Supplemental Information

Supplementary methods:

Primary cell culture

For neocortical cell culture, E13.5 cerebral hemispheres were dissected in Earles Buffered Saline Solution(EBSS) without Ca^{2+} or Mg^{2+} and incubated in EBSS with dispase (1 mg/ml) and DNase 1 for ~10 minutes at room temperature (20-23℃), then the ganglionic eminences were removed with forceps. Isolated cortices were digested in 2 ml of EBSS with 0.01% (w/v) Trypsin and DNase1 for 45 minutes at 37°C. 3 x 10^5 cells were plated in 350 μl defined medium ("Sato's medium";

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. ELISA assay of OLIG2 $^{ph\text{-}S147}$ phosphorylation-specific antibody. OLIG2 peptide (residues 137 to150), either phosphorylated on S147 (red) or not (yellow), were coated onto 96-well microtiter plates. Purified anti-OLIG2$^{ph\text{-}S147}$ antibody was applied at a range of concentrations and optical density (OD) was read at 450 nm.

Supplementary Figure S2. A. S147A mutation did not alter OLIG2 binding to MASH1 or NKX2.2. Myc-tagged OLIG2^{WT} or OLIG2

Supplementary Figure S5. P19-OLIG2^{S147A} and P19-OLIG2^{WT} cells were treated with retinoic acid (RA) and the Sonic hedgehog agonist SHHAg1.2. The OLIG2^{S147A} mutation strongly enhanced expression of the differentiation marker MBP. However, morphological differentiation (formation of multi-process bearing cells) was not observed over the time course of the experiment.

Supplementary Figure S6. NGN2-mediated transcription of a luciferase reporter from the HB9 promoter was repressed by OLIG2. The luciferase assay was performed with cell lysates from transfected P19 cells. pCDNA empty vector was used to normalize the amounts of transfected DNA. Results are the mean ± s.e.m. of three independent experiments. OLIG2^{S147A} displayed increased repressive activity compared to OLIG2^{WT}.

Supplementary Figure S7. Dissociated cells from mouse E13.5 neocortex were cultured in defined medium in the presence of 10 ng/ml FGF2 for three days. Various concentrations of PKA activators forskolin (A-D) or dibutyryl-cAMP (E-H) were added. Cells were fixed and immuno-labelled with anti-NG2 (green) and anti-OLIG2 (red). Cells in two randomly selected fields on each of three microscope slides were counted under the 40X objective. The proportions of labelled cells were plotted as mean ± s.e.m. (I, J). Statistical comparisons were by one-way ANOVA (* = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$). The results shown here were from one of at least two independent experiments, all of which gave similar results.

Supplementary references

Bottenstein,J.E., and Sato,G.H. (1979). Growth of a rat neuroblastoma cell line in serum-free supplemented medium. Proc. Natl. Acad. Sci. USA *76*, 514-517.

Lee,S.K., Lee,B., Ruiz,E.C., and Pfaff,S.L. (2005). Olig2 and Ngn2 function in opposition to modulate gene expression in motor neuron progenitor cells. Genes Dev. *19*, 282-294.