Development 137, 487-497 (2010) doi:10.1242/dev.039800

The transcription factor Foxg1 regulates the competence of telencephalic cells to adopt subpallial fates in mice

Martine Manuel*,§, Ben Martynoga*,†, Tian Yu‡, John D. West, John O. Mason¶ and David J. Price¶

SUMMARY

Foxg1 is required for development of the ventral telencephalon in the embryonic mammalian forebrain. Although one existing hypothesis suggests that failed ventral telencephalic development in the absence of Foxg1 is due to reduced production of the morphogens sonic hedgehog (Shh) and fibroblast growth factor 8 (Fgf8), the possibility that telencephalic cells lacking Foxg1 are intrinsically incompetent to generate the ventral telencephalon has remained untested. We examined the ability of *Foxg1*$^{-/-}$ telencephalic cells to respond to Shh and Fgf8 by examining the expression of genes whose activation requires Shh or Fgf8 in vivo and by testing their responses to Shh and Fgf8 in culture. We found that many elements of the Shh and Fgf8 signalling pathways continue to function in the absence of Foxg1 but, nevertheless, we were unable to elicit normal responses of key ventral telencephalic marker genes in *Foxg1*$^{-/-}$ telencephalic tissue following a range of in vivo and in vitro manipulations. We explored the development of *Foxg1*$^{-/-}$ cells in *Foxg1*$^{-/-}$ *Foxg1*$^{+/+}$ chimeric embryos that contained ventral telencephalon created by normally patterned wild-type cells. We found that *Foxg1*$^{-/-}$ cells contributed to the chimeric ventral telencephalon, but that they retained abnormal specification, expressing dorsal rather than ventral telencephalic markers. These findings indicate that, in addition to regulating the production of ventralising signals, Foxg1 acts cell-autonomously in the telencephalon to ensure that cells develop the competence to adopt ventral identities.

KEY WORDS: Foxg1, Telencephalon, Patterning, Mouse

INTRODUCTION

Two major components of the mammalian forebrain, the cerebral cortex and the basal ganglia, develop from the dorsal and ventral parts of the embryonic telencephalon. The telencephalon arises at the rostral end of the neural tube and its dorsal and ventral domains are patterned by the activities of a number of secreted molecules produced by surrounding signalling centres (Danesin et al., 2009; Hebert, 2005; Hebert and Fishell, 2008; Rallu et al., 2002a; Sur and Rubenstein, 2005; Wilson and Houart, 2004). Sonic hedgehog (Shh) is produced ventrally, fibroblast growth factor 8 (Fgf8) is produced rostrally and a number of bone morphogenetic proteins (Bmps) and Wnt proteins are produced caudo-medially. These signalling molecules activate the graded expression of transcription factors that control many aspects of the subsequent development of telencephalic cells, including their molecular and cellular identities.

The transcription factor Foxg1 is essential for normal telencephalic development. *Foxg1*$^{-/-}$ mutant mice die perinatally with a severely hypoplastic telencephalon comprising dorsal, but not ventral, tissue (Martynoga et al., 2005; Xuan et al., 1995). Previous studies have focused mainly on defects in the residual dorsal telencephalon, revealing defects of progenitor proliferation, cell differentiation, progenitor patterning, signalling molecule expression and specification of neuronal subtypes (Ahlgren et al., 2003; Bourguignon et al., 1998; Dou et al., 1999; Hanashima et al., 2007; Hanashima et al., 2004; Hanashima et al., 2002; Hardcastle and Papalopulu, 2000; Hebert and Fishell, 2008; Muzio and Mallamaci, 2005; Xuan et al., 1995). Examining possible causes of the failure of ventral telencephalic development is much harder because ventral telencephalon is not detectable even at very early ages (Martynoga et al., 2005; Xuan et al., 1995).

Foxg1 is one of the earliest transcription factors to be expressed in the part of the neural plate from which telencephalon develops. One hypothesis proposed to explain the requirement of Foxg1 in ventral telencephalic development is that Foxg1 is needed for normal production of Fgf8 (Hebert and Fishell, 2008). This suggestion is based on research showing that Fgf signalling is required for normal ventral telencephalic development (Gutin et al., 2006; Storm et al., 2006) coupled with the observation that rostral telencephalic Fgf8 expression is reduced in *Foxg1*$^{-/-}$ embryos (Martynoga et al., 2005). In addition, Shh expression, an important player in ventral telencephalic development (Fuccillo et al., 2006a; Fuccillo et al., 2004), is reduced in *Foxg1*$^{-/-}$ telencephalon (Huh et al., 1999). Although it is reasonable to suppose that reduced levels of telencephalic Fgf8 and Shh would selectively impair ventral telencephalic development, the extent to which they account for the phenotype of mouse embryos lacking Foxg1 is unknown. The likelihood that Foxg1 has other actions essential for normal mammalian ventral telencephalic development is strongly supported by a recent study in zebrafish indicating that Foxg1 is required downstream of Hedgehog activity for the acquisition of ventral telencephalic identity (Danesin et al., 2009).

We began by examining the ability of telencephalic cells from *Foxg1*$^{-/-}$ mouse embryos to respond to Shh and Fgf8. We studied their expression of genes known to require Shh or Fgf8 for their

Genes and Development Group, University of Edinburgh, Hugh Robson Building, George Square, Edinburgh EH8 9XD, UK.

*,¶These authors contributed equally to this work
†Present address: National Institute for Medical Research, The Ridgeway, Mill Hill, London, NW7 1AA, UK
‡Present address: Department of Craniofacial Development, King's College, Floor 27, Guy's Tower, London SE1 9RT, UK
§Author for correspondence (martine.manuel@ed.ac.uk)

Accepted 26 November 2009

activation and tested the responses of cultured *Foxg1*$^{-/-}$ telencephalic cells to Shh and Fgf8. We found that the expression of many genes that are targets of Shh and Fgf8 signalling could be induced in *Foxg1*$^{-/-}$ cells but we could not induce the expression of other ventral telencephalic marker genes. We explored the development of *Foxg1*$^{-/-}$ cells in *Foxg1*$^{-/-}$ *Foxg1*$^{+/+}$ chimeric embryos in which the wild-type cells were patterned normally. We found that *Foxg1*$^{-/-}$ cells contributed to the chimeric ventral telencephalon, but the mutant cells were specified abnormally, expressing dorsal rather than ventral telencephalic markers. These findings indicate that, in addition to regulating production of ventralising cues, Foxg1 has a crucial cell-autonomous function, giving telencephalic cells the competence to adopt ventral identities.

MATERIALS AND METHODS

Animals

Foxg1$^{-/-}$ embryos were generated by intercrossing *Foxg1*$^{+/-}$ mice (Martynoga et al., 2005). *Foxg1*$^{-/-}$;*Gli3*$^{+/-}$ embryos were made by crossing *Foxg1*$^{+/-}$ and *Foxg1*$^{+/-}$;*Gli3*$^{+/-}$ mice, whereas *Foxg1*$^{-/-}$;*Pax6*$^{-/-}$ embryos were made by intercrossing *Foxg1*$^{+/-}$;*Pax6*$^{+/-}$ mice.

Production of chimeric animals

Chimeras were produced by morula aggregation. Eight-cell embryos were obtained by crossing *Foxg1*$^{lacZ/+}$ females with *Foxg1*$^{+/Cre}$ males. Embryos obtained from this cross were: *Foxg1*$^{+/+}$, *Foxg1*$^{lacZ/+}$, *Foxg1*$^{+/Cre}$ and *Foxg1*$^{lacZ/Cre}$ (*Foxg1*$^{-/-}$). *Foxg1*$^{+/+}$ embryos for aggregation were from (BALB/c × A/J) F_2 intercrosses. Embryos were collected from superovulated females and aggregated (West and Flockhart, 1994). The two morulae for aggregation differed at the Gpi1 locus (encoding glucose phosphate isomerase). Aggregated embryos were cultured overnight, transferred to recipient pseudopregnant females and recovered at embryonic day 12.5 (E12.5). The genotype of each chimera was determined by PCR (Martynoga et al., 2005). The use of two null-mutant *Foxg1* alleles allowed distinction between chimeras containing *Foxg1*$^{lacZ/Cre}$ (*Foxg1*$^{-/-}$) compound heterozygous cells and those containing heterozygous (*Foxg1*$^{lacZ/+}$ or *Foxg1*$^{+/Cre}$) or wild-type (*Foxg1*$^{+/+}$) cells. The global contribution of each morula to the chimera was estimated using Gpi1 electrophoresis (West and Flockhart, 1994).

Explant cultures

E10.5 embryos were dissected in ice-cold Earle's balanced saline solution (EBSS). *Foxg1*$^{-/-}$ embryos were distinguished by their eye and telencephalic defects. Explants were dissected and cultured on Falcon inserts (0.4 μm pore size, Becton Dickinson) in wells containing defined culture medium with 10% foetal bovine serum. For dose-response experiments, a minimum of three explants were pooled and cultured with 0, 5, 20 or 50 nM Shh (recombinant mouse Shh-N, R&D Systems) or with 0, 0.42 or 4.2 nM Fgf8 (recombinant mouse Fgf8b, R&D Systems). After 24 hours, explants were harvested and RNA was extracted for qRT-PCR analysis.

For induction with Shh-coated beads, 1 hour after placement on an insert, 3-6 Affi-gel blue beads (100-200 mesh, Bio-rad) soaked either in 25 μM Shh-N or 0.1% BSA were placed on the explants, which were then cultured for 24-36 hours. Explants were fixed in 4% paraformaldehyde.

In situ hybridisation

Antisense RNA probes for *Ptc1*, *Fgfr1*, *Fgfr3*, *Sprouty2* (*Spry2* – Mouse Genome Informatics), *Mest* and *Etv5* were digoxigenin-labelled. In situ hybridisations were on 10 μm paraffin sections (Christoffels et al., 2000).

Immunohistochemistry

Immunohistochemistry was carried out as previously described (Martynoga et al., 2005). The primary antibodies used were: anti-Pax6 [Developmental Studies Hybridoma Bank (DSHB)], anti-Ngn2 (Lo et al., 2002), anti-beta-galactosidase (β-gal) (Molecular Probes), anti-Gsh2 (Toresson et al., 2000), anti-Islet1 (DSHB), anti-Mash1 (BD Bioscience), anti-Nkx2.1 (Abcam), anti-calbindin (Sigma), anti-Olig2 (Ligon et al., 2004), anti-Tbr1 and anti-Tbr2 (Englund et al., 2005), anti-pan-Dlx (Hevner et al., 2004), anti-cyclin D1 (Novocastra) and anti-p21^{cip1} (BD Pharmingen).

Western blotting

Western blot analysis of Gli3 expression in the telencephalon of E12.5 control, *Foxg1*$^{-/-}$ and *Gli3*$^{-/-}$ embryos was performed as in Fotaki et al. (Fotaki et al., 2006).

Reverse transcriptase PCR (RT-PCR) and quantitative RT-PCR (qRT-PCR)

RNA was extracted from embryonic tissues (RNEasy Mini Kit, Qiagen). cDNA was synthesised from the RNA template using M-MLV (Promega) or Sensiscript (Qiagen) reverse transcriptase and random hexanucleotide primers. PCR on the cDNA template used gene-specific primers (see Table S1 in the supplementary material). 'No RT' and 'no cDNA' controls were included where appropriate and in all cases were negative.

For qRT-PCR, Quantitect SYBR Green PCR reagents (Qiagen) were used on an Opticon single wavelength PCR machine (MJ Research). All primers spanned introns. Expression levels of genes of interest were normalised to the expression of β-*actin* or *Gapdh*. Following normalisation, expression levels for each gene were expressed as a ratio to normalised levels of a control group (wild-type telencephalon for expression analyses or untreated samples for *Shh* and *Fgf8* induction analyses). For expression analyses, each qRT-PCR reaction was run in triplicate on cDNA from at least three independent RNA extracts. For morphogen dose-response analyses, each qRT-PCR was performed on a minimum of three samples from independent cultures (11-15 control and 6-8 *Foxg1*$^{-/-}$ samples for Shh induction analysis; four control and three *Foxg1*$^{-/-}$ samples for Fgf8 induction analysis)

RESULTS

The hedgehog (Hh)/Gli pathway is active in the *Foxg1*$^{-/-}$ telencephalon

In normal mouse embryogenesis, Shh signalling through the Hh/Gli pathway is essential for the development of the ventral telencephalon: ventral telencephalic cell types are lost in embryos lacking Shh or its obligate cell surface transducer smoothened (Smo) (Chiang et al., 1996; Corbin et al., 2003; Ericson et al., 1995; Fuccillo et al., 2004; Fuccillo et al., 2006b; Hebert and Fishell, 2008; Ohkubo et al., 2002; Rallu et al., 2002b; Zaki et al., 2005). In *Foxg1*$^{-/-}$ embryos, telencephalic exposure to *Shh* is likely to be abnormally low (Huh et al., 1999), but the impact of this and the extent to which *Foxg1*$^{-/-}$ telencephalic cells can respond to Shh are unknown. We tested whether the Shh receptors Ptc1 and Smo are still expressed in *Foxg1*$^{-/-}$ telencephalon. Fig. 1A shows that *Ptc1* and *Smo* are expressed in *Foxg1*$^{-/-}$ telencephalon; in situ hybridisation showed that *Ptc1* is expressed in the ventral half of both the wild-type and *Foxg1*$^{-/-}$ telencephalon (Fig. 1D,E). As Ptc1 expression is dependent on Hh signalling (Goodrich et al., 1996; Marigo and Tabin, 1996; Wijgerde et al., 2002), this finding indicates that at least some ventral Hh signalling is retained in *Foxg1*$^{-/-}$ embryos, although the level of expression of *Ptc1* determined by qRT-PCR is lower than in wild-type ventral telencephalon (Fig. 1B). Gli1, whose expression is also activated by and requires Hh signalling (Bai et al., 2002; Bai et al., 2004; Lee et al., 1997), is expressed in *Foxg1*$^{-/-}$ mutant telencephalon (Fig. 1A). The Hh target *Nkx2.1* (Ruiz i Altaba, 1998; Vokes et al., 2007) is absent in *Foxg1*$^{-/-}$ telencephalon (Fig. 1A). These results indicate that many components of the Hh signalling pathway are active in the *Foxg1*$^{-/-}$ telencephalon, albeit with evidence of impaired activation, whereas others such as Nkx2.1 are lost.

Gli3 processing is altered in *Foxg1*$^{-/-}$ telencephalon but this is unlikely to account for most of the ventral defects

As the telencephalon is likely to be exposed to reduced levels of Shh (Huh et al., 1999), which might explain the reduced expression of *Ptc1* in the *Foxg1*$^{-/-}$ telencephalon (Fig. 1B), we

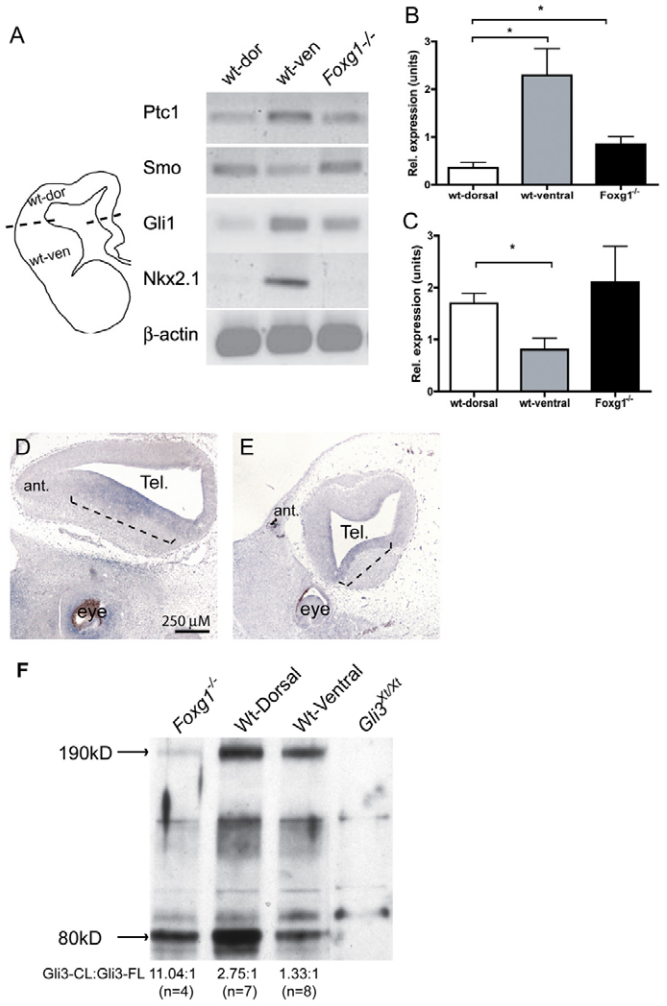

Fig. 1. Evidence of Hh signalling pathway activity and defective expression and post-translational processing of Gli3 in *Foxg1*$^{-/-}$ telencephalon. (**A**) RT-PCR analysis of the expression of components of the Shh signalling pathway and Shh target genes in E12.5 wild-type dorsal (wt-dor), wild-type ventral (wt-ven) and *Foxg1*$^{-/-}$ telencephalon. (**B**) Quantitative RT-PCR shows that *Ptc1* is expressed at very low levels in E12.5 wt-dor telencephalon (white bar), at high levels in wt-ven telencephalon (grey bar) and intermediate levels in *Foxg1*$^{-/-}$ telencephalon (black bar). Expression is significantly greater in wt-ven and in *Foxg1*$^{-/-}$ telencephalon than in wt-dor telencephalon (*t*-test, $P<0.05$, $n=4$ independent extracts). (**C**) qRT-PCR for *Gli3* mRNA in E12.5 wt-dor (white bar) and wt-ven (grey bar) telencephalon and *Foxg1*$^{-/-}$ (black bar) telencephalon. *Gli3* expression in wt-ven telencephalon is significantly lower than in wt-dor telencephalon (*t*-test, $P<0.05$, $n=4$ independent extracts); there is no significant difference between wt-dor and *Foxg1*$^{-/-}$ extracts. (**D**,**E**) In situ hybridisations for *Ptc1* on parasagittal sections showing expression of *Ptc1* (bracketed) in both wt (D) and *Foxg1*$^{-/-}$ (E) E12.5 telencephalon (Tel.); anterior (ant.) is to the left and dorsal is up. (**F**) Western blot of Gli3 protein in *Foxg1*$^{-/-}$, wt-dor, wt-ven, and *Gli3*$^{-/-}$ (*Gli3*$^{Xt/Xt}$) telencephalic extracts. Gli3 is present in full-length (Gli3-FL; ~190 kD) and cleaved (Gli3-CL: ~80 kD) forms in all except *Gli3*$^{-/-}$ extracts; average ratios between the two forms are shown (*n* values are numbers of blots). Scale bar: 250 μm.

predicted alterations in the expression and processing of the transcription factor Gli3. A primary action of Hh signalling in patterning the ventral neural tube involves antagonism of the ability of Gli3 to repress ventral gene expression. This is achieved in two ways. First, exposure to Shh can decrease transcription of *Gli3* (Marigo et al., 1996; Ruiz i Altaba, 1998; Schweitzer et al., 2000). Second, Shh inhibits the post-translational cleavage of the Gli3 protein (Bastida et al., 2004; Wang et al., 2000): the cleaved form (Gli3-CL) is thought to act as a repressor required for development of dorsal telencephalon (Jacob and Briscoe, 2003; Quinn et al., 2009; Rash and Grove, 2007; Wang et al., 2000). Results from wild-type embryos in Fig. 1C,F confirmed lower levels of *Gli3* transcript and Gli3 protein in ventral than in dorsal embryonic telencephalon, supporting previous data (Fotaki et al., 2006; Nery et al., 2001; Sussel et al., 1999), and the abundance of Gli3-CL relative to that of full-length Gli3 (Gli3-FL) was lower in ventral telencephalon (average ratio=1.33:1, $n=8$) than in dorsal telencephalon (average ratio=2.75:1, $n=7$). The level of *Gli3* mRNA in *Foxg1*$^{-/-}$ telencephalon was similar to that in the dorsal part of wild-type telencephalon (Fig. 1C) with an overabundance of Gli3-CL relative to Gli3-FL (average ratio=11.04:1, $n=4$) (Fig. 1F). We went on to test the significance of this for the *Foxg1*$^{-/-}$ telencephalon.

Previous work has shown that the removal of just one copy of *Gli3* in *Shh*$^{-/-}$ and *Smo*$^{-/-}$ embryos is sufficient for a high level of recovery of ventral telencephalic morphology and gene expression. The recovery includes restoration of ventral domains of Mash1, Gsh2 and Nkx2.1 expression; these domains are cleared of abnormally high levels of Pax6 expression, which becomes restricted to a dorsal domain as in wild-type embryos (Rallu et al., 2002b). Given that the post-translational processing of *Gli3* in *Foxg1*$^{-/-}$ telencephalon showed abnormalities that are associated with loss of Shh signalling, we tested whether loss of one copy of *Gli3* restored any aspects of ventral telencephalic development in *Foxg1*$^{-/-}$ embryos. We observed no obvious restoration of ventral telencephalic morphology and little evidence of rescue of ventral patterns of gene expression in *Foxg1*$^{-/-}$;*Gli3*$^{+/-}$ compared with *Foxg1*$^{-/-}$;*Gli3*$^{+/+}$ embryos: there was weak re-expression of Mash1 and a very few Islet1-expressing cells were found in the ventral part of the *Foxg1*$^{-/-}$;*Gli3*$^{+/-}$ telencephalon (see Fig. S1 in the supplementary material). As loss of both copies of *Gli3* in *Foxg1*$^{-/-}$ embryos leads to loss of the telencephalon (Hanashima et al., 2007; Hebert and Fishell, 2008), *Foxg1*$^{-/-}$;*Gli3*$^{-/-}$ embryos were unlikely to be informative in the present context. Our findings suggest that defective antagonism of Gli3-CL is unlikely to account for most of the ventral defects in *Foxg1*$^{-/-}$ telencephalon.

Foxg1$^{-/-}$ telencephalic cells show some abnormal responses to Shh in vitro

Impaired Shh signalling in *Foxg1*$^{-/-}$ embryonic telencephalon might occur because cells are exposed to reduced concentrations of Shh from ventral sources and/or because cells have an abnormal response to Shh. To test the second of these possibilities, we cultured telencephalic explants from E10.5 *Foxg1*$^{-/-}$ embryos with 5, 20 or 50 nM Shh in solution. Explants were harvested after 24 hours in culture and analysed by qRT-PCR (Fig. 2). We used explants from the dorsal telencephalon of wild-type embryos as controls (Fig. 2A,B): Shh application resulted in upregulation of *Ptc1*, *Gli1* and *Olig2* [a gene expressed in ventral telencephalic progenitors that is known to be induced by Shh (Lu et al., 2000)], caused downregulation of *Gli3* and had no effect on expression of *Mash1* [a proneural gene normally expressed at relatively low levels in dorsal telencephalon compared with high levels in ventral telencephalon (Fode et al., 2000)]. In *Foxg1*$^{-/-}$ telencephalic explants, Shh application upregulated *Ptc1*, *Gli1* and *Olig2* and downregulated *Gli3*, as in wild-type explants, but significantly

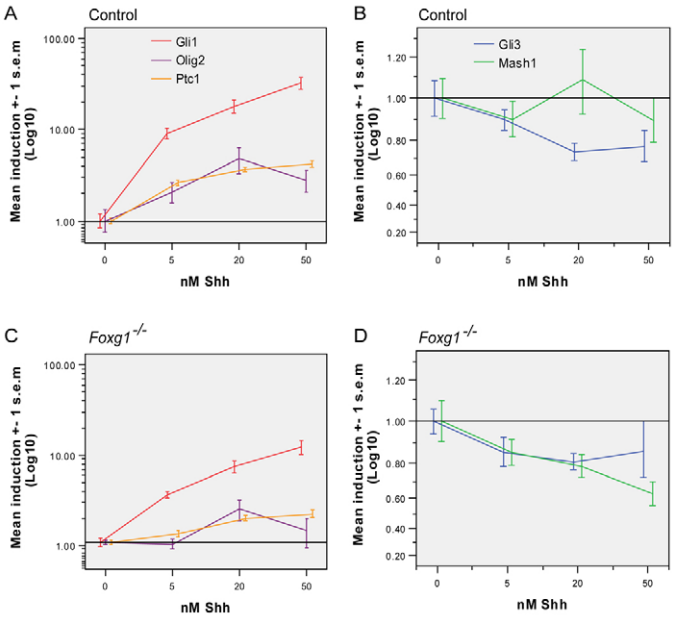

Fig. 2. *Foxg1*$^{-/-}$ telencephalic explants can respond to Shh. (**A-D**) Induction of *Gli1*, *Olig2* and *Ptc1* (A,C) and *Gli3* and *Mash1* (B,D) in wild-type (wt; A,B) and *Foxg1*$^{-/-}$ (C,D) telencephalic explants from E10.5 embryos exposed to 0, 5, 20 or 50 nM Shh, as measured by qRT-PCR. Both wt and *Foxg1*$^{-/-}$ explants significantly upregulate *Ptc1* and *Gli1* expression at all doses of Shh and upregulate *Olig2* expression and downregulate *Gli3* expression at 20 nM Shh (Student's *t*-test, $P<0.05$). Treatment with 20 nM or 50 nM Shh significantly downregulates *Mash1* expression in *Foxg1*$^{-/-}$ (Student's *t*-test, $P<0.05$) but not in wt explants. Means are from 11-15 for wt and 6-8 for *Foxg1*$^{-/-}$ cultures.

downregulated *Mash1* (Fig. 2C,D). Using this experimental paradigm we were unable to induce consistent, statistically significant upregulation of the ventral telencephalic marker *Dlx2* in either wild-type or *Foxg1*$^{-/-}$ explants (data not shown).

We did succeed in inducing Dlx2 and Mash1 expression in wild-type explants when Shh was delivered for 24-36 hours from beads soaked in a 25 μM solution of recombinant Shh (Fig. 3A,D; 17/18 explants showed induction), as has been demonstrated previously (Kohtz et al., 1998; Kuschel et al., 2003). Induction was seen around the beads (Fig. 3D), suggesting that successful induction of these genes in wild-types requires a localised source of Shh delivering a particular concentration or perhaps a concentration gradient. Delivery of Shh in this way failed to induce Mash1 and Dlx expression in *Foxg1*$^{-/-}$ explants (Fig. 3E; 0/16 explants showed induction). In control experiments, no Dlx- and Mash1-positive cells were found in wild-type or *Foxg1*$^{-/-}$ explants treated with BSA-soaked beads (Fig. 3B,C; 0/34 explants showed expression). We used qRT-PCR to confirm that, as in experiments with Shh added in solution, *Ptc1* and *Gli1* were induced in *Foxg1*$^{-/-}$ explants exposed to Shh-soaked beads (Fig. 3F; Student's one sample *t*-test, $P<0.05$, $n=4$ independent experiments in each case). *CyclinD1*, which is also a target of Shh signalling in neural progenitors (Kenney and Rowitch, 2000; Oliver et al., 2003), was significantly upregulated by Shh-soaked beads in *Foxg1*$^{-/-}$ explants (Fig. 3F).

Overall, these in vitro experiments suggested that loss of Foxg1 results in a selective impairment in the response of telencephalic cells to Shh: whereas many Shh targets are upregulated (*Ptc1*, *Gli1*, *Olig2*, *CyclinD1*) or downregulated (*Gli3*) as in wild-types, we were unable to upregulate expression of the ventral telencephalic markers Mash1 and Dlx2 in *Foxg1*$^{-/-}$ explants using a paradigm that did upregulate these markers in wild-type explants. One experiment suggested that Shh might even suppress *Mash1* expression under some conditions when Foxg1 is absent.

Partial rescue of ventral telencephalic gene expression in *Foxg1*$^{-/-}$;*Pax6*$^{-/-}$ double mutants

We considered the possibility that persistent expression of Pax6 throughout the *Foxg1*$^{-/-}$ telencephalon might mediate suppression of ventral telencephalic marker expression, as Pax6 is known to suppress ventral marker expression in normal dorsal telencephalon (Kroll and O'Leary, 2005; Quinn et al., 2007; Stoykova et al., 2000; Toresson et al., 2000; Yun et al., 2001). We generated *Foxg1*$^{-/-}$;*Pax6*$^{-/-}$ embryos in which the coding sequence of *Foxg1* is replaced by a *lacZ* reporter cassette (Xuan et al., 1995). The *Foxg1*-expressing telencephalon labelled by β-galactosidase immunostaining was smaller in *Pax6*$^{-/-}$;*Foxg1*$^{-/-}$ than in *Foxg1*$^{-/-}$ mutants (Fig. 4), consistent with the known role of Pax6 in the regulation of cortical progenitor proliferation (Estivill-Torrus et al., 2002; Quinn et al., 2007). We found stronger Mash1 staining in the *Pax6*$^{-/-}$;*Foxg1*$^{-/-}$ telencephalon compared with *Foxg1*$^{-/-}$ telencephalon and Gsh2 and Olig2 expression was restored in a small number of cells, well below the numbers of Gsh2 and Olig2 expressing cells in wild-type ventral telencephalon (Fig. 4E-O). There was no rescue of Islet1, Nkx2.1 or Dlx2 in *Pax6*$^{-/-}$;*Foxg1*$^{-/-}$ telencephalon (data not shown). The dorsal markers Tbr2 (Eomes – Mouse Genome Informatics) and reelin were expressed throughout the telencephalon in both *Pax6*$^{-/-}$;*Foxg1*$^{-/-}$ and *Foxg1*$^{-/-}$ mutants (Fig. 4P-R). These findings indicate that some defects of ventral telencephalic gene expression in *Foxg1*$^{-/-}$ embryos, most notably the loss of Mash1 expression, might be mediated in part by persistent ventral telencephalic expression of Pax6.

Foxg1$^{-/-}$ telencephalic cells can respond to Fgf proteins

Fgf proteins act downstream of Shh and Gli3 to generate ventral telencephalic cell types (Gutin et al., 2006) and Fgf8 has a prominent role in this process (Storm et al., 2006). Simultaneous deletion of Fgf receptors 1 and 2 (Fgfr1 and Fgfr2) results in a loss of ventral cell types even though Shh remains expressed and, unlike the *Shh*$^{-/-}$ and *Smo*$^{-/-}$ phenotypes, the *Fgfr1*$^{-/-}$;*Fgfr2*$^{-/-}$ phenotype is not rescued by removal of Gli3 (Gutin et al., 2006). We tested whether defects in Fgf responsiveness might contribute to the loss of ventral cell types in *Foxg1*$^{-/-}$ telencephalon. We first examined the expression of Fgf8 signalling pathway components (Fig. 5). We examined the expression of *Fgfr1*, which is thought to be the main Fgf8 receptor in the early telencephalon (Gutin et al., 2006), and that of *Fgfr3* as it is the highest affinity receptor for Fgf8 (Chellaiah et al., 1999; Ornitz et al., 1996), although its function in the early telencephalon is unclear (Gutin et al., 2006); we found that *Fgfr3* (Fig. 5A,B) and *Fgfr1*(Fig. 5G,H) are expressed throughout both wild-type and *Foxg1*$^{-/-}$ telencephalon, with higher levels expressed dorsally for *Fgfr3* (Fig. 5A,B).

We examined the expression of known targets of Fgf8 signalling. The sprouty 2 gene (*Spry2*) is induced by Fgf8 signalling (Chambers and Mason, 2000) and is expressed in the early telencephalon (Zhang et al., 2001), where its expression requires Fgf8 signalling (Storm et al., 2003). We found that *Sprouty2* is expressed throughout the telencephalon of *Foxg1*$^{-/-}$ embryos as in wild-types (Fig. 5I,J). *Mest* (*Peg1*) encodes a highly conserved enzyme of unknown

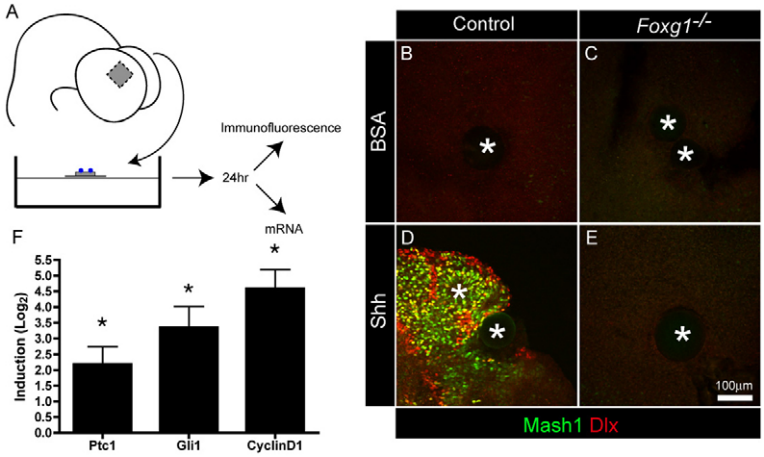

Fig. 3. Shh cannot induce ventral markers in *Foxg1*$^{-/-}$ explants. (**A**) The telencephalic region of E10.5 embryos taken for culture (shaded) and the culture strategy. (**B-E**) Mash1 (green) and pan-Dlx (red) immunofluorescence on control (B,D) and *Foxg1*$^{-/-}$ (C,E) dorsal telencephalic explants treated with bovine serum albumin (BSA)-soaked (B,C) and Shh-soaked (D,E) beads. Asterisks indicate the position of the beads. (**F**) Induction of Hh target gene expression by Shh-soaked beads (relative to BSA-beads) in *Foxg1*$^{-/-}$ telencephalic explants as measured by qRT-PCR; values are means ± s.e.m.; *Ptc1*, *Gli1* and cyclin D1 are significantly induced in *Foxg1*$^{-/-}$ telencephalic cells (Student's *t*-test, *, $P<0.05$, $n=4$ independent cultures for each). Scale bar: 100 µm.

function whose telencephalic expression is induced by and depends on Fgf8 signalling (Sansom et al., 2005). In wild-type telencephalon, *Mest* is expressed in the dorsal lateral ganglionic eminence (dLGE); in *Foxg1*$^{-/-}$ mutants, *Mest* expression is retained in the ventral-most part of the telencephalon (Fig. 5E,F, arrows). The transcription factor Etv5 is another target of Fgf8 signalling whose telencephalic expression is strongly down-regulated in *Fgfr1*$^{-/-}$ embryos (Sansom et al., 2005). In wild-type telencephalon, *Etv5* is expressed in the dLGE; in *Foxg1*$^{-/-}$ embryos, *Etv5* expression is retained in the ventral-most part of the telencephalon (Fig. 5C,D, arrows). The retention of *Spry2*, *Etv5* and *Mest* expression domains in the ventral part of the *Foxg1*$^{-/-}$ telencephalon suggests that the Fgf8 signalling pathway is active in this region.

To test whether the Fgf8 pathway can be activated in *Foxg1*$^{-/-}$ cells we cultured telencephalic explants from *Foxg1*$^{-/-}$ embryos with 0.42 or 4.2 nM Fgf8 in solution. The explants were harvested after 24 hours in culture and analysed by qRT-PCR (Fig. 6). Explants from the dorsal telencephalon of wild-type embryos were used as controls. Exposure to 4.2 nM Fgf8 resulted in upregulation of *Etv5* and *Spry2* mRNA levels in control and in *Foxg1*$^{-/-}$ explants (Fig. 6). These findings, together with our in vivo observations on expression of Fgf receptors and targets, indicate that *Foxg1*$^{-/-}$ telencephalic cells are able to respond to Fgf8.

Foxg1$^{-/-}$ cells can contribute to dorsal and ventral telencephalon in *Foxg1*$^{-/-}$ *Foxg1*$^{+/+}$ chimeras

Our results so far indicated that *Foxg1*$^{-/-}$ telencephalic cells might have a profound cell autonomous inability to adopt ventral fates, even though they are exposed to and show some responses to ventralising signals in *Foxg1*$^{-/-}$ embryos. We pursued this possibility further using an in vivo approach in which *Foxg1*$^{-/-}$ cells and wild-type cells in an individual embryo are exposed to the same ventralising signals. To do this, we generated chimeric embryos comprising *Foxg1*$^{-/-}$ and wild-type cells.

We confirmed that *Foxg1*$^{-/-}$ cells can contribute to both dorsal and ventral telencephalon in chimeras (see Fig. S2 in the supplementary material). In *Foxg1*$^{-/-}$ *Foxg1*$^{+/+}$ chimeras, the telencephalon developed its characteristic morphology, including distinct ganglionic eminences ventrally. Many *Foxg1*$^{-/-}$ cells (recognised by their expression of β-galactosidase) segregated into clusters at all rostrocaudal levels in both dorsal and ventral telencephalon (see Fig. S2A-C in the supplementary material), whereas in *Foxg1*$^{+/-}$ *Foxg1*$^{+/+}$ control chimeras the two cell types were intermingled throughout the telencephalon even in cases where one cell type dominated (see Fig. S2D in the supplementary material). The segregation in experimental chimeras suggested a difference in the identities of the two cell types.

For each chimera, the overall percentages of cells derived from the *Foxg1*$^{-/-}$ embryo (in experimental chimeras) or the *Foxg1*$^{+/-}$ embryo (in control chimeras) were estimated by quantitating the Gpi1 isozyme composition of the upper body (West and Flockhart, 1994), where Foxg1 is not expressed and its absence would be expected to have little or no effect on the contribution of either cell type. The percentage of the Gpi1b isozyme represented the contribution of the *Foxg1*$^{-/-}$ or *Foxg1*$^{+/-}$ cells. Both *Foxg1*$^{-/-}$ and *Foxg1*$^{+/-}$ cells carried one mutant allele in which the coding sequence of *Foxg1* is replaced by a *lacZ* reporter cassette (*Foxg1*^{lacZ}; Xuan et al., 1995), allowing us to detect and count proportions of *Foxg1*$^{-/-}$ and *Foxg1*$^{+/-}$ cells in the telencephalon of chimeras. In control chimeras, the proportion of β-galactosidase-expressing telencephalic cells divided by the percentage of Gpi1b was, as expected, ~1 (see Fig. S3 in the supplementary material). In experimental chimeras, however, proportions of β-galactosidase-expressing *Foxg1*$^{-/-}$ telencephalic cells were about a third of the percentages of Gpi1b (see Fig. S3 in the supplementary material), indicating that although *Foxg1*$^{-/-}$ cells contribute to both ventral and dorsal telencephalon, they are significantly under-represented to similar extents in both.

Telencephalic cells require Foxg1 cell-autonomously to adopt ventral identities

We examined the expression of the transcription factors Olig2, Gsh2 and Mash1, ventral progenitor markers expressed in both LGE and medial ganglionic eminence (MGE), and Nkx2.1, whose expression is restricted to the MGE (Fig. 7A). *Foxg1*$^{-/-}$ cells in chimeric telencephalon consistently failed to express any of these ventral progenitor markers, whereas surrounding wild-type cells in experimental chimeras and wild-type and *Foxg1*$^{+/-}$ cells in control chimeras expressed them appropriately (Fig. 7B-M). We examined the expression of two markers of differentiating neurons derived from the ventral telencephalon, Islet1 and calbindin. Islet1 marks differentiating striatal projection neurons and cholinergic interneurons (Stenman et al., 2003; Wang and Liu, 2001), and calbindin marks GABAergic interneurons (Anderson et al., 1997). The vast majority of Islet1-expressing telencephalic cells in *Foxg1*$^{-/-}$ *Foxg1*$^{+/+}$ experimental chimeras were wild-type (negative for β-galactosidase) (Fig. 7N-P,R-T), but a very small number of Islet1-positive *Foxg1*$^{-/-}$ cells were seen in the MGE of mid-level

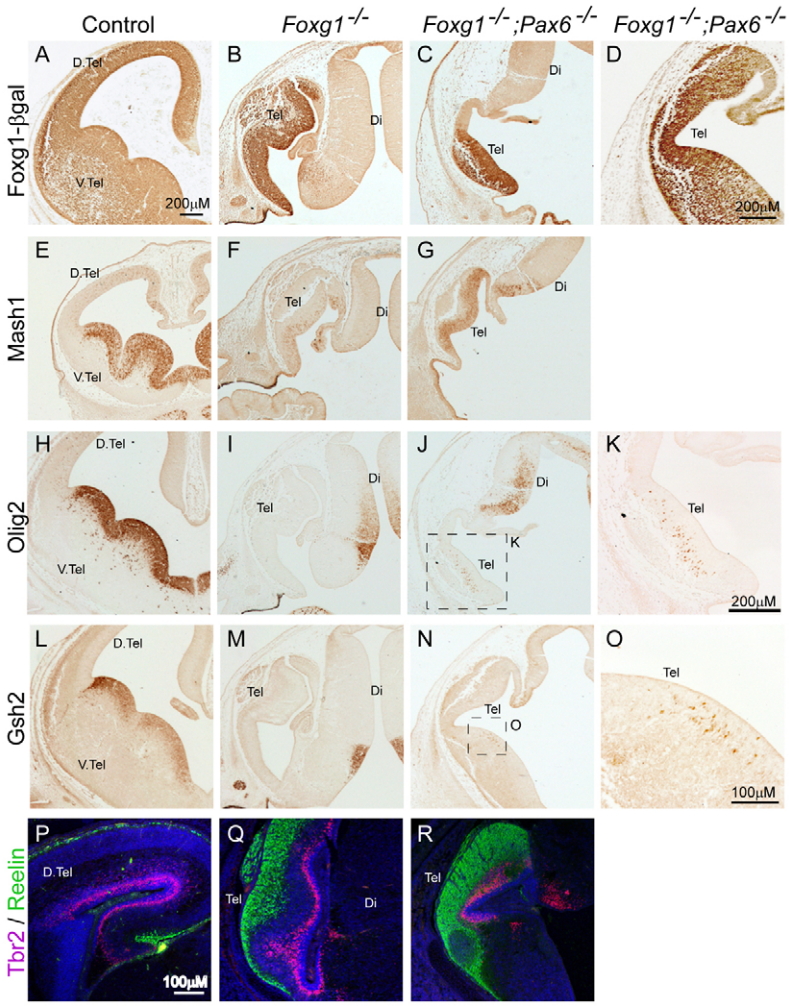

Fig. 4. Limited recovery of Mash1, Olig2 and Gsh2 expression in *Foxg1*$^{-/-}$;*Pax6*$^{-/-}$ telencephalon. (**A-D**) Immunohistochemistry for Foxg1-β-gal marks telencephalic territory in *Foxg1*$^{+/-}$ (control, A), *Foxg1*$^{-/-}$ (B) and *Foxg1*$^{-/-}$;*Pax6*$^{-/-}$ (C,D) E12.5 embryos. (**E-R**) Immunohistochemistry for Mash1 (E-G), Olig2 (H-K), Gsh2 (L-O) and Tbr2 and reelin (P-R) in the telencephalon of control, *Foxg1*$^{-/-}$ and *Foxg1*$^{-/-}$;*Pax6*$^{-/-}$ E12.5 (E-O) and E16.5 (P-R) embryos. (E-G) Telencephalic Mash1 expression appears higher in *Foxg1*$^{-/-}$;*Pax6*$^{-/-}$ than in *Foxg1*$^{-/-}$ embryos. (H-O) Olig2 and Gsh2 expression is absent from the telencephalon of *Foxg1*$^{-/-}$ embryos, but a small number of Olig2- and Gsh2-expressing cells are present in the telencephalon of *Foxg1*$^{-/-}$;*Pax6*$^{-/-}$embryos. Sections in C,G,J,K are from a different *Foxg1*$^{-/-}$;*Pax6*$^{-/-}$embryo and rostrocaudal level than those in D,N,O. (P-R) Dorsal markers Tbr2 (purple) and reelin (green) are expressed throughout the telencephalon in both *Pax6*$^{-/-}$;*Foxg1*$^{-/-}$ and *Foxg1*$^{-/-}$ embryos. D.Tel, dorsal telencephalon; Di, diencephalon; Tel, telencephalon; V.Tel, ventral telencephalon. Scale bars: 200 μm in A,D,K; 100 μm in O,P.

telencephalic sections (Fig. 7T, arrows). Large numbers of *Foxg1*$^{+/-}$ cells in *Foxg1*$^{+/-}$ *Foxg1*$^{+/+}$ control chimeras expressed Islet1 (Fig. 7Q,U, arrows). No calbindin-positive *Foxg1*$^{-/-}$ interneurons were ever observed (Fig. 7V-X), whereas many wild-type and *Foxg1*$^{+/-}$ ventral telencephalic cells in experimental and control chimeras expressed calbindin (Fig. 7W-Y, arrows). The near-complete failure of *Foxg1*$^{-/-}$ cells to express a wide range of ventral markers expressed by their surrounding wild-type cells in the chimeric telencephalon indicates a cell-autonomous requirement for Foxg1 in the acquisition of ventral telencephalic identities.

As *Foxg1*$^{-/-}$ cells in chimeric ventral telencephalon failed to adopt ventral identities, we examined the expression of Ngn2, Pax6, Tbr1 and Tbr2 to see whether they had adopted dorsal identities instead. In wild-type embryos, dorsal telencephalic progenitors express Ngn2 and high levels of Pax6, Tbr2 is found in basal progenitors and early-born neurons of the dorsal telencephalon and Tbr1 is expressed by dorsally derived glutamatergic neurons (Englund et al., 2005; Hevner et al., 2003; Hevner et al., 2001) (Fig. 8A). *Foxg1*$^{-/-}$ cells located in the dorsal telencephalon of experimental chimeras expressed all of these markers, as did their wild-type neighbours, consistent with their expression in the telencephalon of *Foxg1*$^{-/-}$ embryos (data not shown). *Foxg1*$^{-/-}$ cells in the LGE of experimental chimeras expressed Ngn2, Pax6, Tbr2 and Tbr1 (Fig. 8B-F,L,M,O,P). *Foxg1*$^{-/-}$ cells located in the MGE of experimental chimeras expressed Pax6 (Fig. 8H-J), but did not express Ngn2 (Fig. 8B), Tbr1 or Tbr2 (data not shown). Ectopic expression of dorsal markers in the ventral telencephalon was seen only in *Foxg1*$^{-/-}$ cells, indicating that the *Foxg1*$^{-/-}$ cells did not alter the specification of surrounding wild-type cells in a cell-nonautonomous manner. These findings suggest that Foxg1 is required cell-autonomously for ventral telencephalic suppression of Pax6 expression and for suppression of Tbr1 and 2 and Ngn2 expression in the LGE, although not in the MGE.

We also examined expression of cyclin D1 and p21^{cip1} in chimeras. Cyclin D1, an early target of Shh signalling in neural progenitors (Kenney and Rowitch, 2000; Oliver et al., 2003), is expressed in a ventral^{high} to dorsal^{low} gradient in wild-type telencephalon but was not detectable by immunohistochemistry in *Foxg1*$^{-/-}$ telencephalon (see Fig. S4A,B in the supplementary material). In *Foxg1*$^{-/-}$ *Foxg1*$^{+/+}$ chimeras, many *Foxg1*$^{-/-}$ ventral telencephalic cells expressed cyclin D1, as did surrounding wild-type cells (see Fig. S4C-G in the supplementary material). In normal embryonic dorsal telencephalon, p21^{cip1} is expressed in the mantle layer of the medial wall only, whereas in *Foxg1*$^{-/-}$ embryos p21^{cip1} is expressed more widely throughout the mantle layer of the dorsal telencephalon (Seoane et al., 2004). We found that p21^{cip1} is not upregulated in *Foxg1*$^{-/-}$ telencephalic cells in chimeras, but remains restricted to cells in the mantle layer of the medial wall only, as in wild-types (see Fig. S4H-K in the supplementary material).

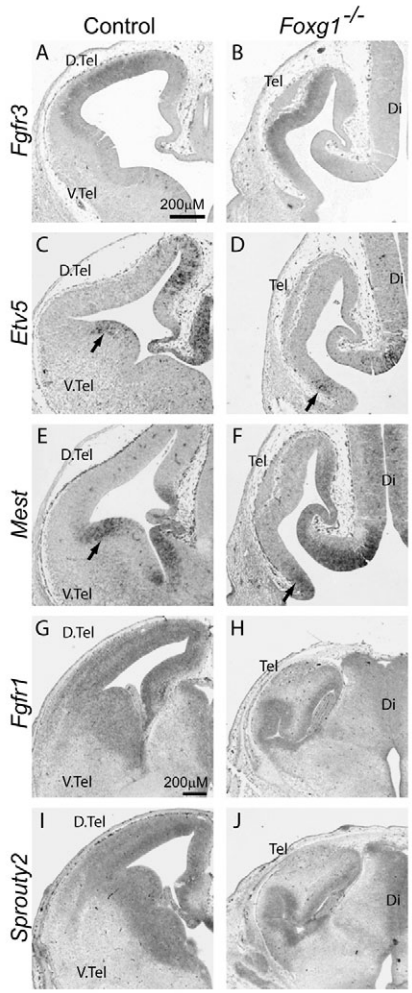

Fig. 5. Components of the Fgf8 signalling pathway are present in the *Foxg1*$^{-/-}$ telencephalon. (**A-J**) RNA in situ hybridisations on coronal sections through the forebrain of control (A,C,E,G,I) or *Foxg1*$^{-/-}$ (B,D,F,H,J) embryos at E12.5 (A-F) and E14.5 (G-J) showing expression of *Fgfr3* (A,B), *Etv5* (C,D), *Mest* (E,F), *Fgfr1* (G,H) and sprouty 2 (I,J). (C-F) Arrows show *Mest* and *Etv5* expression in the lateral ganglionic eminence in control embryos and in the ventral-most part of the telencephalon of *Foxg1*$^{-/-}$ embryos. D.Tel, dorsal telencephalon; Di, diencephalon; Tel, telencephalon; V.Tel, ventral telencephalon. Scale bars: 200 μm.

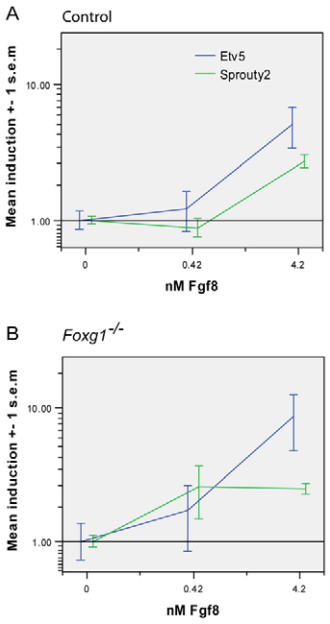

Fig. 6. *Foxg1*$^{-/-}$ telencephalic explants can respond to Fgf8. Induction of *Etv5* and sprouty 2 in wild-type (**A**) and *Foxg1*$^{-/-}$ (**B**) telencephalic explants exposed to 0, 0.42 or 4.2 nM Fgf8, as measured by qRT-PCR. Both control and mutant explants significantly upregulate *Etv5* and sprouty 2 expression in response to 4.2 nM Fgf8 (Student's *t*-test, $P<0.05$). Values plotted are means (Log10) of four wild-type samples and three *Foxg1*$^{-/-}$ cultures.

DISCUSSION

Foxg1 is required for normal embryonic development of the ventral telencephalon (Martynoga et al., 2005; Xuan et al., 1995). Previous work showed that embryos lacking Foxg1 have reduced telencephalic expression of two signalling molecules crucial for the specification of ventral telencephalic cell types, Fgf8 and Shh, and suggested that lack of these molecules might account for the lack of ventral telencephalon in *Foxg1*$^{-/-}$ embryos (Huh et al., 1999; Martynoga et al., 2005; reviewed by Hebert and Fishell, 2008). We have now examined the competence of telencephalic cells lacking Foxg1 to develop ventral cell types. Our new data indicate that telencephalic cells lacking Foxg1 have a cell-autonomous inability to express many ventral marker genes and to repress many dorsal marker genes even though they do show some responses to Shh and Fgf8. These findings imply that the profound loss of ventral cell types in the *Foxg1*$^{-/-}$ telencephalon is due not only to a reduced production of ventralising signals but also to an intrinsic inability of telencephalic cells to express normal ventral molecular phenotypes, as a result of defects downstream of many of the cell responses to Shh and Fgf8. Our findings strongly support recent findings in zebrafish that Foxg1 is required downstream of Shh for the development of ventral telencephalic identities (Danesin et al., 2009).

Telencephalic cells lacking Foxg1 show some responses to Shh and Fgf8

We obtained in vitro and in vivo evidence that telencephalic cells are able to respond to Shh and Fgf8 and that they do so in *Foxg1*$^{-/-}$ embryos. First, *Foxg1*$^{-/-}$ telencephalic cells express key receptors for both Shh and Fgf8. Second, *Foxg1*$^{-/-}$ telencephalic cells express genes whose activation requires Shh and Fgf8 signalling. Third, exposure of *Foxg1*$^{-/-}$ telencephalic explants to Shh or Fgf8 results in induction of known Shh and Fgf8 target genes, including *Ptc1*, *Gli1*, *Olig2*, cyclin D1, *Spry2* and *Etv5*.

Previous work showed that the telencephalic actions of Shh are mediated by Gli3: Shh inhibits the cleavage of Gli3 into its repressor form (Gli3-CL) and Gli3-CL inhibits the expression of Shh targets (reviewed by Fuccillo et al., 2006; Hebert and Fishell, 2008). We found an overabundance of Gli3-CL in the *Foxg1*$^{-/-}$ telencephalon. The most probable explanation for this is the reduced exposure of the telencephalon to Shh in *Foxg1*$^{-/-}$ embryos (Huh et al., 1999). In wild-type embryos, a site of *Shh* expression develops in the ventral telencephalon by E10.5; this does not form in *Foxg1*$^{-/-}$ embryos, although expression of *Shh* appears normal at other sites around the telencephalon (i.e. diencephalon, floor plate, notochord and anterior mesendoderm).

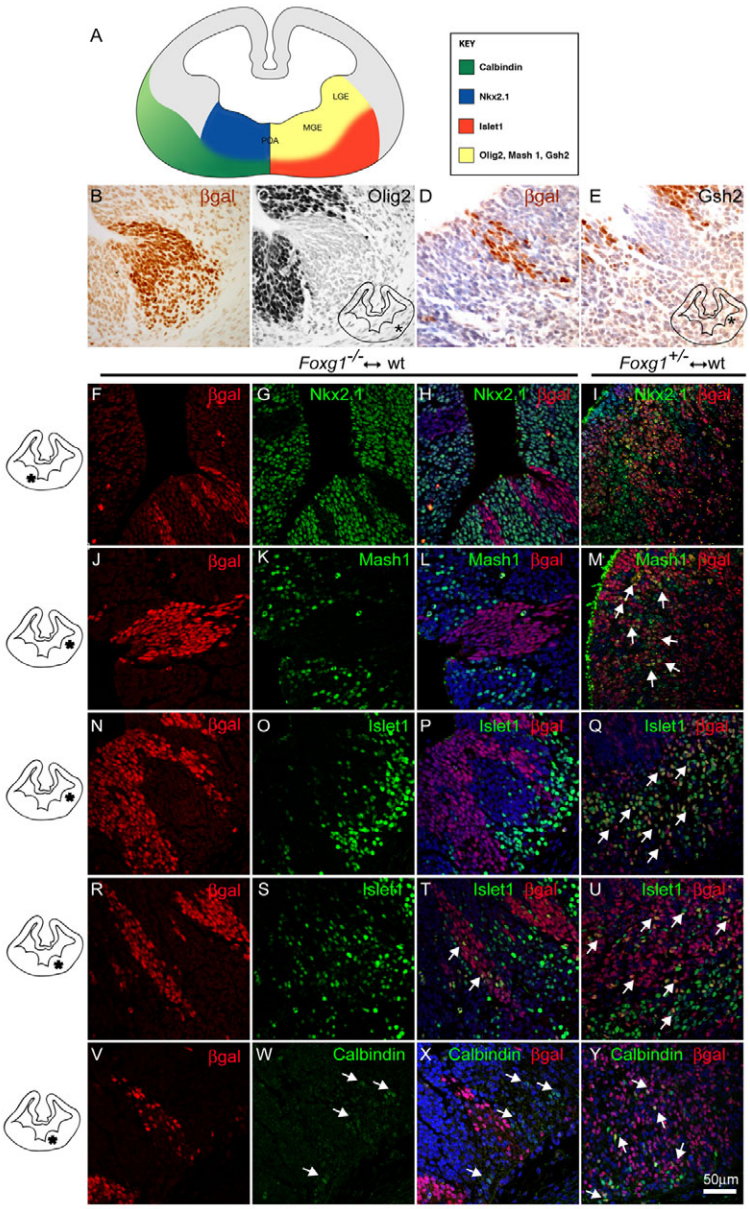

Fig. 7. *Foxg1*$^{-/-}$ cells in chimeric telencephalon do not express markers of ventral telencephalic fate. (**A**) Restricted expression of the ventral markers calbindin, Nkx2.1, Islet1, Olig2, Mash1 and Gsh2 in normal E12.5 telencephalon. (**B**,**C**) Foxg1-β-gal and Olig2 immunohistochemistry on adjacent coronal sections from a *Foxg1*$^{-/-}$ *Foxg1*$^{+/+}$ brain. (**D**,**E**) Foxg1-β-gal and Gsh2 immunohistochemistry on adjacent coronal sections from a *Foxg1*$^{-/-}$ *Foxg1*$^{+/+}$ brain. (**F**-**Y**) Coronal sections from experimental (*Foxg1*$^{-/-}$ *Foxg1*$^{+/+}$) and control (*Foxg1*$^{+/-}$ *Foxg1*$^{+/+}$) chimeras. (F-I) Foxg1-β-gal and Nkx2.1 immunofluorescence. (J-M) Foxg1-β-gal and Mash1 immunofluorescence. (N-U) Foxg1-β-gal and Islet1 immunofluorescence. (V-Y) Foxg1-β-gal and calbindin immunofluorescence. The nuclei of *Foxg1*$^{-/-}$ or *Foxg1*$^{+/-}$ cells are labelled by β-gal immunofluorescence (red). In some cases, nuclei are counterstained with TO-PRO-3 (blue). For each staining, the approximate location of the field of view shown is indicated by the asterisk in the schematic. Arrows highlight individual marker+/β-gal+ co-expressing cells. LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; POA, pre-optic area. Scale bar: 50 μm.

Previous work showed a reduction in the expression of *Fgf8* in its rostro-ventral telencephalic domain in *Foxg1*$^{-/-}$ embryos aged E9.5 and older (Martynoga et al., 2005). Fgf signalling is essential for the generation of ventral telencephalic cell types and there is evidence that it patterns the ventral telencephalon in a dose-dependent manner (Gutin et al., 2006; Shanmugalingam et al., 2000; Shinya et al., 2001; Storm et al., 2006; Walshe and Mason, 2003). Our new results indicate that in *Foxg1*$^{-/-}$ embryos, residual Fgf8 expression is sufficient to activate ventrally-expressed Fgf8 target genes *Spry2*, *Mest* and *Etv5* that depend on Fgf8 for their expression (Sansom et al., 2005; Storm et al., 2003). Overall, these findings indicate that the levels of Fgf8 and Shh available to telencephalic cells in *Foxg1*$^{-/-}$ embryos are sufficient to activate many of their target genes.

Failed ventral telencephalic gene expression in the absence of Foxg1

We tested the possibility that elevated levels of Gli3-CL in *Foxg1*$^{-/-}$ embryos block the expression of ventral telencephalic marker genes by genetically deleting one allele of *Gli3* in *Foxg1*$^{-/-}$ embryos. Unlike in *Shh*$^{-/-}$ and *Smo*$^{-/-}$ telencephalons, where loss of one copy of *Gli3* leads to widespread reactivation of ventral gene expression (Rallu et al., 2002b), *Foxg1*$^{-/-}$;*Gli3*$^{+/-}$ embryos showed no restoration of Nkx2.1, Olig2 and Gsh2, no loss of ectopic Pax6 and Lhx2/9 in ventral telencephalon and only a minor restoration of expression of Mash1 and Islet1. This suggests that loss of Shh signalling is insufficient to explain the majority of the ventral telencephalic defects in *Foxg1*$^{-/-}$ embryos.

Because levels of not just Shh, but also Fgf8, and probably other signalling molecules, are altered in *Foxg1*$^{-/-}$ embryos, we decided that the best way to attempt to rescue ventral telencephalic gene expression in *Foxg1*$^{-/-}$ cells was to embed mutant cells in a wild-type environment capable of delivering combinations and levels of signals sufficient for normal dorso-ventral patterning. We generated *Foxg1*$^{-/-}$ *Foxg1*$^{+/+}$ chimeras in which wild-type cells showed normal dorsoventral telencephalic patterning. *Foxg1*$^{-/-}$ cells were present in the ventral telencephalon, but in lower proportions than in Foxg1-nonexpressing regions of the embryo. This telencephalon-specific under-representation suggests that the proliferative defects in

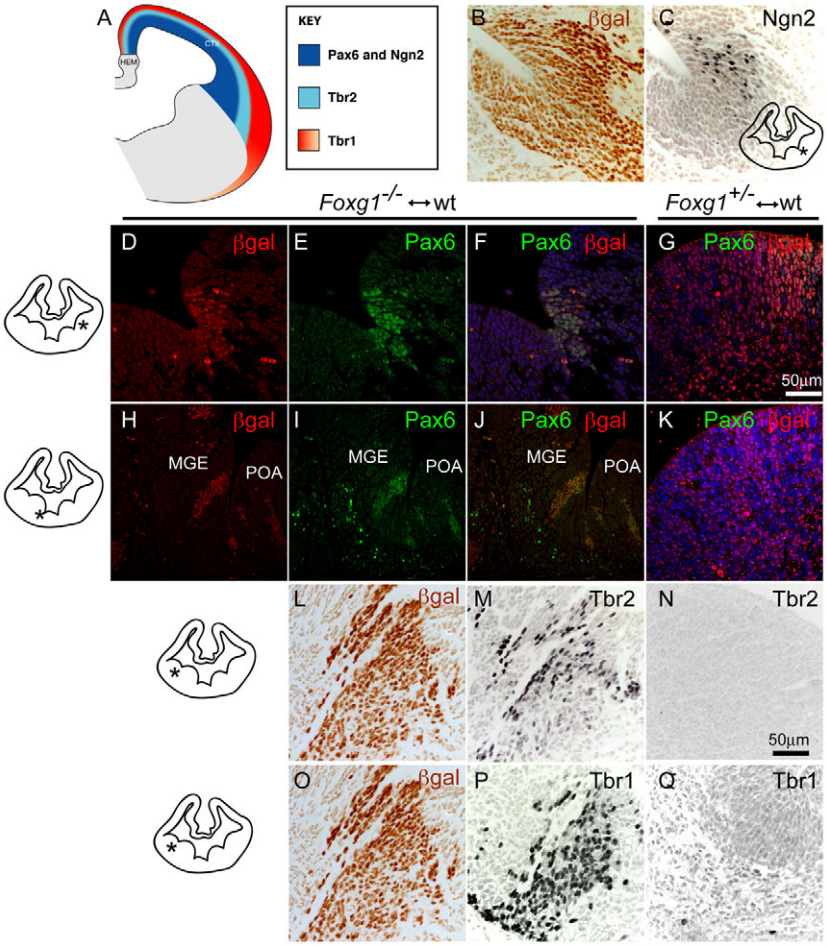

Fig. 8. *Foxg1*$^{-/-}$ cells in the chimeric ventral telencephalon express markers of dorsal telencephalic fate. (**A**) Restricted expression of dorsal markers Pax6, Ngn2, Tbr2 and Tbr1 in wild-type E12.5 telencephalon. (**B**,**C**) Foxg1-β-gal and Ngn2 immunohistochemistry on adjacent coronal sections from a *Foxg1*$^{-/-}$ *Foxg1*$^{+/+}$ brain. (**D-K**) Foxg1-β-gal (red) and Pax6 (green) immunofluorescence on coronal sections from experimental (*Foxg1*$^{-/-}$ *Foxg1*$^{+/+}$) and control (*Foxg1*$^{+/-}$ *Foxg1*$^{+/+}$) chimeras. (**L**,**M**) Foxg1-β-gal and Tbr2 immunohistochemistry on adjacent sections from a *Foxg1*$^{-/-}$ *Foxg1*$^{+/+}$ brain. (**O**,**P**) Foxg1-β-gal and Tbr1 immunohistochemistry on adjacent sections from a *Foxg1*$^{-/-}$ *Foxg1*$^{+/+}$ brain. (**N**) Tbr2 and (**Q**) Tbr1 immunohistochemistry in the lateral ganglionic eminence (LGE) of control chimeras: Tbr2 is absent from the LGE of control chimeras and the small number of Tbr1-expressing cells in the mantle zone are also observed in wild-type LGE and have probably migrated from the dorsal telencephalon (Hevner et al., 2003; Hevner et al., 2001). For each staining, the approximate location of the field of view is indicated by the asterisk in the schematic. CTX, cerebral cortex; HEM, cortical hem; MGE, medial ganglionic eminence; POA, pre-optic area. Scale bar: 50 μm.

Foxg1$^{-/-}$ embryos reported previously (Martynoga et al., 2005; Xuan et al., 1995) have a significant cell-autonomous component. *Foxg1*$^{-/-}$ cells located in the ventral telencephalon of chimeras did not express the ventral markers Nkx2.1, Gsh2, Olig2, Mash1 and calbindin, showed expression of Islet1 in only a very few medially-located cases, did not lose their expression of Pax6 throughout the LGE and MGE and did not lose their expression of Tbr1, Tbr2 and Ngn2 in the LGE. They did, however, regain expression of cyclin D1, an early target of Shh signalling, and did not show ectopic expression of p21^{cip1}. Because the delivery of signals sufficient to pattern surrounding wild-type cells does not restore the normal ventral telencephalic phenotype to *Foxg1*$^{-/-}$ cells, we conclude that they have a cell-autonomous selective block to the expression of ventral telencephalic marker genes.

Why are telencephalic cells that lack Foxg1 unable to adopt normal ventral telencephalic phenotypes?

We examined the possibility that unchecked ventral telencephalic expression of Pax6 resulting from the absence of Foxg1 might prevent *Foxg1*$^{-/-}$ ventral telencephalic cells from expressing ventral telencephalic genes. It is known that Pax6 normally acts cell-autonomously to repress ventral telencephalic gene expression in the dorsal telencephalon and that loss of Pax6 leads to a loss of dorsal telencephalic phenotypes at the expense of ventral telencephalic phenotypes (Kroll and O'Leary, 2005; Quinn et al., 2007; Stoykova et al., 2000; Toresson et al., 2000; Yun et al., 2001). We hypothesised that loss of Pax6 might reverse the effects of loss of Foxg1. We found that it had remarkably limited effect: it caused no ventral re-expression of Nkx2.1, Dlx2 and Islet1, only a slight re-expression of Gsh2 and Olig2 and no ventral loss of ectopic Tbr2 expression. It did, however, cause significant re-expression of Mash1. Previous work showed that Gsh2, Dlx2 and Mash1, but not Nkx2.1, are expressed in *Pax6*$^{-/-}$ cells in the dorsal telencephalon of *Pax6*$^{-/-}$ *Pax6*$^{+/+}$ chimeras and that Tbr2 expression is lost in *Pax6*$^{-/-}$ cells in the dorsal telencephalon of *Pax6*$^{-/-}$ *Pax6*$^{+/+}$ chimeras (Quinn et al., 2007). These results, together with our present findings, indicate that loss of telencephalic Mash1 expression in the absence of Foxg1 might be caused by ectopic ventral Pax6 expression. The abnormalities of other ventral telencephalic marker gene expression in *Foxg1*$^{-/-}$ telencephalon (including loss of expression of genes such as Gsh2 and gain of expression of genes such as Tbr2) are not rescued by the loss of Pax6 from *Foxg1*$^{-/-}$ embryos. These experiments also indicate that the ectopic dorsal upregulation of ventral genes and loss of Tbr2 observed in *Pax6*$^{-/-}$ telencephalon (Kroll and O'Leary, 2005; Quinn et al., 2007) require Foxg1 activity.

Previous work showed that in *Foxg1*$^{-/-}$ mouse embryos, expression of telencephalic *Bmp4* and of a gene activated by Bmp4, *p21*^{cip1}, expands ventrally from the normal dorsally restricted domains (Dou et al., 1999; Hanashima et al., 2002; Seoane et al., 2004). Our finding that p21^{cip1} is not upregulated in *Foxg1*$^{-/-}$ telencephalic cells in chimeras is interesting as it suggests that the Smad3 intracellular signalling pathway is not constitutively active in these cells. This possibility was hypothesised because, in various

cell lines, Foxg1 can bind to the Smad3-Smad4-FoxO complex to inhibit its transcriptional activity, including its activation of *p21*^{cip1} (Seoane et al., 2004). This suggests that the expanded domain of p21^{cip1} expression in *Foxg1*$^{-/-}$ embryos might require higher levels of activation from overexpressed Tgfβ family members than is achieved in the chimeric telencephalon, where many cells are wild-type. Exploration of interspecies differences in the telencephalic actions of Foxg1 is likely to be fruitful as zebrafish embryos do not show telencephalic upregulation of Bmp expression in response to depletion of Foxg1 (Danesin et al., 2009).

Acknowledgements
We thank K. Campbell, D. Anderson, D. Rowitch, J. Kohtz, Y. Sasai, R. Hevner and the Developmental Studies Hybridoma Bank for provision of antibodies; J. Hebert, S. McConnell, E. Lai and L. Shen for provision of transgenic mouse lines; R.E. Hill, G. R. Martin, W. D. Richardson, S. Vainio and F. J. Livesey for in situ probes; J. Hebert and T. Theil for advice on setting up the explant culture system; K. Gillies, R. Smith, M. Molinek, L. Leon and animal house staff for expert technical assistance; and members of the Price and Mason laboratories for advice and encouragement. This work was funded by the Wellcome Trust, MRC and BBSRC. Deposited in PMC for release after 6 months.

Competing interests statement
The authors declare no competing financial interests.

Supplementary material
Supplementary material for this article is available at
http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.039800/-/DC1

References
Ahlgren, S., Vogt, P. and Bronner-Fraser, M. (2003). Excess FoxG1 causes overgrowth of the neural tube. *J. Neurobiol.* **57**, 337-349.
Anderson, S. A., Eisenstat, D. D., Shi, L. and Rubenstein, J. L. (1997). Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. *Science* **278**, 474-476.
Bai, C. B., Auerbach, W., Lee, J. S., Stephen, D. and Joyner, A. L. (2002). Gli2, but not Gli1, is required for initial Shh signaling and ectopic activation of the Shh pathway. *Development* **129**, 4753-4761.
Bai, C. B., Stephen, D. and Joyner, A. L. (2004). All mouse ventral spinal cord patterning by hedgehog is Gli dependent and involves an activator function of Gli3. *Dev. Cell* **6**, 103-115.
Bastida, M. F., Delgado, M. D., Wang, B., Fallon, J. F., Fernandez-Teran, M. and Ros, M. A. (2004). Levels of Gli3 repressor correlate with Bmp4 expression and apoptosis during limb development. *Dev. Dyn.* **231**, 148-160.
Bourguignon, C., Li, J. and Papalopulu, N. (1998). XBF-1, a winged helix transcription factor with dual activity, has a role in positioning neurogenesis in Xenopus competent ectoderm. *Development* **125**, 4889-4900.
Chambers, D. and Mason, I. (2000). Expression of sprouty2 during early development of the chick embryo is coincident with known sites of FGF signalling. *Mech. Dev.* **91**, 361-364.
Chellaiah, A., Yuan, W., Chellaiah, M. and Ornitz, D. M. (1999). Mapping ligand binding domains in chimeric fibroblast growth factor receptor molecules. Multiple regions determine ligand binding specificity. *J. Biol. Chem.* **274**, 34785-34794.
Chiang, C., Litingtung, Y., Lee, E., Young, K. E., Corden, J. L., Westphal, H. and Beachy, P. A. (1996). Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function. *Nature* **383**, 407-413.
Corbin, J. G., Rutlin, M., Gaiano, N. and Fishell, G. (2003). Combinatorial function of the homeodomain proteins Nkx2.1 and Gsh2 in ventral telencephalic patterning. *Development* **130**, 4895-4906.
Danesin, C., Peres, J. N., Johansson, M., Snowden, V., Cording, A., Papalopulu, N. and Houart, C. (2009). Integration of telencephalic Wnt and hedgehog signaling center activities by Foxg1. *Dev. Cell* **16**, 576-587.
Dou, C. L., Li, S. and Lai, E. (1999). Dual role of brain factor-1 in regulating growth and patterning of the cerebral hemispheres. *Cereb. Cortex* **9**, 543-550.
Englund, C., Fink, A., Lau, C., Pham, D., Daza, R. A., Bulfone, A., Kowalczyk, T. and Hevner, R. F. (2005). Pax6, Tbr2, and Tbr1 are expressed sequentially by radial glia, intermediate progenitor cells, and postmitotic neurons in developing neocortex. *J. Neurosci.* **25**, 247-251.
Ericson, J., Muhr, J., Jessell, T. M. and Edlund, T. (1995). Sonic hedgehog: a common signal for ventral patterning along the rostrocaudal axis of the neural tube. *Int. J. Dev. Biol.* **39**, 809-816.
Estivill-Torrus, G., Pearson, H., van Heyningen, V., Price, D. J. and Rashbass, P. (2002). Pax6 is required to regulate the cell cycle and the rate of progression from symmetrical to asymmetrical division in mammalian cortical progenitors. *Development* **129**, 455-466.
Fode, C., Ma, Q., Casarosa, S., Ang, S. L., Anderson, D. J. and Guillemot, F. (2000). A role for neural determination genes in specifying the dorsoventral identity of telencephalic neurons. *Genes Dev.* **14**, 67-80.
Fotaki, V., Yu, T., Zaki, P. A., Mason, J. O. and Price, D. J. (2006). Abnormal positioning of diencephalic cell types in neocortical tissue in the dorsal telencephalon of mice lacking functional Gli3. *J. Neurosci.* **26**, 9282-9292.
Fuccillo, M., Rallu, M., McMahon, A. P. and Fishell, G. (2004). Temporal requirement for hedgehog signaling in ventral telencephalic patterning. *Development* **131**, 5031-5040.
Fuccillo, M., Joyner, A. L. and Fishell, G. (2006a). Morphogen to mitogen: the multiple roles of hedgehog signalling in vertebrate neural development. *Nat. Rev. Neurosci.* **7**, 772-783.
Fuccillo, M., Rutlin, M. and Fishell, G. (2006b). Removal of Pax6 partially rescues the loss of ventral structures in Shh null mice. *Cereb. Cortex* **16 Suppl. 1**, i96-i102.
Goodrich, L. V., Johnson, R. L., Milenkovic, L., McMahon, J. A. and Scott, M. P. (1996). Conservation of the hedgehog/patched signaling pathway from flies to mice: induction of a mouse patched gene by Hedgehog. *Genes Dev.* **10**, 301-312.
Gutin, G., Fernandes, M., Palazzolo, L., Paek, H., Yu, K., Ornitz, D. M., McConnell, S. K. and Hebert, J. M. (2006). FGF signalling generates ventral telencephalic cells independently of SHH. *Development* **133**, 2937-2946.
Hanashima, C., Shen, L., Li, S. C. and Lai, E. (2002). Brain factor-1 controls the proliferation and differentiation of neocortical progenitor cells through independent mechanisms. *J. Neurosci.* **22**, 6526-6536.
Hanashima, C., Li, S. C., Shen, L., Lai, E. and Fishell, G. (2004). Foxg1 suppresses early cortical cell fate. *Science* **303**, 56-59.
Hanashima, C., Fernandes, M., Hebert, J. M. and Fishell, G. (2007). The role of Foxg1 and dorsal midline signaling in the generation of Cajal-Retzius subtypes. *J. Neurosci.* **27**, 11103-11111.
Hardcastle, Z. and Papalopulu, N. (2000). Distinct effects of XBF-1 in regulating the cell cycle inhibitor p27(XIC1) and imparting a neural fate. *Development* **127**, 1303-1314.
Hebert, J. M. (2005). Unraveling the molecular pathways that regulate early telencephalon development. *Curr. Top. Dev. Biol.* **69**, 17-37.
Hebert, J. M. and Fishell, G. (2008). The genetics of early telencephalon patterning: some assembly required. *Nat. Rev. Neurosci.* **9**, 678-685.
Hevner, R. F., Shi, L., Justice, N., Hsueh, Y., Sheng, M., Smiga, S., Bulfone, A., Goffinet, A. M., Campagnoni, A. T. and Rubenstein, J. L. (2001). Tbr1 regulates differentiation of the preplate and layer 6. *Neuron* **29**, 353-366.
Hevner, R. F., Neogi, T., Englund, C., Daza, R. A. and Fink, A. (2003). Cajal-Retzius cells in the mouse: transcription factors, neurotransmitters, and birthdays suggest a pallial origin. *Brain Res. Dev. Brain Res.* **141**, 39-53.
Hevner, R. F., Daza, R. A., Englund, C., Kohtz, J. and Fink, A. (2004). Postnatal shifts of interneuron position in the neocortex of normal and reeler mice: evidence for inward radial migration. *Neuroscience* **124**, 605-618.
Huh, S., Hatini, V., Marcus, R. C., Li, S. C. and Lai, E. (1999). Dorsal-ventral patterning defects in the eye of BF-1-deficient mice associated with a restricted loss of shh expression. *Dev. Biol.* **211**, 53-63.
Jacob, J. and Briscoe, J. (2003). Gli proteins and the control of spinal-cord patterning. *EMBO Rep.* **4**, 761-765.
Kenney, A. M. and Rowitch, D. H. (2000). Sonic hedgehog promotes G(1) cyclin expression and sustained cell cycle progression in mammalian neuronal precursors. *Mol. Cell. Biol.* **20**, 9055-9067.
Kohtz, J. D., Baker, D. P., Corte, G. and Fishell, G. (1998). Regionalization within the mammalian telencephalon is mediated by changes in responsiveness to Sonic Hedgehog. *Development* **125**, 5079-5089.
Kroll, T. T. and O'Leary, D. D. (2005). Ventralized dorsal telencephalic progenitors in Pax6 mutant mice generate GABA interneurons of a lateral ganglionic eminence fate. *Proc. Natl. Acad. Sci. USA* **102**, 7374-7379.
Kuschel, S., Ruther, U. and Theil, T. (2003). A disrupted balance between Bmp/Wnt and Fgf signaling underlies the ventralization of the Gli3 mutant telencephalon. *Dev. Biol.* **260**, 484-495.
Lee, J., Platt, K. A., Censullo, P. and Ruiz i Altaba, A. (1997). Gli1 is a target of Sonic hedgehog that induces ventral neural tube development. *Development* **124**, 2537-2552.
Ligon, K. L., Alberta, J. A., Kho, A. T., Weiss, J., Kwaan, M. R., Nutt, C. L., Louis, D. N., Stiles, C. D. and Rowitch, D. H. (2004). The oligodendroglial lineage marker OLIG2 is universally expressed in diffuse gliomas. *J. Neuropathol. Exp. Neurol.* **63**, 499-509.
Lo, L., Dormand, E., Greenwood, A. and Anderson, D. J. (2002). Comparison of the generic neuronal differentiation and neuron subtype specification functions of mammalian achaete-scute and atonal homologs in cultured neural progenitor cells. *Development* **129**, 1553-1567.
Lu, Q. R., Yuk, D., Alberta, J. A., Zhu, Z., Pawlitzky, I., Chan, J., McMahon, A. P., Stiles, C. D. and Rowitch, D. H. (2000). Sonic hedgehog-regulated oligodendrocyte lineage genes encoding bHLH proteins in the mammalian central nervous system. *Neuron* **25**, 317-329.
Marigo, V. and Tabin, C. J. (1996). Regulation of patched by sonic hedgehog in the developing neural tube. *Proc. Natl. Acad. Sci. USA* **93**, 9346-9351.

Marigo, V., Johnson, R. L., Vortkamp, A. and Tabin, C. J. (1996). Sonic hedgehog differentially regulates expression of GLI and GLI3 during limb development. *Dev. Biol.* **180**, 273-283.
Martynoga, B., Morrison, H., Price, D. J. and Mason, J. O. (2005). Foxg1 is required for specification of ventral telencephalon and region-specific regulation of dorsal telencephalic precursor proliferation and apoptosis. *Dev. Biol.* **283**, 113-127.
Muzio, L. and Mallamaci, A. (2005). Foxg1 confines Cajal-Retzius neuronogenesis and hippocampal morphogenesis to the dorsomedial pallium. *J. Neurosci.* **25**, 4435-4441.
Nery, S., Wichterle, H. and Fishell, G. (2001). Sonic hedgehog contributes to oligodendrocyte specification in the mammalian forebrain. *Development* **128**, 527-540.
Ohkubo, Y., Chiang, C. and Rubenstein, J. L. (2002). Coordinate regulation and synergistic actions of BMP4, SHH and FGF8 in the rostral prosencephalon regulate morphogenesis of the telencephalic and optic vesicles. *Neuroscience* **111**, 1-17.
Oliver, T. G., Grasfeder, L. L., Carroll, A. L., Kaiser, C., Gillingham, C. L., Lin, S. M., Wickramasinghe, R., Scott, M. P. and Wechsler-Reya, R. J. (2003). Transcriptional profiling of the Sonic hedgehog response: a critical role for N-myc in proliferation of neuronal precursors. *Proc. Natl. Acad. Sci. USA* **100**, 7331-7336.
Ornitz, D. M., Xu, J., Colvin, J. S., McEwen, D. G., MacArthur, C. A., Coulier, F., Gao, G. and Goldfarb, M. (1996). Receptor specificity of the fibroblast growth factor family. *J. Biol. Chem.* **271**, 15292-15297.
Quinn, J. C., Molinek, M., Martynoga, B. S., Zaki, P. A., Faedo, A., Bulfone, A., Hevner, R. F., West, J. D. and Price, D. J. (2007). Pax6 controls cerebral cortical cell number by regulating exit from the cell cycle and specifies cortical cell identity by a cell autonomous mechanism. *Dev. Biol.* **302**, 50-65.
Quinn, J. C., Molinek, M., Mason, J. O. and Price, D. J. (2009). Gli3 is required autonomously for dorsal telencephalic cells to adopt appropriate fates during embryonic forebrain development. *Dev. Biol.* **327**, 204-215.
Rallu, M., Corbin, J. G. and Fishell, G. (2002a). Parsing the prosencephalon. *Nat. Rev. Neurosci.* **3**, 943-951.
Rallu, M., Machold, R., Gaiano, N., Corbin, J. G., McMahon, A. P. and Fishell, G. (2002b). Dorsoventral patterning is established in the telencephalon of mutants lacking both Gli3 and Hedgehog signaling. *Development* **129**, 4963-4974.
Rash, B. G. and Grove, E. A. (2007). Patterning the dorsal telencephalon: a role for sonic hedgehog? *J. Neurosci.* **27**, 11595-11603.
Ruiz i Altaba, A. (1998). Combinatorial Gli gene function in floor plate and neuronal inductions by Sonic hedgehog. *Development* **125**, 2203-2212.
Sansom, S. N., Hebert, J. M., Thammongkol, U., Smith, J., Nisbet, G., Surani, M. A., McConnell, S. K. and Livesey, F. J. (2005). Genomic characterisation of a Fgf-regulated gradient-based neocortical protomap. *Development* **132**, 3947-3961.
Schweitzer, R., Vogan, K. J. and Tabin, C. J. (2000). Similar expression and regulation of Gli2 and Gli3 in the chick limb bud. *Mech. Dev.* **98**, 171-174.
Seoane, J., Le, H. V., Shen, L., Anderson, S. A. and Massague, J. (2004). Integration of Smad and forkhead pathways in the control of neuroepithelial and glioblastoma cell proliferation. *Cell* **117**, 211-223.
Shanmugalingam, S., Houart, C., Picker, A., Reifers, F., Macdonald, R., Barth, A., Griffin, K., Brand, M. and Wilson, S. W. (2000). Ace/Fgf8 is required for forebrain commissure formation and patterning of the telencephalon. *Development* **127**, 2549-2561.
Shinya, M., Koshida, S., Sawada, A., Kuroiwa, A. and Takeda, H. (2001). Fgf signalling through MAPK cascade is required for development of the subpallial telencephalon in zebrafish embryos. *Development* **128**, 4153-4164.
Stenman, J., Yu, R. T., Evans, R. M. and Campbell, K. (2003). Tlx and Pax6 co-operate genetically to establish the pallio-subpallial boundary in the embryonic mouse telencephalon. *Development* **130**, 1113-1122.
Storm, E. E., Rubenstein, J. L. and Martin, G. R. (2003). Dosage of Fgf8 determines whether cell survival is positively or negatively regulated in the developing forebrain. *Proc. Natl. Acad. Sci. USA* **100**, 1757-1762.
Storm, E. E., Garel, S., Borello, U., Hebert, J. M., Martinez, S., McConnell, S. K., Martin, G. R. and Rubenstein, J. L. (2006). Dose-dependent functions of Fgf8 in regulating telencephalic patterning centers. *Development* **133**, 1831-1844.
Stoykova, A., Treichel, D., Hallonet, M. and Gruss, P. (2000). Pax6 modulates the dorsoventral patterning of the mammalian telencephalon. *J. Neurosci.* **20**, 8042-8050.
Sur, M. and Rubenstein, J. L. (2005). Patterning and plasticity of the cerebral cortex. *Science* **310**, 805-810.
Sussel, L., Marin, O., Kimura, S. and Rubenstein, J. L. (1999). Loss of Nkx2.1 homeobox gene function results in a ventral to dorsal molecular respecification within the basal telencephalon: evidence for a transformation of the pallidum into the striatum. *Development* **126**, 3359-3370.
Toresson, H., Potter, S. S. and Campbell, K. (2000). Genetic control of dorsal-ventral identity in the telencephalon: opposing roles for Pax6 and Gsh2. *Development* **127**, 4361-4371.
Vokes, S. A., Ji, H., McCuine, S., Tenzen, T., Giles, S., Zhong, S., Longabaugh, W. J., Davidson, E. H., Wong, W. H. and McMahon, A. P. (2007). Genomic characterization of Gli-activator targets in sonic hedgehog-mediated neural patterning. *Development* **134**, 1977-1989.
Walshe, J. and Mason, I. (2003). Fgf signalling is required for formation of cartilage in the head. *Dev. Biol.* **264**, 522-536.
Wang, B., Fallon, J. F. and Beachy, P. A. (2000). Hedgehog-regulated processing of Gli3 produces an anterior/posterior repressor gradient in the developing vertebrate limb. *Cell* **100**, 423-434.
Wang, H. F. and Liu, F. C. (2001). Developmental restriction of the LIM homeodomain transcription factor Islet-1 expression to cholinergic neurons in the rat striatum. *Neuroscience* **103**, 999-1016.
West, J. D. and Flockhart, J. H. (1994). Genotypically unbalanced diploid<==>diploid foetal mouse chimaeras: possible relevance to human confined mosaicism. *Genet Res.* **63**, 87-99.
Wijgerde, M., McMahon, J. A., Rule, M. and McMahon, A. P. (2002). A direct requirement for Hedgehog signaling for normal specification of all ventral progenitor domains in the presumptive mammalian spinal cord. *Genes Dev.* **16**, 2849-2864.
Wilson, S. W. and Houart, C. (2004). Early steps in the development of the forebrain. *Dev. Cell* **6**, 167-181.
Xuan, S., Baptista, C. A., Balas, G., Tao, W., Soares, V. C. and Lai, E. (1995). Winged helix transcription factor BF-1 is essential for the development of the cerebral hemispheres. *Neuron* **14**, 1141-1152.
Yun, K., Potter, S. and Rubenstein, J. L. (2001). Gsh2 and Pax6 play complementary roles in dorsoventral patterning of the mammalian telencephalon. *Development* **128**, 193-205.
Zaki, P. A., Martynoga, B., Delafield-Butt, J. T., Fotaki, V., Yu, T. and Price, D. J. (2005). Loss of Gli3 enhances the viability of embryonic telencephalic cells in vitro. *Eur. J. Neurosci.* **22**, 1547-1551.
Zhang, S., Lin, Y., Itaranta, P., Yagi, A. and Vainio, S. (2001). Expression of Sprouty genes 1, 2 and 4 during mouse organogenesis. *Mech. Dev.* **109**, 367-370.

Table S1. RT-PCR primer sequences used in this study

	Forward Primer	Reverse Primer
Beta-actin	CCTGTCAGCAATGCCTGGGT	CCAGCCTTCCTTCTTGGGTA
Gapdh	GGGTGTGAACCACGAGAAAT	CCTTCCACAATGCCAAAGTT
Ptc1	CTGGGAGGAAATGCTGAATA	GTTTTCCAGTGGCATTCTTG
Smo	GTTCGTGGTCCTCACCTATG	TCACGGAGTCTCCATCTACC
Gli1	GTTATGGAGCAGCCAGAGAG	GAGTTGATGAAAGCCACCAG
Gli3	ACACAGCCCTCCTCTCATC	CATCAGGCTTGATCTTGGAC
cyclin D1	CGCCCTCCGTATCTTACTTC	CTCTTCTTCAAGGGCTCCA
Nkx2.1	GAAAGACAGCATCAGCTTCC	CCATGCCCACTTTCTTGTAG
sprouty 2	AGAAGAGGATTCAAGGGAGA	AGACAAGACGTGTACCTGCT
Etv5	GGTTAGCTGAAGCACAAGTT	TTGTAGAGGCACTTTTCTCC
Mash1	CTGGACTTTACCAACTGGTTC	ATGCAGAGACACTGTTGGAG
Dlx2	CCTCAACAATGTCTCCTACTC	CTGGAAACTGGAGTAGATGGT