Published in final edited form as:
Neuron. 2014 February 5; 81(3): 574–587. doi:10.1016/j.neuron.2013.11.024.

Olig1 function is required to repress *Dlx1/2* and interneuron production in mammalian brain

John C. Silbereis[1,2,3,6], **Hiroko Nobuta**[1,2,6], **Hui-Hsin Tsai**[1,2,6], **Vivi M. Heine**[1,2], **Gabriel L. McKinsey**[3,4], **Dimphna H. Meijer**[5], **MacKenzie A. Howard**[2], **Magda A. Petryniak**[1,^], **Gregory B. Potter**[1,^], **John A. Alberta**[5], **Scott C. Baraban**[2], **Charles D. Stiles**[5], **John L.R. Rubenstein**[4], and **David H. Rowitch**[1,2,6,*]

[1]Department of Pediatrics, Eli and Edythe Broad Institute for Stem Cell Research and Regeneration Medicine

[2]Department of Neurosurgery, University of California San Francisco, 513 Parnassus Avenue, San Francisco, CA, 94143 USA

[3]Neuroscience Graduate Program, University of California San Francisco, 513 Parnassus Avenue, San Francisco, CA, 94143 USA

[4]Department of Psychiatry, University of California San Francisco, 513 Parnassus Avenue, San Francisco, CA, 94143 USA

[5]Department of Cancer Biology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MS 02115

[6]Howard Hughes Medical Institute, 4000 Jones Bridge Road, Chevy Chase, MD 20815

Summary

Abnormal GABAergic interneuron density, and imbalance of excitatory versus inhibitory tone, is thought to result in epilepsy, neurodevelopmental disorders and psychiatric disease. Recent studies indicate that interneuron cortical density is determined primarily by the size of the precursor pool in the embryonic telencephalon. However, factors essential to regulate interneuron allocation from telencephalic multipotent precursors are poorly understood. Here we report that *Olig1* represses production of GABAergic interneurons throughout the mouse brain. *Olig1* deletion in mutant mice results in ectopic expression and upregulation of *Dlx1/2* genes in the ventral medial ganglionic eminences and adjacent regions of the septum resulting in a ~30% increase in adult cortical interneuron numbers. We show that Olig1 directly represses the *Dlx1/2 I12b* intergenic enhancer and that *Dlx1/2* functions genetically downstream of *Olig1*. These findings establish *Olig1* as an essential repressor of *Dlx1/2* and interneuron production in developing mammalian brain.

[*]Author for correspondence. David H. Rowitch, MD, PhD, Departments of Pediatrics and Neurosurgery and Howard, Hughes Medical Institute, University of California San Francisco, 533 Parnassus Avenue, San Francisco, CA, 94143, Tele: (415) 476-7242; rowitchd@peds.ucsf.edu.
[#]Present address: Department of Child Neurology, Center for Neurogenomics and Cognitive Research, VU University Medical Center, Amsterdam, NL
[^]Present address: Department of Pediatrics, Oregon Health & Science University, P.O. Box 574Mail Code L481, Portland, OR 97239

Keywords

Olig1; interneuron; Dlx2; Down Syndrome; CNS development; GABA; telencephalon; cerebral cortex; pattern formation; oligodendrocyte

Introduction

The balance between excitatory and inhibitory tone in the cerebral cortex is mediated largely by relative activity of excitatory glutamatergic pyramidal cells and inhibitory gamma-aminobutyric acid-containing (GABAergic) local circuit neurons, also known as interneurons (IN). GABAergic INs regulate sensory fields, plasticity and the frequency and tone of cortical oscillatory activity (Alonso and Swadlow, 2005; Kehrer et al., 2008; Lehmann et al., 2012; Lewis et al., 2005; Llinas et al., 2005; Schiller and Tehovnik, 2005). Disruption of excitatory/inhibitory balance is linked to epilepsy, neurodevelopmental and psychiatric disorders (Ben-Ari, 2006; Cobos et al., 2005; Corbin et al., 2001; Han et al., 2012; Hashimoto et al., 2008; Hashimoto et al., 2003; Kehrer et al., 2008; Rossignol, 2011; Rubenstein, 2010; Rubenstein and Merzenich, 2003; Yizhar et al., 2011).

A recent study suggests that the size of the cortical IN population is determined primarily in the early embryo at time of specification, rather than by neurotrophic competition, and programmed cell death later in development (Southwell et al., 2012). Transplanted IN precursors are capable of functional integration into the adult brain (Alvarez-Dolado et al., 2006; Southwell et al., 2010) and can attenuate seizures in rodent models of epilepsy (Baraban et al., 2009; Hunt et al., 2013). Increased IN population size also induces and extends critical periods for ocular dominance plasticity (Southwell et al., 2010). Thus, generating the appropriate number of cortical neurons during development is crucial. However, the factors that normally limit the size of the IN progenitor pool are poorly understood and essential repressors of IN developmental programs have not been described.

Specification of cortical inhibitory neurons from multipotent precursors in the embryonic brain is complex, involving: (1) patterning of spatially discrete progenitor pools for specific subtypes, (2) temporal regulation of multiphase neurogenesis and (3) mechanisms of neuron versus oligodendroglial (OL) cell fate acquisition (Butt et al., 2005; Kessaris et al., 2006; Marin, 2012; Wonders et al., 2008). Cortical inhibitory neurons are produced from E10 to E17 in the medial ganglionic eminence (MGE), anterior entopeduncular area (AEP; a ventral region of the MGE), caudal ganglionic eminence (CGE), and preoptic areas (POA) of the ventral telencephalon; they then migrate tangentially into the cerebral cortex (Anderson et al., 1997; Corbin et al., 2001; Miyoshi et al., 2007; Wonders and Anderson, 2006). Parvalbumin (PV) and calretinin (CR) positive cells are derived relatively late in embryogenesis from progenitor domains that produce both OLs and INs, whereas neuropeptide-Y (NPY) and somatostatin (SST) subtypes are born prior to the onset of OL specification (Kessaris et al., 2006; Miyoshi et al., 2007; Taniguchi et al., 2013; Wonders et al., 2008). In contrast, the adjacent regions of the lateral ganglionic eminence and the telencephalic septum generate neurons of the ventral forebrain and olfactory bulb, but are not thought to give rise to cortical INs (He et al., 2001; Kessaris et al., 2006; Petryniak et al., 2007; Rubin et al., 2010). *Dlx1/2* function is necessary for the establishment of IN cell production within these regions and differentiation into GABAergic INs (Anderson et al., 1997). Though the mechanisms that control OL versus IN fate are poorly understood, we have shown that *Dlx1/2* function is required in the MGE and AEP to control the neuron-glial switch, promoting neurogenesis at the expense of OLs through repression of *Olig2* (Petryniak et al., 2007). In contrast, *Olig2*-null animals show no abnormalities in early IN development (Petryniak et al., 2007; Furusho et al., 2006; Ono et al., 2008).

Olig1 is expressed in the embryonic neuroepithelium of the ventral forebrain (Petryniak et al., 2007), which can give rise to INs and OLs (Mukhopadhyay et al., 2009; Samanta et al., 2007). However, *Olig1* function is generally thought to be limited to late stages of OL development to promote differentiation (Lu et al., 2002; Xin et al., 2005) and remyelination (Arnett et al., 2004). Here we show a surprising role for *Olig1* as an upstream repressor of *Dlx1/2* and GABAergic IN production in the embryonic brain, establishing that *Olig1* functions in the regulation of the neuron-glial switch. Loss of *Olig1* de-represses production of late CR and PV IN subtypes in ventral MGE, AEP, and regions of the MGE connected to the septum, resulting in a 30% excess of INs in adult cortex. Postnatally, *Olig1*-null neural progenitors produced excessive numbers of INs and are deficient in OL production. We show Olig1 directly binds and represses the *I12b* enhancer element, a known *Dlx1/2* intergenic *cis*-acting DNA regulatory sequence, and using a newly generated floxed conditional *Dlx1/2* knockout allele, we show that *Dlx1/2* lies genetically downstream of *Olig1*. Together, these findings demonstrate that *Olig1* is an essential repressor of GABAergic neuron production in the mammalian brain.

Results

Inhibitory IN numbers are increased in the cortex of *Olig1*-null animals

To assess *Olig1*-dependent regulation of IN production, we first analyzed IN markers in the adult (P50) motor and somatosensory cortex of *Olig1*-null and controls by immunohistochemistry (IHC) (Figure 1a). Evaluation of IN subtypes in motor and somatosensory cortex demonstrated that there was a ~35% increase in parvalbumin+ (PV) and calretinin+ (CR) IN subtypes, but not somatostatin+ (SST) or neuropeptide Y+ (NPY) subtypes (Figure 1 b, i–k and m). We also observed an ~ 30% increase in cells expressing the pan-IN lineage markers GABA and GAD67 (Figure 1 g–h & Figure S1 k–l). We next determined if the laminar distribution of INs was abnormal. Increased numbers of PV+ and CR+ INs were present throughout the cortical layers. We did not find any difference in the laminar distribution of SST+ and NPY+ cells (Figure 1 n–q). SST+ and NPY+ neurons are generated early in telencephalic neurogenesis before E13. In contrast, CR+ neurons are generated at later stages and the PV+ subtype is generated throughout embryogenesis coinciding with the onset of OL specification (Kessaris et al., 2006; Miyoshi et al., 2007; Taniguchi et al., 2013; Wonders et al., 2008). Thus, *Olig1* acts to limit late born INs generated simultaneously with OLs, but not early born INs. Normal numbers of glutamatergic and cholinergic neurons were observed in *Olig1*-null animals (data not shown).

To confirm our findings, we conducted unbiased stereological analysis of the number of GAD67+ cells throughout the cortex and determined that the density and estimated total number of GAD67+ cells was increased by ~25% throughout the cortex (Figure S1 k–l). Cortical volume was unchanged in *Olig1*-null mice (Figure 1b & Figure S1m). To ensure that our results are not due to misexpression of IN markers with other cell types, we performed IHC for PV and GAD67 with markers of pyramidal cells (Tbr1), OLs (Olig2), microglia (Iba1) and astrocytes (GFAP). As shown (Figure S1g–j), we found no instance of abnormal IN marker expression in *Olig1* −/− brains.

Inhibitory PV+ INs synapse on the soma of cortical pyramidal cells, whereas CR+ neurons synapse mainly on the soma of other INs (Caputi et al., 2009; Freund and Buzsaki, 1996; Gonchar and Burkhalter, 1999). In keeping with the counts described above, we found a ~30% increase of PV+ puncta on the soma of layer 2/3 and 5/6 pyramidal neurons of somatosensory and motor cortex (Figure 1 c–d). Moreover, such puncta also expressed vesicular GABA transporter (VGAT) (Figure 1e), a marker of inhibitory synapses (Bragina et al., 2007). Quantification of VGAT+ puncta in dendritic fields revealed no differences in

the number of inhibitory synapses on dendrites, consistent with our finding that SST+ cell numbers are not affected in *Olig1*−/− mice (Figure 1 e–f).

Increased IN number does not alter inhibitory events on cortical pyramidal cells: Evidence of postsynaptic Gephyrin mediated compensation

There are a myriad of cell intrinsic and synaptic homeostatic mechanisms that control inhibition in cortical circuits (Pozo and Goda, 2010; Turrigiano, 2011). *Olig1* −/− mice provide a here-to-fore unique system to determine if increases in endogenously derived INs are sufficient to enhance inhibition in the adult cortex. To test this possibility we performed voltage-clamp analysis of inhibitory postsynaptic currents in layer 5 pyramidal cells in acute cortical slices derived from P35 mice. As a functional measure of inhibitory tone we analyzed both spontaneous and miniature inhibitory postsynaptic potentials (sIPSPs & mIPSPs). We found no significant increase in inhibitory activity onto pyramidal cells in terms of event frequency, amplitude or kinetics (Figure S2 A–B and data not shown). Because, we observed more presynaptic vGAT puncta, expressed at the soma of cortical neurons, we hypothesized that a postsynaptic compensatory mechanism might regulate inhibition in *Olig1*−/− mice. Gephyrin, a scaffolding protein that regulates recruitment, stability and clustering of GABA receptors at the postsynapse is downregulated in response to increased GABAergic activity (Poulopoulos et al., 2009; Prior et al., 1992; Saiepour et al., 2010; Tretter et al., 2008; Tretter et al., 2012). As demonstrated (Figure S2 C), we observed normal numbers of Gephyrin puncta identified by IHC at the postsynapse. We also noted that some CR+ interneurons make inhibitory connections with other interneurons, and thus excess CR+ may also limit the activity of other IN subtypes in *Olig1* −/− mice (Caputi et al., 2009; Freund and Buzsaki, 1996; Gonchar and Burkhalter, 1999).

Olig1 represses neurogenesis in the cerebellum and olfactory bulb

We next assessed *Olig1* function in the cerebellum (CB) and olfactory bulb (OB), brain areas that exhibit protracted neurogenesis (Maricich and Herrup, 1999; Schuller et al., 2006). As shown (Figure 2 a–c & d), we observed a ~30% surplus of AP2Beta+ and Pax2+ cerebellar INs at P7 and P21, respectively. Robust neurogenesis and neural cell turnover persists in the olfactory bulb (OB) throughout life and is regulated by Dlx1/2 (Alvarez-Buylla et al., 2002; Long et al., 2007). To assess neurogenesis in the OB, we conducted birth dating assays by injecting the thymidine analogue Bromodeoxyuridine (BrdU) intraperitoneally into P2 pups and analyzing olfactory bulbs in tissue harvested by perfusion at P50. These mice exhibited approximately 2-fold increases in the numbers of BrdU+ cells in the granule layer and glomerular layer (Figure 2 f–j). In summary, these findings provide evidence that *Olig1* has a general role in repressing IN production, including in the neocortex (PV+ and CR+ subtypes), cerebellum (Pax2+ / AP2Beta+), and perinatal olfactory bulb.

Olig1−/− mice produce fewer numbers of oligodendrocytes

Given previous evidence for common precursor domains for INs and OLs in the embryonic telencephalon, perinatal cerebellum and olfactory bulb throughout life (Goldman et al., 1997; Menn et al., 2006; Petryniak et al., 2007; Silbereis et al., 2009; Zhang and Goldman, 1996), we assessed the impact of *Olig1* loss-of-function on the OL population in the adult cerebral cortex and cerebellum by histological analysis. The numbers of cells expressing the pan-OL marker Olig2, as well as the mature OL markers *PLP* and APC are all reduced in the corpus callosum, motor cortex, and cerebellar white matter of the P21 and P50 mouse brain (Figure S1 a–f).

Olig1 is expressed in multipotent telencephalic progenitors that produce cortical IN

GABAergic INs of somatosensory and motor cortex develop from the ventral embryonic telencephalon under control of *Dlx1/2* and other transcriptional programs (Anderson et al., 1997; Wonders and Anderson, 2006). As shown (Figure 3 a–d), we detected *Olig1* mRNA transcripts in the AEP and ventral MGE telencephalic regions that express *Dlx1/2* (Petryniak et al., 2007), as well as caudal/dorsal regions of embryonic septum, which produces OLs but is not thought to produce cortical INs (Rubin et al., 2010). *Nkx2.1* is a hedgehog responsive gene critical for establishing progenitors of ventral identity that derive both forebrain OLs and INs (Butt et al., 2008; Elias et al., 2008; Kessaris et al., 2006; Maricich and Herrup, 1999). As shown (Figure 3 e–f), we found that Olig1+ cells co-labeled with Nestin and Nkx2.1.

A second line of evidence assigning *Olig1* expression to IN progenitors was provided by fate mapping with *Olig1*-cre. Our analysis in the adult (P50) neocortex, consistent with previous studies (Mukhopadhyay et al., 2009; Samanta et al., 2007), showed that *Olig1-cre* precursors fate mapped to ~35% of GABAergic cells and ~45% of PV+ INs, but fewer INs of other subtypes. In contrast, we found no labeling of glutamatergic cortical neurons (Figure 3 g–h, data not shown). Together, these findings indicate *Olig1* is expressed in multipotent precursor cells for GABAergic INs, particularly the PV+ subtype.

Olig1 represses telencephalic IN genetic programs

We next used *in situ* hybridization (ISH) to assess expression of *Lhx6, Dlx1* and *Dlx2*, genes necessary for the genesis of INs from MGE, AEP, CGE and preoptic area (POA) of wild type and *Olig1*−/− E15 embryonic brain. *Olig1* mutants showed expansion of *Lhx6, Dlx1* and *Dlx2* expression into the ventral MGE, the AEP and the caudal septum (Figure 4 a–h). To quantify this upregulation and assess the expression of *Vax1* and *Sp8* (two additional genes associated with IN production in the telencephalon), we dissected the caudal septum, AEP and ventral MGE from wild type and *Olig1*-null embryos and performed qPCR (Anderson et al., 1997; Taglialatela et al., 2004; Waclaw et al., 2006). We observed 2-4-fold increased expression of *Dlx2, Vax1* and *Sp8* (Figure 4i). These data indicate that loss of *Olig1* function results in upregulated expression of key transcription factors that drive IN cell fate acquisition.

Previous, gain-of-function studies have shown that Olig1 promotes OL specification from neural progenitors (Kim et al., 2011; Lu et al., 2001; Lu et al., 2000; Maire et al., 2010). To assess potential changes in embryonic OPC production, we assessed *PDGFRa*+ cells by ISH and quantified Sox10+ OPCs by IHC in *Olig1*−/− mutant and wild type E15 embryos in the mantle of the ventral telencephalon. This showed a reduction in OPC number (Figure S3 a–c). In contrast, we observed no significant change in levels of the mitotic cell marker phospho-Histone3 (PH3) in the septum, MGE, and AEP (Figure S3 d–f). Together, these findings suggest that *Olig1* function is required to promote OPC production at the expense of INs in the ventral telencephalon, but that it does not regulate IN precursor proliferation. Further, this shows a unique function of *Olig1* as a repressor of IN development, because *Olig2-null* mice, which lack OPCs, show normal expression of *Dlx2* (Petryniak et al., 2007) and IN precursor numbers identified by expression of GAD67 mRNA in the ventral telencephalon (Figure S4)(Furusho et al., 2006; Ono et al., 2008).

To confirm the birthdate of ectopic cortical INs in the *Olig1* mutant embryonic forebrain we injected Bromodeoxyuridine (BrdU) into pregnant dams at E16. BrdU co-labeling analyses with PV and GABA revealed an approximately 30% increase in the number of INs generated at these ages (Figure 4 j–o). Interestingly, at P0 we observed enhanced expression of *Dlx2* in the subventricular zone (SVZ) of *Olig1*-null animals (Figure S5 a–d), raising the

possibility of persistent IN production. However, BrdU birth dating at P2 ruled this out (Figure S5 e–f). Together, these findings indicate that *Olig1* regulates neuron-glial fate choice in the embryonic telencephalon.

Postnatal roles for *Olig1* in suppression of IN production

The finding of increased olfactory bulb neurogenesis perinatally and ectopic *Dlx2* expression in *Olig1*–null dorsal SVZ at P0 suggested there may be persistent roles for *Olig1* in neural stem cells. To test if *Olig1* regulates cell fate in defined culture conditions, we harvested progenitors from P3 anterior SVZ and then cultured progenitor cells as neurospheres or adherent monolayers of neural stem cells (NSCs). Neurospheres were expanded in EGF and FGF and then transferred to factor-free medium overnight to induce differentiation markers. Western blot analysis of total proteins demonstrated increased levels of neuron-specific Tuj1 and Dlx2 expression in Olig1-null neurospheres compared to wild type; in contrast, Olig2 levels were dramatically reduced (Figure 5a). In keeping with these findings, *Olig1*-null spheres showed enhanced capacity to produce young doublecortin (DCX)+ neurons (Figure 5b). As shown (Figure 5c–e), *Olig1* loss-of-function enhanced GABAergic IN production from NSC monolayer cultures. By contrast, monolayers derived from *Olig1*-null progenitors were deficient in production of NG2+, O4+ and GalC+ OL lineage cells (Figure 5f–i), which respectively label OPCs, premyelinating OLs and myelinating OLs. GFAP+ astroglial production was unaffected (data not shown). Thus, *Olig1* function is required in cultured postnatal neural progenitors to repress IN production and preserve oligodendrogenesis.

Evidence that Olig1 is a direct repressor of the *Dlx1/2 I12b* intergenic enhancer

Olig1 acts as a transcriptional repressor (Lee et al., 2005; Novitch et al., 2001; Sun et al., 2003). Thus, we hypothesized that *Olig1* may directly repress *Dlx1* and/or *Dlx2*, which colocalize within 10 kb of each other on mouse chromosome 2. This potential hierarchy is consistent with the observations that (1) *Olig1-cre* fate mapping labels 30% of cortical GABAergic neurons, (2) Olig1 protein shows segregated expression from Dlx2 in ventral telencephalon (Figure 6a), and (3) the previous finding that *Dlx1/2-cre* fate mapping fails to label Olig1-positive cells (Potter et al., 2009).

We tested whether *Olig1* might regulate *cis*-acting DNA regulatory sequences in the intergenic region of *Dlx1/2*. Activity of the *I12b* enhancer drives expression of *Dlx1/2* in the embryonic ventral telencephalon (Ghanem et al., 2007; Park et al., 2004; Poitras et al., 2007). We determined that the *I12b* enhancer contains three E-box sites, the canonical binding sequences for bHLH transcription factors including *Olig1* (Figure 6b, Figure S6a–b). We then used electrophoretic mobility shift assays (EMSA) to test *Olig1* binding to *Dlx1/2 I12b* E-box sites *in vitro*. As shown in Figure 6c–d, purified *Olig1* proteins shifted E-boxes 1 and 3, with the highest affinity for E-Box 1. Binding to E-box 1 was dose-dependent and was abrogated by DNA mutation of E-box sites within the *I12b* enhancer (Figure 6c–d, Figure S6b). Specificity of *Olig1* binding was further tested by supershift assays, which demonstrated that treatment of an antibody against Olig1, but not treatment with a control IgG antibody, inhibits binding of Olig1 protein to enhancer DNA sequences (Figure 6e–f).

To confirm that *Olig1* acts as a repressor of *Dlx1/2*, we next used a luciferase assay by cloning the *I12b* enhancer into the *pGL4* luciferase construct (Promega) and transfecting it into P19 cells. Because *Dlx1/2* are positive feedback regulators of their own expression via the *I12b* locus (Potter et al., 2009), we transfected a *Dlx2* expression construct to induce *I12b*-dependent luciferase activity. When an *Olig1* expression construct was transfected into these cells it induced a nearly three-fold reduction in luciferase activity (Figure 6g).

Together, these data provide biochemical evidence that *Olig1* functions upstream of *Dlx1/2* as a transcriptional repressor of the *Dlx1/2-I12b* enhancer.

Genetic functions of *Dlx1/2* downstream of *Olig1*

We next tested whether *Dlx1/2* function lies genetically downstream of *Olig1*. We generated a conditional floxed allele that removes *Dlx1* exons 2 and 3, the intergenic region and *Dlx2* exons 2 and 3 upon exposure to cre recombinase (Figure 7a). Targeted ES cells produced chimeras that passed the allele through the germline (Figure 7b–c).

We first sought to determine whether the increase in cortical IN density in *Olig1*-null mice was *Dlx1/2*-dependent *in vivo*. To test specific requirements of *Dlx1/2* in the O*lig1* lineage, we crossed our *Olig1*-null *cre* knockin mice ($Olig1^{cre(KI)/cre(KI)}$), in which the *Olig1* coding sequence has been replaced with a cre recombinase gene (Lu et al., 2002), to the *Dlx1/2* floxed mice. By using these cre knockin mice, we are able to confine *Dlx* gene excision in *Olig1*-null animals to the *Olig1* expression domain. $Olig1^{cre(KI)/cre(KI)}\times Dlx1/2^{fl/fl}$ animals failed to thrive, typically died in the neonatal period and never survived past P21, precluding analysis of PV populations in the adult cortex. However, $Olig1^{\mathrm{cre(KI)/cre(KI)}}\times Dlx1/2^{fl/+}$ animals were viable into adulthood, at which point analysis of the cortices showed normalization of INs identified by IHC for GAD67 and PV (Figure 7d–g & Figure S7a–d).

To further establish *Dlx1/2* functions downstream of *Olig1*, we derived NSC monolayers from the ventral telencephalon of E14 $Olig1^{cre(KI)/cre(KI)}\times Dlx1/2^{fl/fl}$, $Olig1^{cre(KI)/cre(KI)}\times Dlx1/2^{+/+}$ and wild typemice. As shown (Figure 7h–i & k–l), we observed that the increased IN production characteristic of $Olig1^{cre(KI)/cre(KI)}$ NSCs was normalized in $Olig1^{cre(KI)/cre(KI)}\times Dlx1/2^{fl/fl}$ NSCs. Conversely, we observed complete rescue of OL specification in $Olig1^{\mathrm{cre(KI)/cre(KI)}}$; $Dlx1/2^{fl/fl}$ NSCs (Figure 7j & m). Taken together, these genetic findings support a model in which *Olig1* acts as an essential repressor of *Dlx1/2* to limit IN pool size and promote oligodendrogliogenesis (Figure 8a).

Discussion

Recent studies indicate that the number of adult cortical INs is determined primarily at time of specification in the embryonic telencephalon, rather than through later neurotrophic competition, and developmental cell death (Southwell et al., 2012). Thus, limiting the number of cortical neurons produced during development is crucial. Here we show that *Olig1* represses *Dlx1/2* and IN production while preserving the potential to generate oligodendrocytes from common progenitors of the developing brain (Figure 8a).

Olig1 functions as an essential repressor of IN production in mammalian brain

We identified *Olig1* as a determinant of IN precursor pool size and IN numbers in the adult murine cortex. We observed a ~30% expansion of the total IN population, confined to the PV+ and CR+ IN cell types and a similar increase in PV/VGAT synapse density. This is consistent with previous findings that the maximum increase in density after transplantation of similar MGE progenitors into cortex is ~30% above normal (Baraban et al., 2009; Southwell et al., 2010; Southwell et al., 2012). Based on these studies, it was unclear whether increased endogenous generation of INs in *Olig1*−/− mice would affect inhibitory activity on pyramidal cells. Indeed, we found that increased IN cortical density in *Olig1*-null mice did not induce changes in the number of inhibitory potentials on pyramidal cells in adult mouse cortex. This may reflect the increase in CR+ cells, which make inhibitory synapses on other INs (Caputi et al., 2009; Freund and Buzsaki, 1996; Gonchar and Burkhalter, 1999). It is also notable that expression of the postsynaptic scaffolding protein Gephyrin is unaltered in *Olig1-null* mice. Gephyrin regulates the recruitment, stability and

clustering of GABA receptors at the postsynapse and is downregulated by increased inhibitory activity (Langosch et al., 1992; Poulopoulos et al., 2009; Prior et al., 1992; Saiepour et al., 2010; Tretter et al., 2008; Tretter et al., 2012; Vlachos et al., 2012). These data suggest that the increased interneuron number in *Olig1*−/− mice might result in Gephyrin-dependent postsynaptic compensation. We further demonstrated that *Olig1* is necessary to limit IN production in the cerebellum and olfactory bulb. Though numerous genes are required for IN specification and expansion, this is, to our knowledge, the first example of a transcription factor that represses cortical IN number.

Olig1 regulates neuron-glial fate choice

The expansion of cortical IN number in *Olig1*−/− animals suggested a critical role in regulating embryonic neurogenesis. *Olig1* is not robustly expressed in forebrain until E12.5 (Lu et al., 2002), a time point that coincides with the onset of oligodendrocyte specification (He et al., 2001; Kessaris et al., 2006). Indeed, several lines of evidence support the hypothesis that *Olig1* regulates the neuron-glial switch. First, *Olig1* is co-expressed in Nkx2.1+ and Nestin+ multipotent radial glia. Second, we observed upregulation of pro-IN gene expression in ventral MGE, AEP of *Olig1*−/− animals (e.g., *Lhx6*, *Dlx1/2*), coupled with decreased OPC production in the ventral telencephalon in the absence of excessive proliferation. A surprising finding of the study was that the septum appears competent to produce INs in the absence of *Olig1* function (Figure 8b). Finally, *Olig1* limits production of PV+ and CR+ cells, which are derived late in embryogenesis from progenitor domains that produce both OLs and INs, but not NPY+ and SST+ cells, which are born prior to the onset of OL specification (Kessaris et al., 2006; Miyoshi et al., 2007; Taniguchi et al., 2013; Wonders et al., 2008). In support of broad roles for Olig1 in neuron-glial fate choice, we found enhanced neurogenesis in the cerebellum and SVZ / olfactory bulb. Taken together, our findings suggest that *Olig1* acts in regions of protracted neurogenesis to limit IN production and promote OPC specification in several brain regions.

Olig1 regulates cell fate choice in multipotent progenitors through repressive interactions with *Dlx1/2*

DNA binding and luciferase assays suggest that *Olig1* is a direct repressor of the *Dlx1/2* locus acting through E-boxes in the *I12b* intergenic enhancer (Ghanem et al., 2003; Ghanem et al., 2007; Park et al., 2004; Poitras et al., 2007). This model is supported by our mouse genetic experiments in which enhanced IN genesis in *Olig1*−/− is rescued by conditional removal of *Dlx1/2* from the *Olig1* expression domain. Together, our findings indicate *Olig1* is a repressor of *Dlx1/2*. Future studies will probe interactions of *Olig1* with genes that control interneuron production in the cerebellum and other brain regions.

Despite similar structural features, Olig1 and Olig2 are functionally distinct in many respects (Meijer et al., 2012), including expression pattern, post-translational modification, cofactors, and transcriptional targets (Li et al., 2007; Li and Richardson, 2008; Lu et al., 2012). Our data show another unique role of Olig1 as an essential repressor of IN development. Prior studies show that forced *Olig1* overexpression results in ectopic OPC specification from neural progenitors (Kim et al., 2011; Lu et al., 2001; Lu et al., 2000; Maire et al., 2010) Although, *Olig2* shows more robust expression than *Olig1* in the MGE and binds E-boxes in the *Dlx I12b* enhancer (Mazzoni et al., 2011), this binding evidently is dispensable for IN genesis because we did not detect ectopic expression of *Dlx2* or *GAD67* in *Olig2*−/− animals despite upregulation of *Olig1* (Figure S4) (Petryniak et al., 2007, Furusho et al., 2006; Ono et al., 2008). Thus, *Olig1* shows a unique function in IN repression compared to *Olig2*.

Potential roles for Olig1 in human brain development and disease related to interneuron numbers and inhibitory tone

In the human fetal brain, OLIG1 proteins are expressed in primitive neuroepithelia that can give rise to INs (Jakovcevski and Zecevic, 2005), consistent with our findings. *OLIG1* and *OLIG2* are colocalized to human chromosome 21 in the Down syndrome (DS) critical region and several studies report they are overexpressed in DS (Bhattacharyya et al., 2009; Chakrabarti et al., 2010). Certain behavioral and psychiatric disorders are associated with abnormal IN numbers, including Tourette's syndrome (Kalanithi et al., 2005; Kataoka et al., 2010) and Schizophrenia (Hashimoto et al., 2008; Hashimoto et al., 2003; Lewis et al., 2008). Our findings raise the possibility that *OLIG1* expression becomes dysregulated in certain pathological conditions.

Transplantation of progenitors for cortical INs deriving from the MGE can confer increased seizure threshold and alter plasticity (Baraban et al., 2009; Southwell et al., 2010). Recently, methods to derive human INs and OLs capable of transplantation, widespread migration and functional integration into mammalian brain have been established (Maroof et al., 2013; Nicholas et al., 2013). IN transplants attenuate symptoms in rodent models of epilepsy (Baraban et al., 2009; Hunt et al., 2013), Parkinson's Disease (Martinez-Cerdeno et al., 2010), and neuropathic pain (Braz et al., 2012). Based on its properties to repress IN formation in cultured neural progenitors, reducing *Olig1* expression (*e.g.*, siRNA) might provide a method to augment IN production for such potential therapeutic applications. Future studies will determine if increased IN number in *Olig1*−/− mice leads to differences in inhibitory tone during development, learning and memory tasks, and pathologies such as seizures.

Experimental Procedures

Animals

All experimental procedures were approved by the Institutional Animal Care and Use Committee and Laboratory Animal Resource Center at the University of California San Francisco (UCSF). Mouse colonies were maintained at UCSF in accordance with National Institutes of Health and UCSF guidelines. The *Olig1*$^{cre/cre}$ (Lu et al., 2002) and *Caggs-EGFP* (Nakamura et al., 2006) reporter mice have been previously described. The Dlx1/2$^{fl/fl}$ mice were generated as described in the supplemental experimental procedures.

In situ hybridization and immunohistochemistry

ISH and IHC were performed using standard protocols. Table S1 lists details of antibodies and protocols for ISH, IHC and BrdU labeling are provided in the supplemental experimental procedures.

rtPCR

RNA was isolated (Trizol extraction followed by RNeasy; Qiagen) from MGE plus septum, reversed transcribed and assayed for gene expression by SYBR-Green technology on a Lightcycler 480 (Roche). Primer sequences and details of analytical methods and statistics can be found in the supplemental experimental procedures.

Neural progenitor cultures

Neurosphere and neural stem cell monolayer cultures were derived from E14 ventral telencephalon or P3 SVZ by standard methods (Ahlenius and Kokaia, 2010). Details of culture preparations and Western blot analysis of these cultures are provided in the supplemental experimental procedures.

Microscopy, cell counting and statistical analyses

Cell populations were quantified *in vivo* from micrographs of identical field size of anatomically matched regions of somatosensory, motor cortex and corpus callosum. *In vitro* cell populations cultured in 8 well culture slides were sampled at defined points within each slide well using a Nikon 80i microscope equipped with a motorized stage. Cell counts were conducted by a researcher blinded to genotype using ImageJ and Nikon Elements software. Statistical significance was determined using unpaired, 2 tailed Student's T-tests.

DNA binding and luciferase assays

These methods are detailed in the supplemental experimental procedures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to Michael Wong and Sandra Chang for expert technical help. J. S. acknowledges support from training grant T32 GM007449-36 from the NIGMS and the Ruth Kirschstein NRSA fellowship F31 NS076254-03 from the NINDS. G.P. and H.N acknowledge postdoctoral fellowship support from the European Leukodystrophy Association. This work has been supported by grants to C.D.S. (NS047572) and D.H.R. (NS040511) from the NINDS, to J.L.R.R. (MH049428) from NIMH and to S.C.B (R01-NS- 048528) from NINDS. D.H.R. is an HHMI Investigator.

References

Ahlenius H, Kokaia Z. Isolation and generation of neurosphere cultures from embryonic and adult mouse brain. Methods in molecular biology. 2010; 633:241–252. [PubMed: 20204633]

Alonso JM, Swadlow HA. Thalamocortical specificity and the synthesis of sensory cortical receptive fields. J Neurophysiol. 2005; 94:26–32. [PubMed: 15985693]

Alvarez-Buylla A, Seri B, Doetsch F. Identification of neural stem cells in the adult vertebrate brain. Brain research bulletin. 2002; 57:751–758. [PubMed: 12031271]

Alvarez-Dolado M, Calcagnotto ME, Karkar KM, Southwell DG, Jones-Davis DM, Estrada RC, Rubenstein JL, Alvarez-Buylla A, Baraban SC. Cortical inhibition modified by embryonic neural precursors grafted into the postnatal brain. J Neurosci. 2006; 26:7380–7389. [PubMed: 16837585]

Anderson SA, Eisenstat DD, Shi L, Rubenstein JL. Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. Science. 1997; 278:474–476. [PubMed: 9334308]

Baraban SC, Southwell DG, Estrada RC, Jones DL, Sebe JY, Alfaro-Cervello C, Garcia-Verdugo JM, Rubenstein JL, Alvarez-Buylla A. Reduction of seizures by transplantation of cortical GABAergic interneuron precursors into Kv1.1 mutant mice. Proc Natl Acad Sci U S A. 2009; 106:15472–15477. [PubMed: 19706400]

Ben-Ari Y. Seizures beget seizures: the quest for GABA as a key player. Crit Rev Neurobiol. 2006; 18:135–144. [PubMed: 17725516]

Bhattacharyya A, McMillan E, Chen SI, Wallace K, Svendsen CN. A critical period in cortical interneuron neurogenesis in down syndrome revealed by human neural progenitor cells. Dev Neurosci. 2009; 31:497–510. [PubMed: 19738365]

Bragina L, Candiracci C, Barbaresi P, Giovedi S, Benfenati F, Conti F. Heterogeneity of glutamatergic and GABAergic release machinery in cerebral cortex. Neuroscience. 2007; 146:1829–1840. [PubMed: 17445987]

Braz JM, Sharif-Naeini R, Vogt D, Kriegstein A, Alvarez-Buylla A, Rubenstein JL, Basbaum AI. Forebrain GABAergic neuron precursors integrate into adult spinal cord and reduce injury-induced neuropathic pain. Neuron. 2012; 74:663–675. [PubMed: 22632725]

Butt SJ, Fuccillo M, Nery S, Noctor S, Kriegstein A, Corbin JG, Fishell G. The temporal and spatial origins of cortical interneurons predict their physiological subtype. Neuron. 2005; 48:591–604. [PubMed: 16301176]

Butt SJ, Sousa VH, Fuccillo MV, Hjerling-Leffler J, Miyoshi G, Kimura S, Fishell G. The requirement of Nkx2-1 in the temporal specification of cortical interneuron subtypes. Neuron. 2008; 59:722–732. [PubMed: 18786356]

Caputi A, Rozov A, Blatow M, Monyer H. Two calretinin-positive GABAergic cell types in layer 2/3 of the mouse neocortex provide different forms of inhibition. Cerebral cortex. 2009; 19:1345–1359. [PubMed: 18842664]

Chakrabarti L, Best TK, Cramer NP, Carney RS, Isaac JT, Galdzicki Z, Haydar TF. Olig1 and Olig2 triplication causes developmental brain defects in Down syndrome. Nat Neurosci. 2010; 13:927–934. [PubMed: 20639873]

Cobos I, Calcagnotto ME, Vilaythong AJ, Thwin MT, Noebels JL, Baraban SC, Rubenstein JL. Mice lacking Dlx1 show subtype-specific loss of interneurons, reduced inhibition and epilepsy. Nat Neurosci. 2005; 8:1059–1068. [PubMed: 16007083]

Corbin JG, Nery S, Fishell G. Telencephalic cells take a tangent: non-radial migration in the mammalian forebrain. Nat Neurosci. 2001; 4(Suppl):1177–1182. [PubMed: 11687827]

Elias LA, Potter GB, Kriegstein AR. A time and a place for nkx2-1 in interneuron specification and migration. Neuron. 2008; 59:679–682. [PubMed: 18786351]

Freund TF, Buzsaki G. Interneurons of the hippocampus. Hippocampus. 1996; 6:347–470. [PubMed: 8915675]

Furusho M, Ono K, Takebayashi H, Masahira N, Kagawa T, Ikeda K, Ikenaka K. Involvement of the Olig2 transcription factor in cholinergic neuron development of the basal forebrain. Developmental biology. 2006; 293:348–357. [PubMed: 16537079]

Ghanem N, Jarinova O, Amores A, Long Q, Hatch G, Park BK, Rubenstein JL, Ekker M. Regulatory roles of conserved intergenic domains in vertebrate Dlx bigene clusters. Genome Res. 2003; 13:533–543. [PubMed: 12670995]

Ghanem N, Yu M, Long J, Hatch G, Rubenstein JL, Ekker M. Distinct cis-regulatory elements from the Dlx1/Dlx2 locus mark different progenitor cell populations in the ganglionic eminences and different subtypes of adult cortical interneurons. J Neurosci. 2007; 27:5012–5022. [PubMed: 17494687]

Goldman JE, Zerlin M, Newman S, Zhang L, Gensert J. Fate determination and migration of progenitors in the postnatal mammalian CNS. Dev Neurosci. 1997; 19:42–48. [PubMed: 9078432]

Gonchar Y, Burkhalter A. Connectivity of GABAergic calretinin-immunoreactive neurons in rat primary visual cortex. Cerebral cortex. 1999; 9:683–696. [PubMed: 10554991]

Han S, Tai C, Westenbroek RE, Yu FH, Cheah CS, Potter GB, Rubenstein JL, Scheuer T, de la Iglesia HO, Catterall WA. Autistic-like behaviour in Scn1a+/− mice and rescue by enhanced GABA-mediated neurotransmission. Nature. 2012; 489:385–390. [PubMed: 22914087]

Hashimoto T, Arion D, Unger T, Maldonado-Aviles JG, Morris HM, Volk DW, Mirnics K, Lewis DA. Alterations in GABA-related transcriptome in the dorsolateral prefrontal cortex of subjects with schizophrenia. Mol Psychiatry. 2008; 13:147–161. [PubMed: 17471287]

Hashimoto T, Volk DW, Eggan SM, Mirnics K, Pierri JN, Sun Z, Sampson AR, Lewis DA. Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. J Neurosci. 2003; 23:6315–6326. [PubMed: 12867516]

He W, Ingraham C, Rising L, Goderie S, Temple S. Multipotent stem cells from the mouse basal forebrain contribute GABAergic neurons and oligodendrocytes to the cerebral cortex during embryogenesis. J Neurosci. 2001; 21:8854–8862. [PubMed: 11698597]

Hunt RF, Girskis KM, Rubenstein JL, Alvarez-Buylla A, Baraban SC. GABA progenitors grafted into the adult epileptic brain control seizures and abnormal behavior. Nat Neurosci. 2013

Jakovcevski I, Zecevic N. Olig transcription factors are expressed in oligodendrocyte and neuronal cells in human fetal CNS. J Neurosci. 2005; 25:10064–10073. [PubMed: 16267213]

Kalanithi PS, Zheng W, Kataoka Y, DiFiglia M, Grantz H, Saper CB, Schwartz ML, Leckman JF, Vaccarino FM. Altered parvalbumin-positive neuron distribution in basal ganglia of individuals

with Tourette syndrome. Proc Natl Acad Sci U S A. 2005; 102:13307–13312. [PubMed: 16131542]

Kataoka Y, Kalanithi PS, Grantz H, Schwartz ML, Saper C, Leckman JF, Vaccarino FM. Decreased number of parvalbumin and cholinergic interneurons in the striatum of individuals with Tourette syndrome. J Comp Neurol. 2010; 518:277–291. [PubMed: 19941350]

Kehrer C, Maziashvili N, Dugladze T, Gloveli T. Altered Excitatory-Inhibitory Balance in the NMDA-Hypofunction Model of Schizophrenia. Front Mol Neurosci. 2008; 1:6. [PubMed: 18946539]

Kessaris N, Fogarty M, Iannarelli P, Grist M, Wegner M, Richardson WD. Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. Nat Neurosci. 2006; 9:173–179. [PubMed: 16388308]

Kim HM, Hwang DH, Choi JY, Park CH, Suh-Kim H, Kim SU, Kim BG. Differential and cooperative actions of Olig1 and Olig2 transcription factors on immature proliferating cells after contusive spinal cord injury. Glia. 2011; 59:1094–1106. [PubMed: 21538562]

Lee SK, Lee B, Ruiz EC, Pfaff SL. Olig2 and Ngn2 function in opposition to modulate gene expression in motor neuron progenitor cells. Genes Dev. 2005; 19:282–294. [PubMed: 15655114]

Lehmann K, Steinecke A, Bolz J. GABA through the ages: regulation of cortical function and plasticity by inhibitory interneurons. Neural Plast. 2012; 2012:892784. [PubMed: 22792496]

Lewis DA, Hashimoto T, Morris HM. Cell and receptor type-specific alterations in markers of GABA neurotransmission in the prefrontal cortex of subjects with schizophrenia. Neurotox Res. 2008; 14:237–248. [PubMed: 19073429]

Lewis DA, Hashimoto T, Volk DW. Cortical inhibitory neurons and schizophrenia. Nat Rev Neurosci. 2005; 6:312–324. [PubMed: 15803162]

Li H, Lu Y, Smith HK, Richardson WD. Olig1 and Sox10 interact synergistically to drive myelin basic protein transcription in oligodendrocytes. J Neurosci. 2007; 27:14375–14382. [PubMed: 18160645]

Li H, Richardson WD. The evolution of Olig genes and their roles in myelination. Neuron glia biology. 2008; 4:129–135. [PubMed: 19737433]

Llinas R, Urbano FJ, Leznik E, Ramirez RR, van Marle HJ. Rhythmic and dysrhythmic thalamocortical dynamics: GABA systems and the edge effect. Trends Neurosci. 2005; 28:325–333. [PubMed: 15927689]

Long JE, Garel S, Alvarez-Dolado M, Yoshikawa K, Osumi N, Alvarez-Buylla A, Rubenstein JL. Dlx-dependent and -independent regulation of olfactory bulb interneuron differentiation. J Neurosci. 2007; 27:3230–3243. [PubMed: 17376983]

Lu J, Lian G, Zhou H, Esposito G, Steardo L, Delli-Bovi LC, Hecht JL, Lu QR, Sheen V. OLIG2 over-expression impairs proliferation of human Down syndrome neural progenitors. Hum Mol Genet. 2012; 21:2330–2340. [PubMed: 22343408]

Lu QR, Cai L, Rowitch D, Cepko CL, Stiles CD. Ectopic expression of Olig1 promotes oligodendrocyte formation and reduces neuronal survival in developing mouse cortex. Nat Neurosci. 2001; 4:973–974. [PubMed: 11574831]

Lu QR, Sun T, Zhu Z, Ma N, Garcia M, Stiles CD, Rowitch DH. Common developmental requirement for Olig function indicates a motor neuron/oligodendrocyte connection. Cell. 2002; 109:75–86. [PubMed: 11955448]

Lu QR, Yuk D, Alberta JA, Zhu Z, Pawlitzky I, Chan J, McMahon AP, Stiles CD, Rowitch DH. Sonic hedgehog--regulated oligodendrocyte lineage genes encoding bHLH proteins in the mammalian central nervous system. Neuron. 2000; 25:317–329. [PubMed: 10719888]

Maire CL, Wegener A, Kerninon C, Nait Oumesmar B. Gain-of-function of Olig transcription factors enhances oligodendrogenesis and myelination. Stem cells. 2010; 28:1611–1622. [PubMed: 20672298]

Maricich SM, Herrup K. Pax-2 expression defines a subset of GABAergic interneurons and their precursors in the developing murine cerebellum. J Neurobiol. 1999; 41:281–294. [PubMed: 10512984]

Marin O. Brain development: The neuron family tree remodelled. Nature. 2012; 490:185–186. [PubMed: 23060186]

Maroof AM, Keros S, Tyson JA, Ying SW, Ganat YM, Merkle FT, Liu B, Goulburn A, Stanley EG, Elefanty AG, et al. Directed differentiation and functional maturation of cortical interneurons from human embryonic stem cells. Cell Stem Cell. 2013; 12:559–572. [PubMed: 23642365]

Martinez-Cerdeno V, Noctor SC, Espinosa A, Ariza J, Parker P, Orasji S, Daadi MM, Bankiewicz K, Alvarez-Buylla A, Kriegstein AR. Embryonic MGE precursor cells grafted into adult rat striatum integrate and ameliorate motor symptoms in 6-OHDA-lesioned rats. Cell Stem Cell. 2010; 6:238–250. [PubMed: 20207227]

Meijer DH, Kane MF, Mehta S, Liu H, Harrington E, Taylor CM, Stiles CD, Rowitch DH. Separated at birth? The functional and molecular divergence of OLIG1 and OLIG2. Nat Rev Neurosci. 2012; 13:819–831. [PubMed: 23165259]

Menn B, Garcia-Verdugo JM, Yaschine C, Gonzalez-Perez O, Rowitch D, Alvarez-Buylla A. Origin of oligodendrocytes in the subventricular zone of the adult brain. J Neurosci. 2006; 26:7907–7918. [PubMed: 16870736]

Miyoshi G, Butt SJ, Takebayashi H, Fishell G. Physiologically distinct temporal cohorts of cortical interneurons arise from telencephalic Olig2-expressing precursors. J Neurosci. 2007; 27:7786–7798. [PubMed: 17634372]

Mukhopadhyay A, McGuire T, Peng CY, Kessler JA. Differential effects of BMP signaling on parvalbumin and somatostatin interneuron differentiation. Development. 2009; 136:2633–2642. [PubMed: 19592576]

Nakamura T, Colbert MC, Robbins J. Neural crest cells retain multipotential characteristics in the developing valves and label the cardiac conduction system. Circ Res. 2006; 98:1547–1554. [PubMed: 16709902]

Nicholas CR, Chen J, Tang Y, Southwell DG, Chalmers N, Vogt D, Arnold CM, Chen YJ, Stanley EG, Elefanty AG, et al. Functional Maturation of hPSC-Derived Forebrain Interneurons Requires an Extended Timeline and Mimics Human Neural Development. Cell Stem Cell. 2013; 12:573–586. [PubMed: 23642366]

Novitch BG, Chen AI, Jessell TM. Coordinate regulation of motor neuron subtype identity and pan-neuronal properties by the bHLH repressor Olig2. Neuron. 2001; 31:773–789. [PubMed: 11567616]

Ono K, Takebayashi H, Ikeda K, Furusho M, Nishizawa T, Watanabe K, Ikenaka K. Regional- and temporal-dependent changes in the differentiation of Olig2 progenitors in the forebrain, and the impact on astrocyte development in the dorsal pallium. Developmental biology. 2008; 320:456–468. [PubMed: 18582453]

Park BK, Sperber SM, Choudhury A, Ghanem N, Hatch GT, Sharpe PT, Thomas BL, Ekker M. Intergenic enhancers with distinct activities regulate Dlx gene expression in the mesenchyme of the branchial arches. Developmental biology. 2004; 268:532–545. [PubMed: 15063187]

Petryniak MA, Potter GB, Rowitch DH, Rubenstein JL. Dlx1 and Dlx2 control neuronal versus oligodendroglial cell fate acquisition in the developing forebrain. Neuron. 2007; 55:417–433. [PubMed: 17678855]

Poitras L, Ghanem N, Hatch G, Ekker M. The proneural determinant MASH1 regulates forebrain Dlx1/2 expression through the I12b intergenic enhancer. Development. 2007; 134:1755–1765. [PubMed: 17409112]

Potter GB, Petryniak MA, Shevchenko E, McKinsey GL, Ekker M, Rubenstein JL. Generation of Cre-transgenic mice using Dlx1/Dlx2 enhancers and their characterization in GABAergic interneurons. Mol Cell Neurosci. 2009; 40:167–186. [PubMed: 19026749]

Poulopoulos A, Aramuni G, Meyer G, Soykan T, Hoon M, Papadopoulos T, Zhang M, Paarmann I, Fuchs C, Harvey K, et al. Neuroligin 2 drives postsynaptic assembly at perisomatic inhibitory synapses through gephyrin and collybistin. Neuron. 2009; 63:628–642. [PubMed: 19755106]

Pozo K, Goda Y. Unraveling mechanisms of homeostatic synaptic plasticity. Neuron. 2010; 66:337–351. [PubMed: 20471348]

Prior P, Schmitt B, Grenningloh G, Pribilla I, Multhaup G, Beyreuther K, Maulet Y, Werner P, Langosch D, Kirsch J, et al. Primary structure and alternative splice variants of gephyrin, a putative glycine receptor-tubulin linker protein. Neuron. 1992; 8:1161–1170. [PubMed: 1319186]

Rossignol E. Genetics and function of neocortical GABAergic interneurons in neurodevelopmental disorders. Neural Plast. 2011; 2011:649325. [PubMed: 21876820]

Rubenstein JL. Three hypotheses for developmental defects that may underlie some forms of autism spectrum disorder. Curr Opin Neurol. 2010; 23:118–123. [PubMed: 20087182]

Rubenstein JL, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav. 2003; 2:255–267. [PubMed: 14606691]

Rubin AN, Alfonsi F, Humphreys MP, Choi CK, Rocha SF, Kessaris N. The germinal zones of the basal ganglia but not the septum generate GABAergic interneurons for the cortex. J Neurosci. 2010; 30:12050–12062. [PubMed: 20826668]

Saiepour L, Fuchs C, Patrizi A, Sassoe-Pognetto M, Harvey RJ, Harvey K. Complex role of collybistin and gephyrin in GABAA receptor clustering. The Journal of biological chemistry. 2010; 285:29623–29631. [PubMed: 20622020]

Samanta J, Burke GM, McGuire T, Pisarek AJ, Mukhopadhyay A, Mishina Y, Kessler JA. BMPR1a signaling determines numbers of oligodendrocytes and calbindin-expressing interneurons in the cortex. J Neurosci. 2007; 27:7397–7407. [PubMed: 17626200]

Schiller PH, Tehovnik EJ. Neural mechanisms underlying target selection with saccadic eye movements. Prog Brain Res. 2005; 149:157–171. [PubMed: 16226583]

Schuller U, Kho AT, Zhao Q, Ma Q, Rowitch DH. Cerebellar 'transcriptome' reveals cell-type and stage-specific expression during postnatal development and tumorigenesis. Mol Cell Neurosci. 2006; 33:247–259. [PubMed: 16962790]

Silbereis J, Cheng E, Ganat YM, Ment LR, Vaccarino FM. Precursors with glial fibrillary acidic protein promoter activity transiently generate GABA interneurons in the postnatal cerebellum. Stem cells. 2009; 27:1152–1163. [PubMed: 19418461]

Southwell DG, Froemke RC, Alvarez-Buylla A, Stryker MP, Gandhi SP. Cortical plasticity induced by inhibitory neuron transplantation. Science. 2010; 327:1145–1148. [PubMed: 20185728]

Southwell DG, Paredes MF, Galvao RP, Jones DL, Froemke RC, Sebe JY, Alfaro-Cervello C, Tang Y, Garcia-Verdugo JM, Rubenstein JL, et al. Intrinsically determined cell death of developing cortical interneurons. Nature. 2012; 491:109–113. [PubMed: 23041929]

Sun T, Dong H, Wu L, Kane M, Rowitch DH, Stiles CD. Cross-repressive interaction of the Olig2 and Nkx2.2 transcription factors in developing neural tube associated with formation of a specific physical complex. J Neurosci. 2003; 23:9547–9556. [PubMed: 14573534]

Taglialatela P, Soria JM, Caironi V, Moiana A, Bertuzzi S. Compromised generation of GABAergic interneurons in the brains of Vax1−/− mice. Development. 2004; 131:4239–4249. [PubMed: 15280216]

Taniguchi H, Lu J, Huang ZJ. The spatial and temporal origin of chandelier cells in mouse neocortex. Science. 2013; 339:70–74. [PubMed: 23180771]

Tretter V, Jacob TC, Mukherjee J, Fritschy JM, Pangalos MN, Moss SJ. The clustering of GABA(A) receptor subtypes at inhibitory synapses is facilitated via the direct binding of receptor alpha 2 subunits to gephyrin. J Neurosci. 2008; 28:1356–1365. [PubMed: 18256255]

Tretter V, Mukherjee J, Maric HM, Schindelin H, Sieghart W, Moss SJ. Gephyrin, the enigmatic organizer at GABAergic synapses. Frontiers in cellular neuroscience. 2012; 6:23. [PubMed: 22615685]

Turrigiano G. Too many cooks? Intrinsic and synaptic homeostatic mechanisms in cortical circuit refinement. Annual review of neuroscience. 2011; 34:89–103.

Vlachos A, Reddy-Alla S, Papadopoulos T, Deller T, Betz H. Homeostatic Regulation of Gephyrin Scaffolds and Synaptic Strength at Mature Hippocampal GABAergic Postsynapses. Cerebral cortex. 2012

Waclaw RR, Allen ZJ 2nd, Bell SM, Erdelyi F, Szabo G, Potter SS, Campbell K. The zinc finger transcription factor Sp8 regulates the generation and diversity of olfactory bulb interneurons. Neuron. 2006; 49:503–516. [PubMed: 16476661]

Wonders CP, Anderson SA. The origin and specification of cortical interneurons. Nat Rev Neurosci. 2006; 7:687–696. [PubMed: 16883309]

Wonders CP, Taylor L, Welagen J, Mbata IC, Xiang JZ, Anderson SA. A spatial bias for the origins of interneuron subgroups within the medial ganglionic eminence. Developmental biology. 2008; 314:127–136. [PubMed: 18155689]

Xin M, Yue T, Ma Z, Wu FF, Gow A, Lu QR. Myelinogenesis and axonal recognition by oligodendrocytes in brain are uncoupled in Olig1-null mice. J Neurosci. 2005; 25:1354–1365. [PubMed: 15703389]

Yizhar O, Fenno LE, Prigge M, Schneider F, Davidson TJ, O'Shea DJ, Sohal VS, Goshen I, Finkelstein J, Paz JT, et al. Neocortical excitation/inhibition balance in information processing and social dysfunction. Nature. 2011; 477:171–178. [PubMed: 21796121]

Zhang L, Goldman JE. Generation of cerebellar interneurons from dividing progenitors in white matter. Neuron. 1996; 16:47–54. [PubMed: 8562089]

Highlights

- *Olig1* is the first known transcriptional repressor of cortical interneuron genesis
- Adult *Olig1*-null mutants show a 30% increased density of cortical interneurons
- Olig1 directly represses the Dlx1/2 intergenic enhancer
- Targeting *Olig1* could enhance interneuron production for therapy

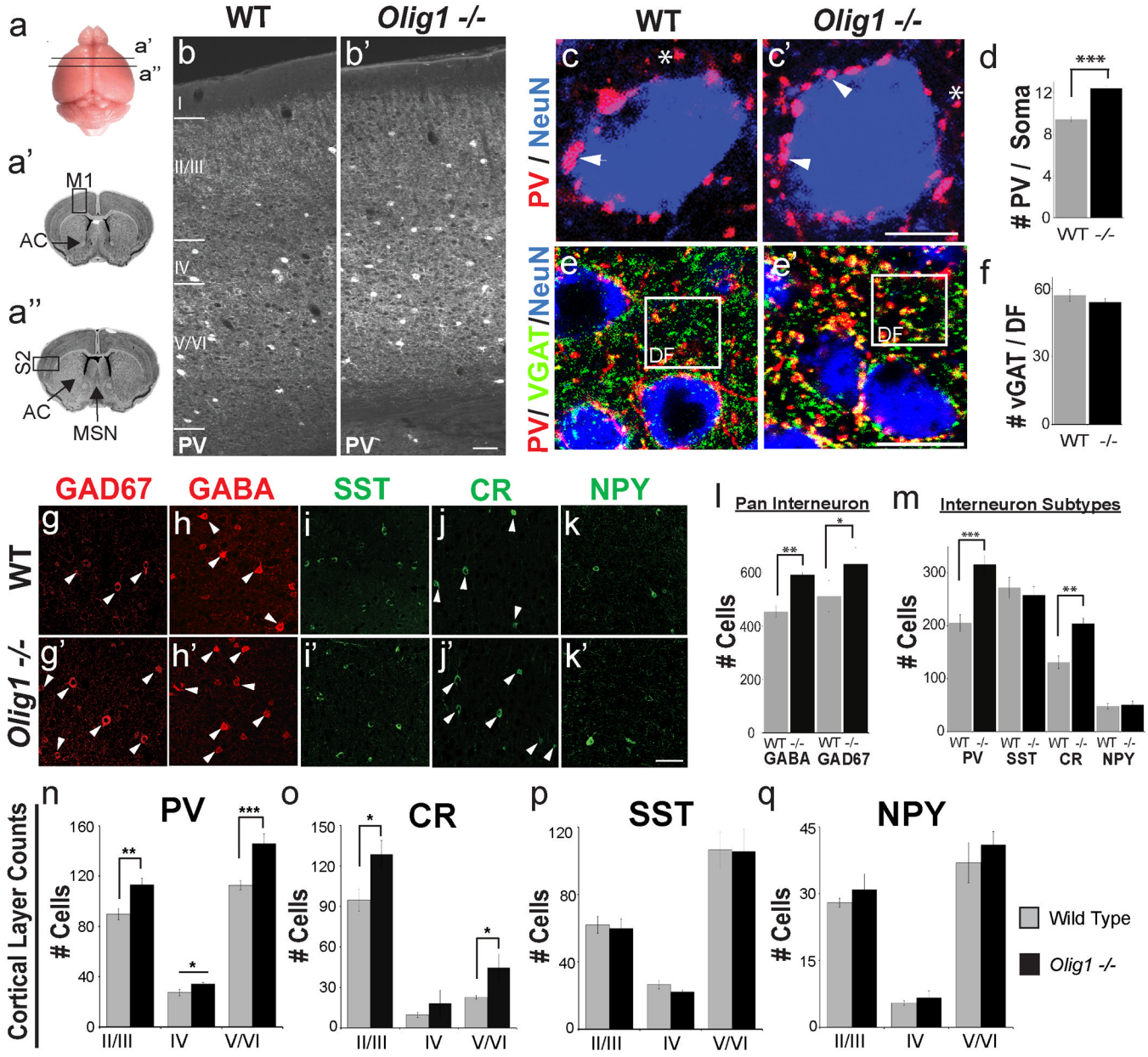

Figure 1. Increase in interneuron numbers in the cerebral cortex of adult *Olig1*-null mutant mice

(a) Representation of regions of secondary somatosensory cortex (S2) and primary motor cortex (M1) in which INs were quantified. (b) Representative images of parvalbumin (PV) in wild type (WT) versus *Olig1*-null (*Olig1*−/−) motor cortex. Note the increased number of PV+ cell bodies. (c) Representative image showing increases in PV+ (red) synaptic puncta localized to NeuN+ (blue) soma in *Olig1*−/− vs. WT M1. Arrowheads point to soma localized PV+ puncta, asterisks denote non soma localized puncta. (d) Quantification of PV + synaptic puncta colocalizing NeuN positive soma in M1 (e) Representative image showing VGAT (green), PV (red), and NeuN (blue). As shown in the boxed region VGAT+ (green) / PV- (red) synaptic puncta in dendritic fields (DF) are identical in *Olig1*−/− vs. WT M1. Note that PV+ (red) puncta colocalize VGAT confirming they label GABAergic synapses (f) Quantification of the number of VGAT+ puncta in dendritic fields demonstrating that there is not a significant increase in the number of VGAT+ neuronal synapses in dendritic

fields of *Olig1*−/− cortex vs. WT. (g –k) Representative images of INs in *Olig1*−/− and Wild Type cortex. Arrows point to cell bodies of cell types for which significant differences were observed. (g–h) 2 μm confocal projections of pan IN markers GAD67 (g) and GABA (h). (i–k) 2 μm confocal projections of IN subtype markers Somatostatin (SST) (i), Calretinin (CR) (j), and Neuropeptide Y (NPY) (k). (l–m) Quantification of the number of cells expressing the pan IN markers (l) and IN subtypes (m). Cell counts were taken from micrographs of S2 and M1 in 2 anterior to posterior serial coronal sections as shown in (a). (n–q) Quantification of the number of cells expressing IN subtypes within distinct lamina of the cortex (II/III, IV, V/VI) as demonstrated in panel (b). Cell counts were taken from micrographs of S2 and primary M1 (For all quantifications: mean +/− SEM, n = 3–4; *p<.05, **p<.01, ***p<.005; 2 tailed unpaired student's t test). (b',e',k") Scale bar = 50 μm, (c') scale bar = 5 μm (k"), (e') scale bar = 15 μm. Abbreviations: AC, anterior commissure; DF, dendritic field; IN, interneuron; M1, primary motor cortex; MSN, medial septal nucleus; S2, secondary somatosensory cortex. See also Figures S1 and S2.

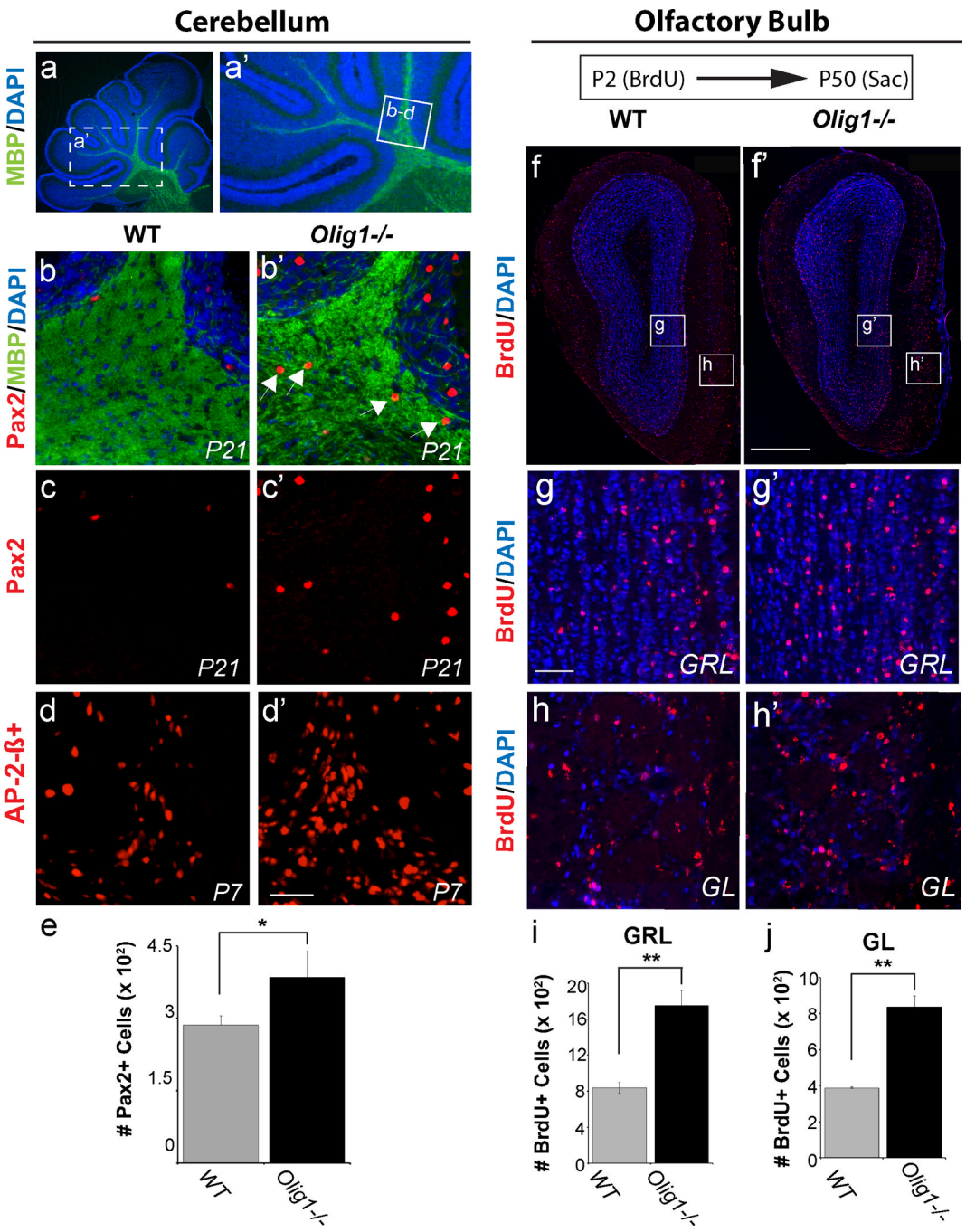

Figure 2. Increase in the number of interneurons in *Olig1*−/− cerebellum and olfactory bulb

(a) Low magnification imagea of MBP (green) and DAPI (blue) of juvenile cerebellum. The box represents the region where the images in b–d were taken. (b) Image taken of P21 cerebellum showing Pax2+ INs (red) and anatomy of cerebellar lobules with white matter defined by dense MBP immunoreactivity (green) and the granule layer defined by dense DAPI staining (blue). Note the ectopic presence of Pax2+ INs in the white matter (arrows) in the *Olig1*−/− mice. (c) Single channel image of Pax2 (red) staining in panel a demonstrating increased numbers of Pax2+ INs in *Olig1*−/− mice. (d) Representative images of immunohistochemistry showing increased numbers of cerebellar IN precursor cells

expressing AP-2ß in *Olig1*−/− versus WT mice at P7. (e) Quantification of Pax2+ cells in the P21 cererbellum demonstrating a statistically significant increase in Pax2+ cells *Olig1*−/− versus WT mice. (f) Low magnification image of the olfactory bulbs of WT (f) vs *Olig1*−/− (f') mice injected with BrdU at P2 stained for BrdU (red) and DAPI (blue). (g) Higher magnification images of the granule cell layer corresponding to the box inset labeled (g & g') in panel (f). (h) Higher magnification images of the glomerular layer corresponding to the box inset labeled (h & h') in panel (f). (i–j) Quantification of the number of BrdU+ cells in the P50 granule cell layer (GRL) and glomerular layer (GL) respectively, following BrdU injection at P2 demonstrating a statistically significant increase in BrdU+ cells in *Olig1*−/− versus WT mice. (c') scale bar = 50 μm, (f) scale bar = 500 μm, (g) scale bar = 50 μm. (For all quantifications: mean +/− SEM, n = 3; *p<.05, **p<.01, 2 tailed unpaired student's t test. Abbreviation: GRL = Granule layer, GL = Glomerular layer. See also Figure S1.

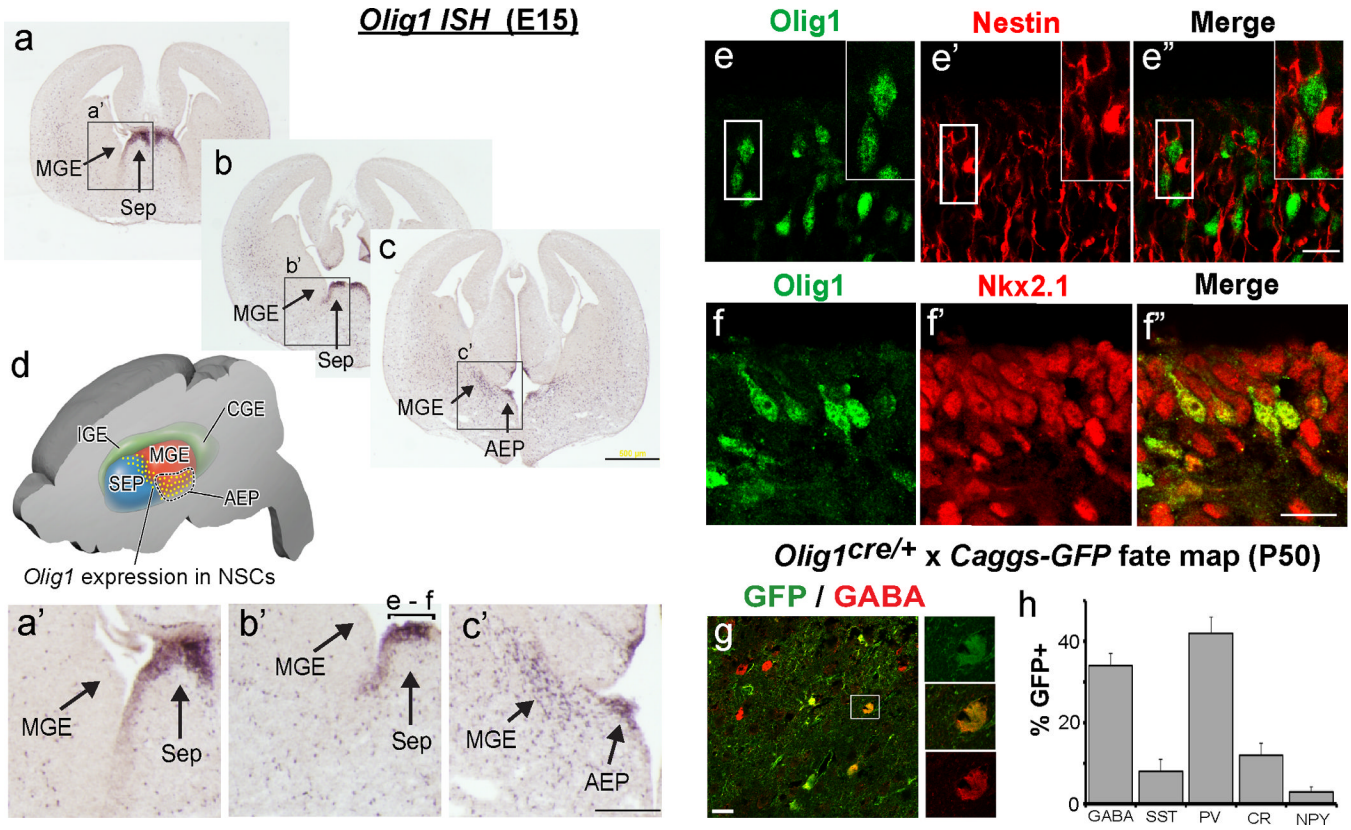

Figure 3. *Olig1* is expressed in ventral telencephalic progenitors for interneurons

(a–c) Anterior to posterior serial sections of *in situ* hybridization for *Olig1* demonstrating expression in the ventricular zone (VZ) of dorsal embryonic septum (sep), ventral medial ganglionic eminence (vMGE) and anterior enteropeduncular area (AEP). (d) A cartoon of the domain in which *Olig1* is expressed in the ventricular zone. (a'–c') Higher magnification view of the regions expressing *Olig1*. These regions are denoted by the boxes and arrows in panels a–c. The bracket labeled e-f in image b' defines the regions shown in panels e–f. (e) Confocal projections showing that Olig1 (green, e) colocalizes the radial glia protein Nestin (red, e'; merged image e"). (f) Confocal projections showing that Olig1 (green, f) colocalizes Nkx2.1+ progenitors (red, f'; merged image f") which are known to give rise to both INs and OLs. (g) Representative image of fate mapping in cerebral cortex from *Olig1*$^{cre/+}$ mice crossed to the *Caggs-Gfp* reporter mouse, showing approximately ~35% of GABA+ INs (red) are derived from *Olig1*+ progenitors as defined by the expression of the GFP+ (green) reporter protein. (h) Quantification of the proportion of a panel of IN markers (GABA, PV, SST, CR, or NPY) colabeling GFP (percentage +/− SEM). Note the preferential labeling of PV+ subtypes. (c) scale bar = 500 μm, (c') scale bar = 200 μm, (e",f") scale bar = 20 μm. Additional abbreviations: lge, lateral ganglionic eminence; cge, caudal ganglionic eminence.

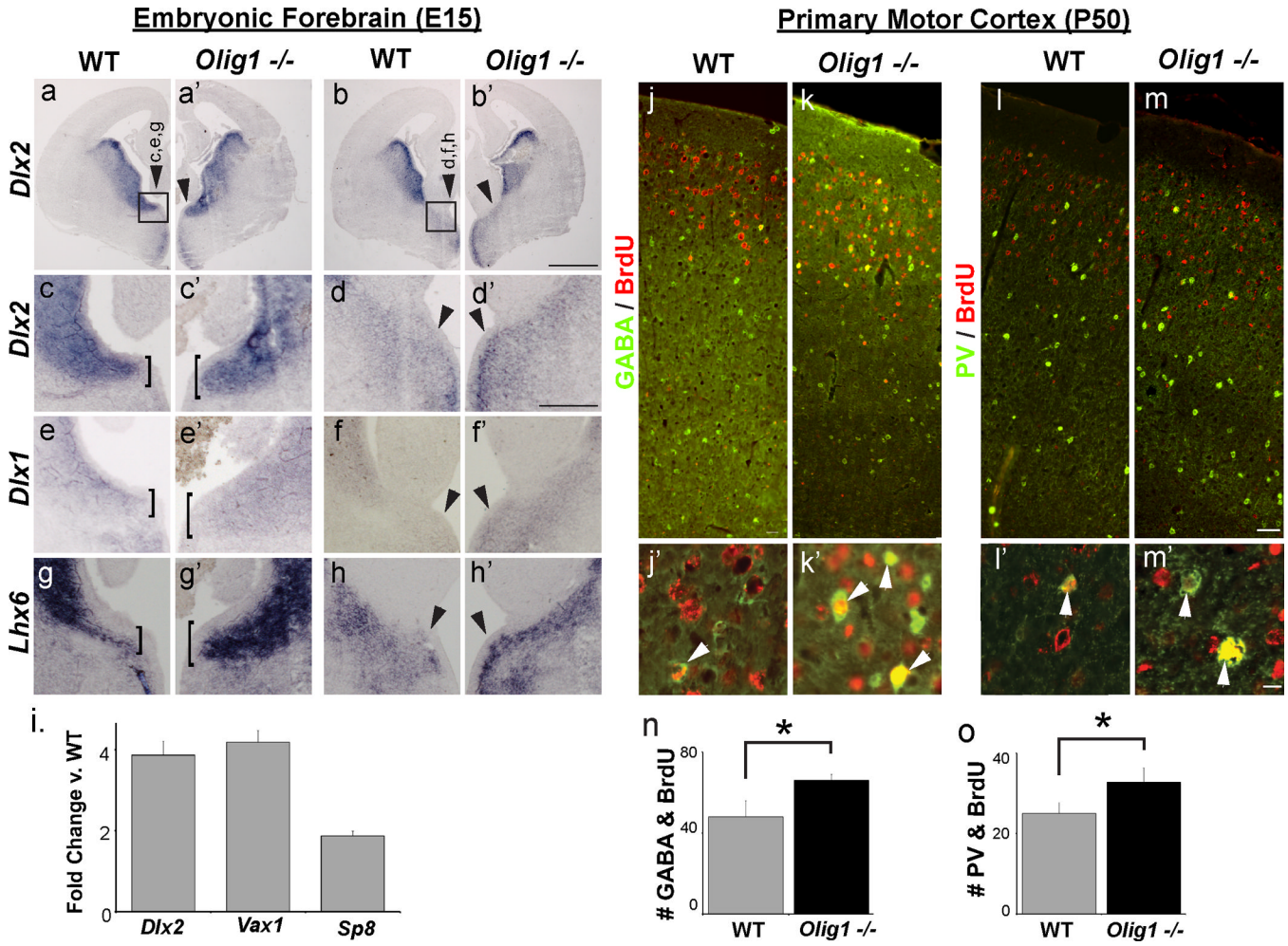

Figure 4. *Olig1* represses prointerneuron genetic programs in embryonic brain

(a–b) Representative images of *In situ* hybridization for *Dlx2* in two anterior to posterior sections of E15.5 forebrain showing upregulation of *Dlx2* in the AEP and ventral MGE (a' and b' respectively) denoted by the box and arrowheads respectively. (c–d) High magnification of *Dlx2* expression delineated in the boxed region (a–b). The brackets in (c) emphasize the expansion of the domain expressing *Dlx2* in the AEP and the arrowheads denote increased expression in ventral MGE. (e–f) High magnification images showing similar upregulated expression of *Dlx1* and (g–h) the proneural gene *Lhx6*. (i) Graph showing quantitative PCR results of cDNA samples derived from RNA samples taken from the E15 ventral forebrain of *Olig1*-null and WT mice. Note upregulation of the proneural genes *Dlx2*, *Vax1* and *Sp8*. (j–k) Representative images of GABA (green) and BrdU (red) birth dating analysis in P50 cortex demonstrating that more GABA+ INs are labeled by BrdU injected at E16. Higher magnification images demonstrating colabeling are shown in j'-k'. (l–m) Representative images of PV (green) and BrdU (red) birth dating analysis in P50 cortex demonstrating that more PV+ INs are labeled by BrdU injected at E16. Higher magnification images demonstrating colabeling are shown in l'–m'. (n–o) Quantification of GABA+ (n) or PV+(o) cells colocalizing BrdU. BrdU was injected at E16. (mean +/− SEM, n = 3; *p<.05, 2 tailed unpaired student's t test). (b') scale bar = 500 μm, (d') scale bar = 200 μm, (m,m') scale bar = 50 μm. See also Figures S3, S4, and S5

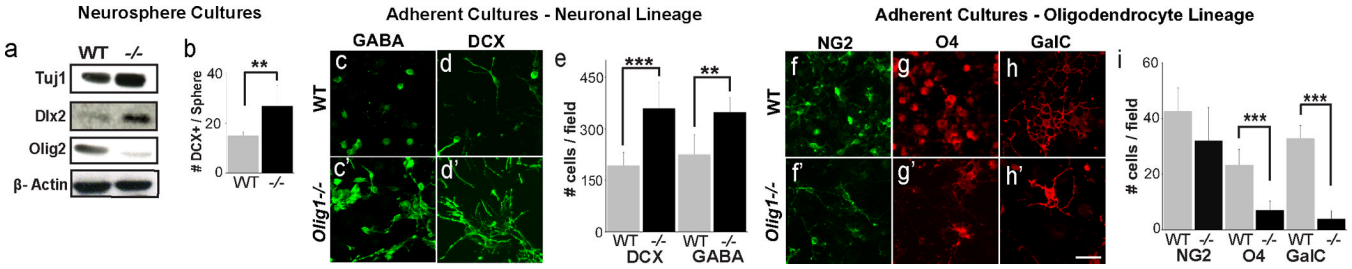

Figure 5. *Olig1* regulates interneuron versus oligodendrocyte cell fate in neural stem cell cultures

(a) Western blots from WT and *Olig1*−/− neurospheres for the neuronal protein Tuj1, Dlx2, and Olig2 showing increased expression of neuronal proteins and decreased expression of Olig2. (b) Quantification of number of DCX+ cells per neurosphere identified by immunohistochemistry (c–d) Representative images of neural progenitor monoloyer cultures derived from P3 WT and *Olig1*−/− SVZ, differentiated for 1 week and stained for DCX (c) and GABA (d). (e) Quantification of the number of DCX and GABA cells captured at 3 defined coordinates in chamber slide wells reveals increased numbers of DCX and GABA+ cells in *Olig1*−/− versus wild type. (f–h) Representative images of neural progenitor monolayer cultures derived from P3 WT and *Olig1*−/− SVZ, differentiated for 1 week and stained for NG2 (g), O4 (h) and GalC. (i) Quantification of the number of NG2, O4 and GalC cells captured at 3 defined coordinates in chamber slide wells reveals decreased numbers of O4 and GalC+ cells in *Olig1*−/− versus wild type. (For all quantifications: mean +/− SEM, n = 3 experiments, 4 slide wells per experiment; *p<.05, **p<.01, ***p<.005, 2 tailed unpaired student's t test). (h) scale bar = 50 μm. See also Figure S5.

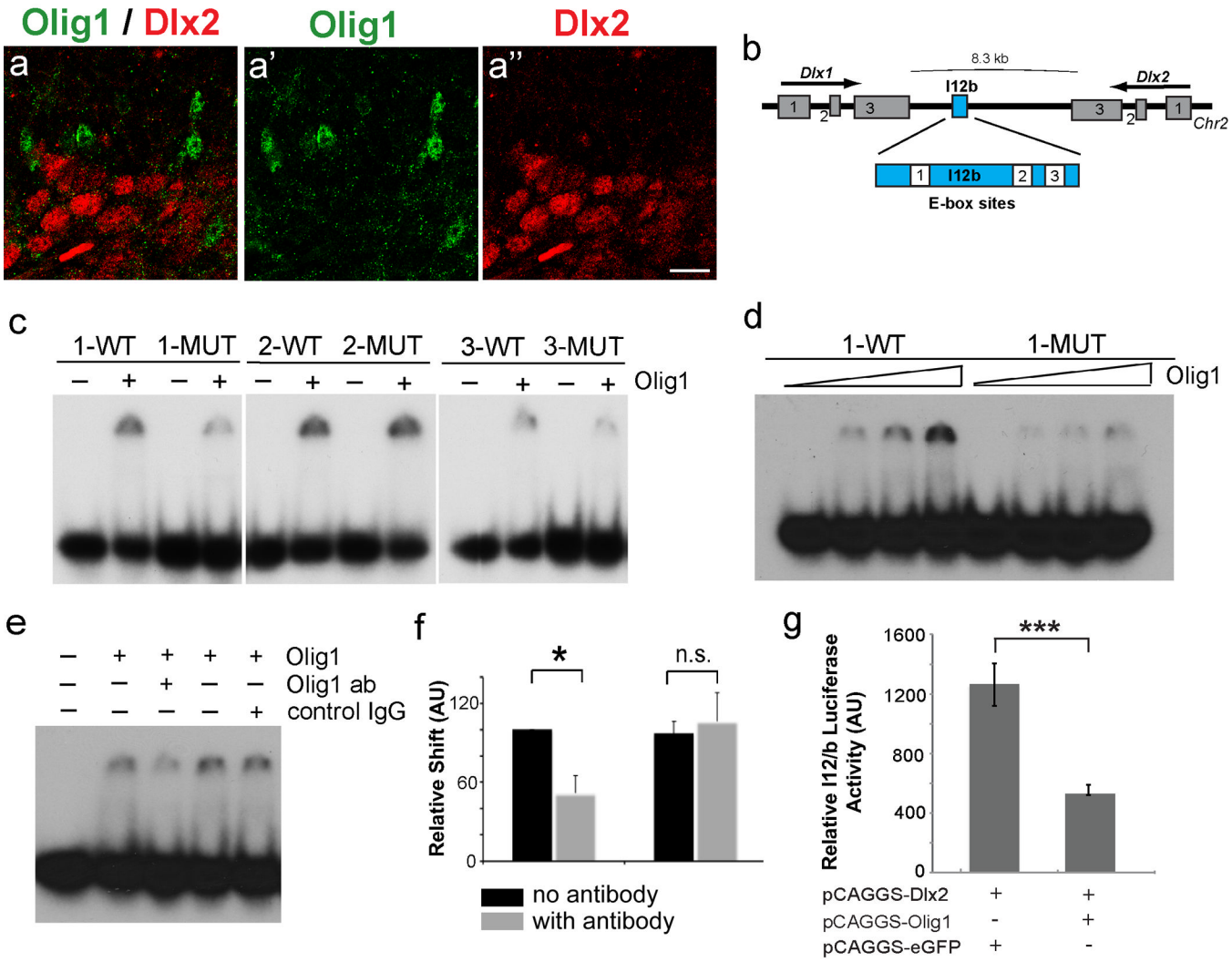

Figure 6. *Olig1* is a direct repressor of *Dlx1/2* at the *I12B* intergenic enhancer

(a) 1 μm confocal projection demonstrating that Olig1 (green, a') and Dlx2 (red, a") does not colocalize Olig1 in VZ. (b) Schematic of the *Dlx1/2* bigenic region showing location of *I12B* intergenic enhancer and 3 E-box sites. (c) Images of gels from electrophoretic mobility shift assays (EMSA) for the 3 *I12B* E-boxes (WT, wildtype and MUT, mutated) in presence or absence of *Olig1* protein. Note the strongest and most specific affinity for E-box site 1. (d) Increasing concentrations of *Olig1* protein show dose-dependent affinity of *Olig1* for E-box 1 WT, but not for E-box 1 MUT. (e) Supershift assay demonstrates that Olig1 antibody, but not control IgG antibody inhibits binding of *Olig1* protein to E-box site 1. (f) Quantification by densiometry of inhibition of DNA binding by incubation of Olig1 protein with Olig1 or control antibody (student T test * $p < 0.05$) (g) Luciferase assays demonstrate that Olig1 is a transcriptional repressor capable of reducing *Dlx2* induced *I12B* luciferase activity to 40% control levels (student's t test ***$p < 0.001$). (a") scale bar = 50 μm. See also Figures S5 and S6.

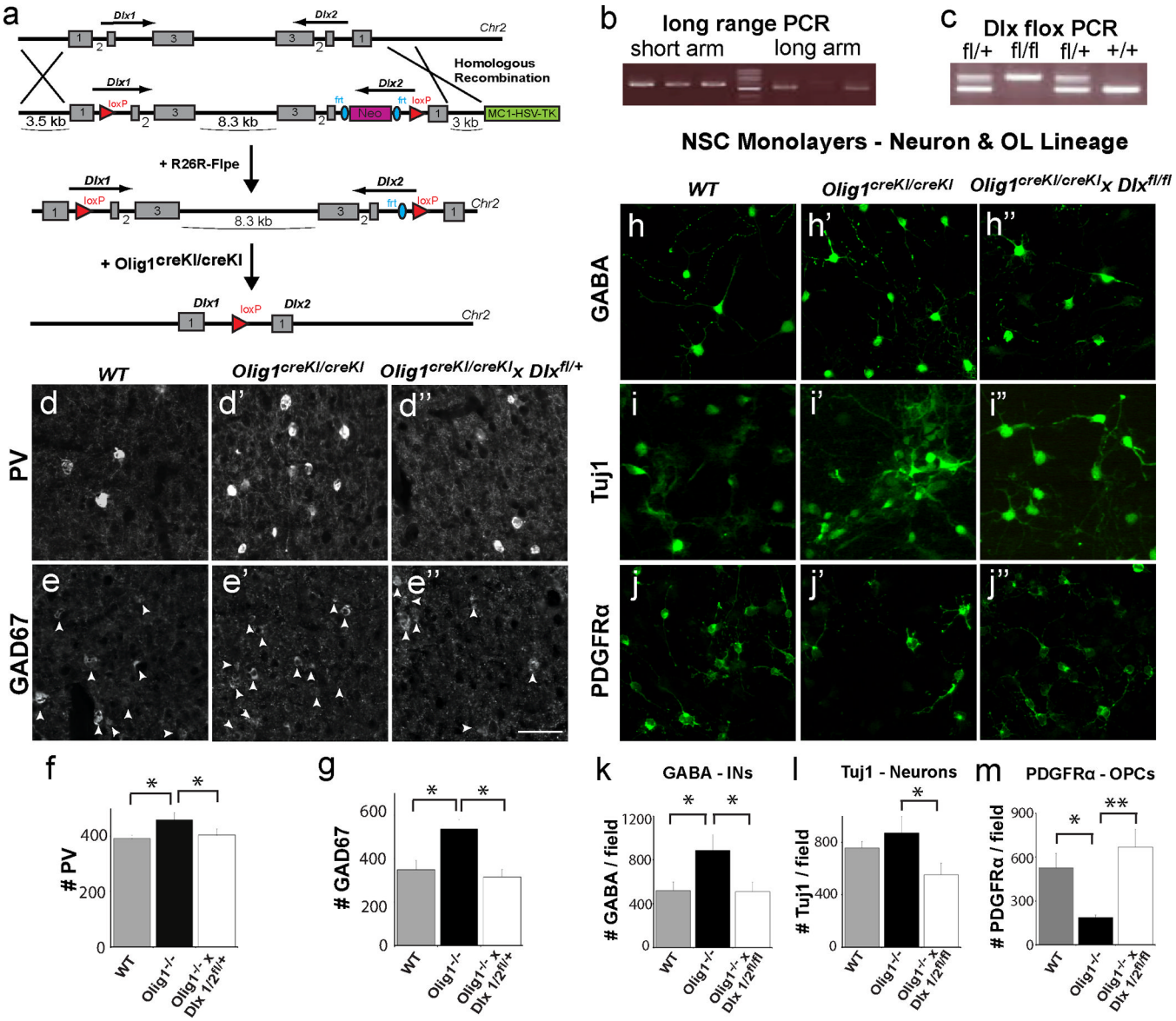

Figure 7. Increased interneuron production in *Olig1*-null animals requires *Dlx1/2* function *in vitro* and *in vivo*

(a) Schema illustrating the *Dlx1/2* flox targeting vector and strategy to knockout *Dlx1/2* within the *Olig1* lineage by generating *Dlx1/2* floxed mice ($Dlx1/2^{fl/fl}$) and crossing them to *Olig1-cre* knockin mice ($Olig1^{cre(KI)/cre(KI)}$). (b) Long range PCR for the *Dlx1/2* floxed allele confirming successful homologous recombination and integration of the full targeting construct into founder (F1) mice. (c) Representative image of PCR for the wild type and *Dlx1/2* floxed alleles demonstrating successful derivation of floxed homozygous mice. (d) Representative images of PV+ cells in cerebral cortex of P60 WT, $Olig1^{cre(KI)/cre(KI)}$, and $Olig1^{cre/cre}\times Dlx1/2^{fl/+}$ mice demonstrating that heterozygosity for *Dlx1/2* in the *Olig1* lineage is sufficient to rescue the increase in PV INs in *Olig1*-null mutants *in vivo*. (e) Representative images of GAD67+ cells in cerebral cortex of P60 WT, $Olig1^{cre(KI)/cre(KI)}$, and $Olig1^{cre(KI)/cre(KI)}\times Dlx1/2^{fl/+}$ mice demonstrating that heterozygosity for *Dlx1/2* in the *Olig1* lineage is sufficient to rescue the increase in GAD67 INs in *Olig1*-null mutants *in vivo*. (f–g) Quantification of the number of PV and GAD67 expressing cells, respectively, in

combined counts of motor and somatosensory cortex. (mean +/− SEM, n = 3, *p<.05, 2 tailed unpaired student's t test). Representative images (h–i) of GABA+ and Tuj1+ cells, respectively, generated by neural stem cell monolayer cultures derived from MGE of E14 WT, *Olig1*$^{cre/cre}$ and *Olig1*$^{cre(KI)/cre(KI)}$×*Dlx1/2*$^{fl/fl}$ mice, demonstrating that genetic ablation of *Dlx1/2* in the *Olig1* lineage is sufficient to rescue the increase in GABAergic INs in *Olig1*-null mutants *in vitro*). (j) Representative images of PDGFRa+ OPCs, demonstrating that *Dlx1/2* deletion in *Olig1* lineage cells rescues the diminution of the OL population observed in *Olig1* knockouts. (k–m) Quantification of the number of GABA+, Tuj1+, and PDGFRa+ cells respectively in NSC monolayer cultures (mean +/− SEM, n = 3 experiments, 2 slide wells per experiment; *p<.05, **p<.01, 2 tailed unpaired student's t test). (e") scale bar = 50 μm. See also Figure S7.

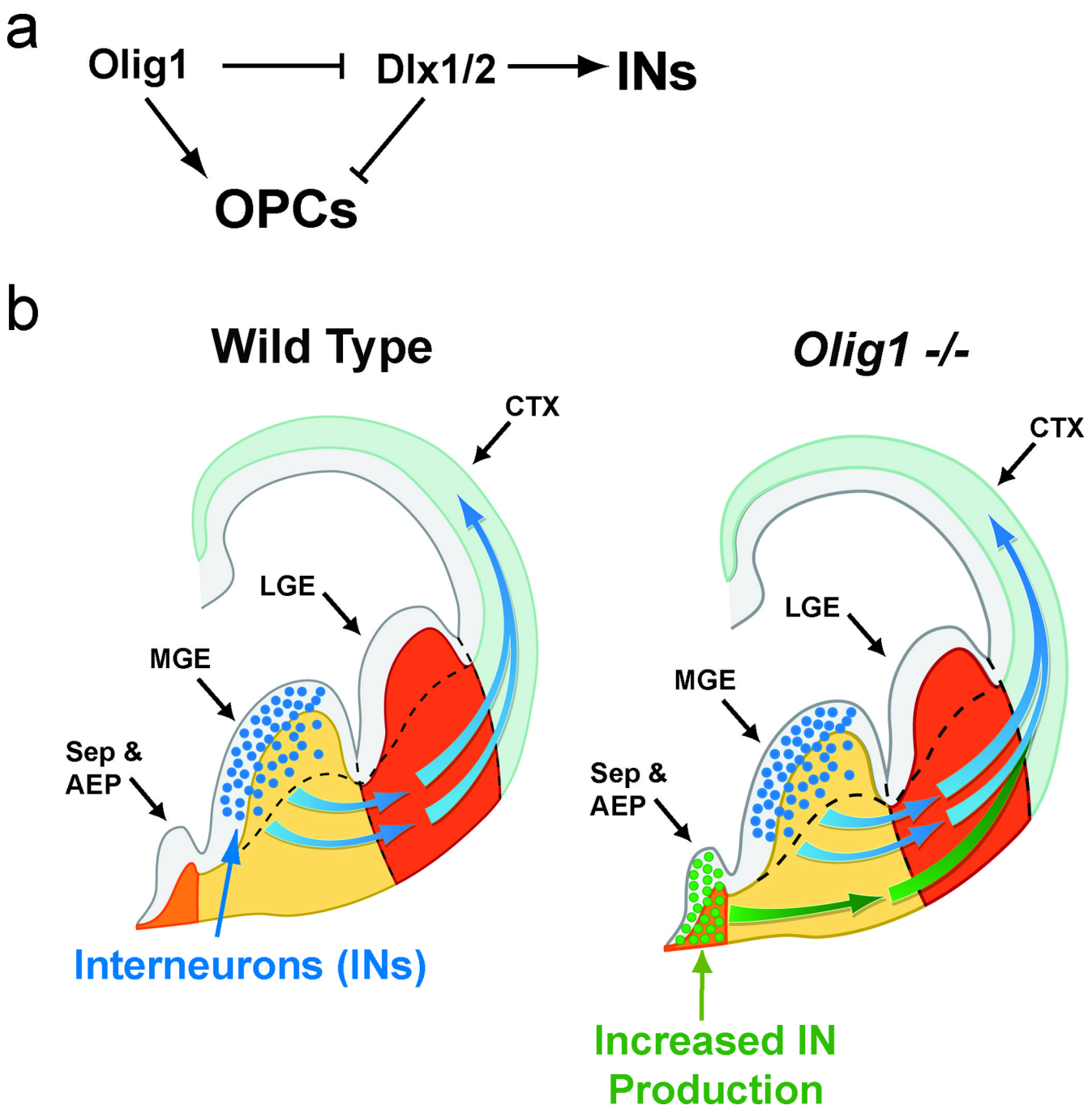

Figure 8. Model of the mechanism of *Olig1* function in the ventral telencephalon

(a) Schematic demonstrating genetic interaction between *Olig1* and *Dlx1/2* control specification of INs versus OLs. *Olig1* inhibits *Dlx1/2*, which are necessary for the production of interneurons and inhibit OL specification. (b) *Olig1* inhibits production of INs from the vMGE, AEP & septum.