Supplementary Figure 1: Neurospheres induced by SHH from E9.5 mouse CNS and generated from the chick CNS are multipotent.

a, E6 telencephalon (DT) and spinal cord (SC) neurospheres from chick embryos were differentiated for 5 days then assayed by immunohistochemistry for the indicated molecular markers. Multipotentiality was confirmed by the presence of neurons, oligodendrocytes and astrocytes. b, Mouse E9.5 DT tissue cultured in the presence of SHH generated neurospheres. After differentiation, immunohistochemistry confirmed the presence of neurons, oligodendrocytes and astrocytes, consistent with the presence of multipotent NSC in these neurospheres. In both a and b, neurons were identified by TuJ1, oligodendrocytes by CNPase and astrocytes by GFAP. c, Quantitation of the differentiated cell types generated in the indicated cultures. At E9.5, no neurospheres form from mouse DT and the differentiation of single cells was assayed. At E10.5, small neurospheres form, but the potential of each neurosphere was mainly neurogeneic. At E11.5 onwards, neurospheres were multipotent and generated large numbers of glia cells.
Supplementary Figure 2: SOX9 is expressed in NS5 cells and neurospheres, whereas SOX10 is expressed in neurospheres but not in NS5 cells.

a, Immunohistochemistry of NS5 cells revealed that these cells express SOX9 and SOX2, but do not express SOX10. b, SOX9 and SOX10 are expressed within proliferating E14.5 dorsal telencephalon (DT) neurospheres.
Supplementary Figure 3: The expression of SoxE mRNA coincides with the timing of neurosphere formation in the dorsal telencephalon.

In situ hybridization for Sox2, Sox8, Sox9 and Sox10 was carried out on dorsal telencephalon sections between E9.5 and E14.5. Sox2 was expressed at all stages. Sox9 and Sox10 were expressed in a small number of cells at E10.5 and then strongly expressed after E11.5. Sox8 was expressed from E11.5 onwards.
Supplementary Figure 4: SOX9 is co-expressed with SOX2 after E10.5 in the developing CNS.

Immunohistochemistry on horizontal sections for SOX2 and SOX9 revealed that SOX2 was expressed at all stages from E9.5-18.5 in the dorsal telencephalon (top is rostral, and bottom is caudal) (a) and spinal cord (top is dorsal and bottom is ventral) (b). SOX9 was absent at E9.5 and expressed in only a small number of cells at E10.5, but in many progenitors from E11.5. From E10.5, many of SOX2+ cells co-express SOX9 (a, b).
Supplementary Figure 5: The pZ/Sox9 expression construct.
The pCAGGS enhancer/promoter drives ubiquitous expression of downstream sequences. The construct contains βgeo followed by three copies of the SV40 polyadenylation signal, all flanked by loxP sites. This is removed by Cre excision, which then allows expression of the second gene comprising mouse Sox9 followed by IRES2-EGFP.
Supplementary Figure 6: Sox10 expression is upregulated in neurospheres generated from Sox9 null CNS. SoxE genes are sufficient to induce precocious neurosphere generation.

a, Embryos lacking Sox9 were generated by cre-mediated recombination. RT-PCR was carried out from cultures 10 days after dissection of E14.5 Sox9 null mutants (null) and wild type litter mates (WT). No Sox9 was expressed after dissection of null mutants but Sox10 expression was increased. b, At E11.5 the dorsal telencephalon and spinal cord were dissected from Sox9 null mutants (null) and wild type (WT) littermates. After 10 days in culture, neuropheres were differentiated for 5 days and assayed by immunohistochemistry. As expected, SOX9 was absent from cultures derived from null mutants, however the proportion of SOX10-positive cells was markedly increased. c, In ovo electroporation of E3 chick spinal cord with control pCAGGS-IRES-nls-GFP (GFP) or a Sox9 construct lacking the transactivation domain (d/n S9) generated few if any neurospheres. d, In ovo electroporation of E3 chick spinal cord with pCAGGS-Sox9-IRES-nls-GFP (Sox9), pCAGGS-Sox10-IRES-nls-GFP (Sox10), pCAGGS-Sox8-IRES-nls-GFP (Sox8) or a version of Sox9 containing the heterologous VP16 transactivation domain (VP16-S9) was sufficient to induce precocious neurosphere formation. (Student’s t-test; *P<0.003, **P<0.002, ***P<0.003, ****P<0.003)
Supplementary Figure 7: Sox9 is necessary for neurosphere formation.

a, b, Schematic of the Flox-Sox9 engineered allele. Null Sox9 embryos were generated by Cre-mediated recombination in the parental germlines. Spinal cord (SC) and dorsal telencephalon (DT) were dissected at E11.5. Sox9 null tissue from both regions gave rise to fewer neurospheres than controls and many cells attached to the culture dish and differentiated (b), scale bars = 50μm. c, Quantitation of the number of neurospheres generated. In DT cultures, fewer than half the WT number of neurospheres were generated from Sox9 null cultures (P<0.008). These also gave far fewer secondary neurospheres. In Sox9 null SC cultures there was a seven-fold decrease in primary neurosphere generation versus WT (P<0.001), and a four-fold decrease in secondary neurosphere formation (n=5). There was also a significant effect in Sox9 heterozygote embryos. Bars indicate standard error.
Supplementary Figure 8: *Sox9* is required for multipotentiality in vivo and in vitro. Embryos lacking *Sox9* throughout the developing CNS were generated by nestin Cre-mediated recombination. a, *In vivo* quantitation of the indicated markers at E18.5 in the cortex of *Sox9* null embryos showed a complete loss of GFAP+ astrocytes and a reduction, compared to wild type littermates (WT), of TuJ1 positive neurons in the subependymal zone. By contrast, PSA-NCAM+ neuroblasts were increased in null brains (null). b, Quantitation of the indicated makers of *in vitro* DT cultures generated from *Sox9* null DT tissue after 5 days of differentiation. Cultures from *Sox9* null DT generated markedly reduced numbers of GFAP+ astrocytes, CNPase+ oligodendrocytes and TuJ1+ neurons compared to wild type littermates (WT).
Supplementary Figure 9: Proliferation is decreased in CNS tissue lacking Sox9. Embryos null for Sox9 throughout the developing CNS were generated by nestin Cre-mediated recombination. a, The number of Ki67 positive cells were reduced in the dorsal telencephalon of both E14.5 and E18.5 Flox-Sox9\textsuperscript{hom};Nestin Cre (null) mice compared to wild type (WT) littermates. There was no difference in the number of PARP positive cells between the null and WT mice at either developmental stage. Scale bars = 20µm. b, Quantification of the percentage of DAPI positive cells, expressing either Ki67 or PARP at E14.5 and E18.5. (Student’s t-test; *P<0.015, **P<0.45, ***P<0.013, ****P<0.43, n=5).
Supplementary Figure 10: SHH signaling induces SoxE genes.
RT-PCR demonstrates the induction of SoxE genes in E9.5 DT (a) and SC (b) cells after the addition of SHH for 18 (ii) or 24 (iii) hours compared to those exposed only to a control protein (i). Sox2 was expressed with or without addition of SHH. Actb (Actin) was used as a loading control for input RNA levels.
Supplementary Figure 11: Sox9 is necessary for the maintenance of ependymal cells in vivo in the adult.

Lateral ventricles of R26R<sup>EYFP</sup> and Flox-Sox9<sup>hom</sup>; R26R<sup>EYFP</sup> adult mice injected with an adenovirus expressing Cre were analysed 4 weeks post infection. GFP positive cells identify cells in which Cre-mediated recombination had taken place. a, Cells in the rostral migratory stream (RMS). GFP cells in the R26R<sup>EYFP</sup> mice gave rise to a few PSA-NCAM and DCX expressing neuroblasts. By contrast, GFP cells in the Flox-Sox9<sup>hom</sup>; R26R<sup>EYFP</sup> mice gave rise to many PSA-NCAM and DCX positive neuroblasts. Scale bars = 10µm.

b, The ciliary marker Arl13b was detected on very few GFP positive ependymal cells in the Flox-Sox9<sup>hom</sup>; R26R<sup>EYFP</sup> mice, whereas the majority of GFP positive ependymal cells in control R26R<sup>EYFP</sup> mice expressed Arl13b. Scale bars = 20µm. c, Quantification of the proportion of PSA-NCAM neuroblasts that express GFP in the SEZ and ependymal regions of R26R<sup>EYFP</sup> and Flox-Sox9<sup>hom</sup>; R26R<sup>EYFP</sup> (hom) mice. In R26R<sup>EYFP</sup> mice, all cells that co-express GFP and PSA-NCAM were located in the SEZ, whereas in the hom mouse many more GFP, PSA-NCAM double positive neuroblasts were located in the ependymal zone. d, GFP positive cells lining the lateral ventricle 2 weeks after injection with the cre virus in R26R<sup>EYFP</sup> mice.
Supplementary Figure 12: Sox9 is necessary for ependymal cell formation. Embryos in which Sox9 had been deleted throughout the developing CNS were generated by nestin Cre-mediated recombination of the Flox-Sox9 targeted allele. Coronal sections were analysed at E18.5 (a) and P0 (b). Ependymal cells were detected by CD133 immunoreactivity. At E18.5 and P0 ependymal cells were found surrounding the lateral ventricle (demarcated by the white dotted line) in the wild type (WT) mice, however CD133 positive cells were not detected in the Flox-Sox9^{hom}; Nestin Cre littermates. Scale bars = 10µm.
Supplementary Figure 13: There is no significant difference between the types of unrecombined cells in the R26REYFP and Flox-Sox9hom; R26REYFP olfactory bulbs after Cre-mediated recombination. R26REYFP and Flox-Sox9hom; R26REYFP adult (8-10 week) mice were injected in one lateral ventricle with an adenovirus expressing Cre. BrdU injections were carried out 3 weeks after the initial injection with the Cre virus. The olfactory bulbs of these mice were analysed 6 weeks after the injection. Confocal images were taken through a section of the olfactory bulbs to assess the overall numbers of BrdU label-retaining cells as well as mature neurons (NeuN) and neuroblasts (PSA-NCAM) throughout the granule cell layer (a). b, the percentage of each cell type was calculated in each section of the olfactory bulb. There was no significant difference between the overall numbers of BrdU label-retaining cells, NeuN positive cells or PSA-NCAM positive cells between sections taken from R26REYFP and Flox-Sox9hom; R26REYFP (hom) mice (Student’s t-test: *P< 0.6, ** P< 0.9, ***P< 0.6). n= 5, error bars indicate standard error. c, Illustration of a sagittal section showing the rostral migratory stream (RMS) from the lateral ventricle (LV) to the olfactory bulb. Coronal section through the olfactory bulb (vertical line in c) is represented in d.