a schematic representation of the *Gcm1* 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 *Gcm1* promoter region containing the CRE-binding site (+1,555 to +1,681) after immunoprecipitation with indicated antibodies. NPCs were transfected with the expression vectors for the N-terminus of mouse OASIS, CREB4, Luman and GFP. GFP was used as a control. (**d**) Western blotting (OASIS, CREB4, Luman and ATF4) and RT-PCR (*Xbp1*) analysis in NPCs treated with LIF and BMP2 for the indicated times. Both full-length OASIS and CREB4 and their N-termini were upregulated from day 2 to 6 after treatment of NPCs with LIF and BMP2, synchronizing with the transient upregulation of ATF4 and spliced form of *Xbp1* (*Xbp1-s*). Luman N-terminus was upregulated after day 8 in these cells. *Xbp-1-u*: unspliced form of *Xbp1*. (**e**) Quantitative analysis of the expression of N-terminus of OASIS, CREB4 and Luman in (**d**). (**f**) Immunoprecipitation by anti-OASIS antibody followed by western blotting with the indicated antibodies using NPCs treated with LIF and BMP2 for the indicated times. OASIS N-terminus was robustly bound to CREB4 N-terminus on day 4. OASIS, and Luman N-terminus formed a complex on day 10. Input indicates cell lysates before immunoprecipitation. Full, Full length; N, N-terminus; IP, immunoprecipitation; IB, immunoblotting. All bars represent the mean values ±s.d. of three experiments. Significant difference was determined by One-way ANOVA followed by Tukey's *post hoc* test. $^{*}P<0.05$, among all indicated samples (OASIS-N, CREB4-N and OASIS-N + CREB4-N) or the samples named OASIS-N, OASIS-N + CREB4-N and OASIS-N + Luman-N (OASIS-N + Luman-N).

of NPCs into astrocytes, and that the impairment of demethylation of the *Gfap* promoter disturbs astrocyte differentiation[38]. However, the regulatory mechanisms underlying demethylation of the *Gfap* promoter have remained unclear. In this study, we found that Gcm1, a direct target of OASIS, regulates the methylation status of the *Gfap* promoter followed by accelerating astrocyte differentiation. Interestingly, Gcm1 expression was fine-tuned by the dynamic interaction among OASIS family members that are activated by ER stress, during astrocyte differentiation. Thus, the spatio-temporal control of Gcm1 expression could contribute to production of functional and mature astrocytes. In this context, this report is the first to show that UPR signalling is linked to astrocyte differentiation. It is well known that not only epigenetic regulation but also the activation of Stat3 and Smads is essential for differentiation of NPCs into astrocytes[32,35,37]. However, the activation of these transcription factors was not affected in *Oasis*$^{-/-}$ mice (Fig. 2c,d). These findings strongly suggest that OASIS-Gcm1 signalling specially acts to regulate demethylation of the *Gfap* promoter, during astrocyte differentiation.

We found that *Gcm1* is a target gene of OASIS in the CNS. This conclusion is supported by the fact that (1) *Gcm1* is significantly downregulated in the cerebral cortices and primary cultured NPCs of *Oasis*$^{-/-}$ mice; (2) a CRE-binding site that OASIS can bind to exist in the *Gcm1* promoter region; (3) overexpression of the OASIS N-terminus upregulated *Gcm1* promoter activities; (4) *Gcm1* promoter activities were drastically decreased by mutation or deletion of the CRE-binding site; and (5) OASIS directly binds to the *Gcm1* promoter. *Gcm* homologues have been shown to be involved as master regulators in key steps of differentiation processes[39]. In addition to astrocyte differentiation, Gcm1 is also essential for the differentiation of trophoblasts into syncytiotrophoblasts[39]. The promoter region of *Gcm1* contains several CRE-binding sites containing the OASIS-binding site, we showed in this study. The effects of various