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Original Article

Sox11 modulates neocortical development by regulating the proliferation and neuronal differentiation of cortical intermediate precursors

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Neural precursor cells play important roles in the neocortical development, but the mechanisms of neural progenitor proliferation, neuronal differentiation, and migration, as well as patterning are still unclear. Sox11, one of SoxC family members, has been reported to be essential for embryonic and adult neurogenesis. But there is no report about the roles of Sox11 in corticogenesis. In order to investigate Sox11 function during cortical development, loss of function experiment was performed in this study. Knockdown of Sox11 by Sox11 siRNA constructs resulted in a diminished neuronal differentiation, but enhanced proliferation of intermediate progenitors. Accompanied with the high expression of Sox11 in the postmitotic neurons, but low expression of Sox11 in the dividing neural progenitors, all the observations indicate that Sox11 induces neuronal differentiation during the neocortical development.

Keywords neocortical development; neural stem cell; proliferation; neuronal differentiation; Sox11

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Introduction

During the development of mammalian neocortex, neural precursor cells (NPCs), including radial glial cells and intermediate precursor (IP) cells, have been proven to play essential roles in neurogenic processes, such as proliferation, neuronal differentiation, neuronal migration, lamination, and patterning of neocortex [1–5]. In the ventricular zone (VZ) of the cortical telencephalon where the cerebral cortex forms, radial glial cells divide symmetrically to maintain the neural progenitor population, or divide asymmetrically to give birth to one neural progenitor and one immature neuron; during the cortical neurogenesis, radial glial cells give rise to IP cells which migrate into the subventricular zone (SVZ) and the intermediate zone (IZ) where IP cells either directly differentiate into neurons and migrate radially into cortical plate (CP), or divide once or twice before differentiating into immature neurons [5–8]. Although many transcription factors and signaling pathways have been proven to play important roles during these processes, there are still many uncovered mechanisms related to neocortical development.

SRY-box (Sox) proteins are a family of transcription factors, which includes 20 family members defined as SoxA, B, C, D, E, F, G, H subpopulations [9,10]. Among all Sox family members, SoxB and SoxC family members have been reported to be essential for NPC proliferation and differentiation [11–13]. Sox11, together with Sox4 and Sox12, belongs to SoxC subfamily, and is highly expressed in some subtypes of precursors and post-mitotic neurons [14,15]. By *in ovo* electroporation in chicken neural tube, overexpression of Sox4 or Sox11 led to an induction of neuronal markers, whereas knockdown of Sox11 expression repressed the endogenous expression of these neuron-specific markers [14]. In adult murine hippocampus, Sox11 has been demonstrated to be expressed in the doublecortin (DCX)-expressing precursor cells and immature neurons from adult neurogenic niche, and is required for neuronal differentiation in adult hippocampal neurogenesis [15,16]. Interestingly, Sox4 and Sox11 are also required for reprogramming of astroglia into neurons [16]. Furthermore, Sox4 and Sox11 have been identified as survival factors during spinal cord development [17].

Although Sox11 has been proven as an important transcription factor during the neural development and neurogenesis, there is no report about its function during neocortical formation and development. In this study, *in situ* hybridization analysis of embryonic mouse brains showed that Sox11 was highly expressed in cortical post-mitotic neurons during embryonic development. Knockdown of Sox11 expression by electroporating Sox11 siRNA construct during cortical neurogenesis resulted in an impaired neuronal differentiation

and enhanced proliferation of IP cells, suggesting its critical roles in the cortical development.

Materials and Methods

Animal

Fifteen CD1 wild-type mice (Laboratory Animal Center, Harbin Veterinary Research Institute) were used in this study. For embryo staging, the midday of vaginal plug formation was regarded as embryonic day 0.5 (E0.5). All animal procedures used in this study were conducted under the Animal Protocol of Harbin Medical University.

In situ hybridization

Mouse Sox11 mRNA fragment was amplified according to Sox11 mRNA open reading frame (ORF) using the following primer pair: 5′-GCTGGAAGATGCTGAAGGAC-3′ and 5′-GCTGCTTGGTGATGTTCTTG-3′ (amplification product size: 582 bp). Digoxygenin (DIG)-labeled antisense mRNA probe was produced by *in vitro* transcription. *In situ* hybridization on cryosections prepared by Leica cryostat (Leica, Wetzlar, Germany) was performed as described [18]. Briefly, the sections were hybridized with hybridization buffer (1 × SSC, 50% formamide, 0.1 mg/ml salmon sperm DNA solution, 1 × Denhart, 5 mM EDTA, pH 7.5) at 65°C overnight and washed with wash buffer (1 × SSC, 50% formamide, 0.1% Tween-20). After blocking for 2 h with blocking buffer (1 × MABT, 2% blocking solution, 20% heat-inactivated sheep serum), sections were labeled with anti-DIG antibody (1:1500; Roche Diagnostics, Mannheim, Germany) at 4°C overnight and washed with 1 × MABT and staining buffer (0.1 M NaCl, 5 mM $MgCl_2$, 0.1 M Tris-HCl, pH 9.5), stained with BM purple (Roche) at room temperature until ideal intensity. The images of *in situ* hybridization were collected using a Leica digital camera under a dissection scope (Leica).

Sox11 siRNA construct cloning

The siRNA oligos against Sox11 were designed according to mouse Sox11 ORF and the protocol of pSilencer^{TM} 1.0-U6 siRNA Expression Vector (Ambion, Austin, USA). After annealing, siRNA oligos were inserted into the pSilencer1.0 vector. Sox11 siRNA constructs were mixed equally as cocktail for further *in utero* electroporation.

In utero electroporation

In utero electroporation was performed as described [19]. Briefly, electroporation was conducted on E13.5 embryo cortex, and the tissues were harvested 24 h later at E14.5 or 4 days later at E17.5. Plasmid DNAs were prepared using EndoFree Plasmid Maxi Kit (Qiagen, Valencia, USA) according to the manufacturer's instructions, and diluted to 2.5 μg/μl with loading dye. DNA solution was injected into the lateral ventricle of the cerebral cortex, and electroporated for five 50-ms pulses at 35 V using an ECM830 electrosquareporator (BTX, Hawthorn, USA). The following constructs were used: pSilencer and pSilencer–mSox11–siRNA cocktail.

Western blot analysis

Protein samples were harvested from the brain tissues electroporated with Sox11 siRNA and empty pSilencer vector (only the green region) 4 days after electroporation at E17.5, and lysed with RIPA lysis buffer with protease inhibitor mixture at 4°C for 1 h. The protein samples were boiled in sodium dodecyl sulfate (SDS) loading buffer for 5 min before loading onto 10% SDS-polyacrylamide electrophoresis gel at 20 μg/lane. After running the gel, proteins were transferred onto nitrocellulose membrane. For immunoblotting, membrane was blocked with 5% non-fat milk powder in PBST [phosphate-buffered saline (PBS) with 0.05% Tween-20, pH 7.4] and incubated at 4°C overnight with primary anti-rabbit antibodies against Sox11 (1:1000; Abcam, Cambridge, USA) and Actin (1:500; Sigma-Aldrich, St Louis, USA). After washing with PBST, membrane was incubated with specific horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature and followed by extended washes with PBST. Immunoblot reactions were visualized using chemiluminescent substrate on the BioMax light films (Kodak, Rochester, USA).

BrdU incorporation

To access proliferation of NPCs in developing cortex, one dose of BrdU (50 μg/g body weight) was administrated by intraperitoneal (i.p.) injection to pregnant mice 1 h before sacrifice.

Tissue preparation and immunohistochemistry

Mouse brains were *in utero* electroporated at E13.5 and collected 1 or 4 days after electroporation at E14.5 or E17.5. After fixed in 4% paraformaldehyde in PBS at 4°C overnight, brain tissues were dehydrated by incubating in 30% sucrose in PBS, and embedded in Tissue-Tek (Sakura, Torrance, USA) and stored at −80°C until use. Brains were coronally sectioned (10 μm/section for immunohistochemistry; 16 μm/section for *in situ* hybridization) by Leica cryostat (Leica).

For immunohistochemistry, sections were incubated in heated (95–100°C) antigen recovery solution (1 mM EDTA, 5 mM Tris, pH 8.0) for 15–20 min for antigen recovery, and cooled down for 20–30 min on ice. Before applying antibodies, sections were blocked in 10% normal goat serum in PBS with 0.1% Tween-20 for 1 h. Sections were incubated with primary antibodies at 4°C overnight and visualized using goat anti-rabbit IgG-Alexa-Fluor-488,

goat anti-chicken IgG-Alexa-Fluor-488 and/or goat anti-mouse IgG-Alexa-Fluor-546 (1:200; Molecular Probes, Grand Island, USA) for 2 h at room temperature. 4′,6-diamidino-2-phenylindole was used to fluorescently stain cell nuclei. Images were captured using a Leica digital camera under a fluorescent microscope (Leica). Following primary antibodies were used: anti-chicken antibody anti-GFP (1:1000; Abcam); anti-rabbit antibodies anti-GFP (1:1000; Rockland, Gilbertsville, USA), anti-Ki67 (1:500; Abcam), anti-Tbr2 (1:500, Abcam), anti-Tbr1 (1:500; Abcam), anti-Ctip2 (1:1000; Abcam); anti-mouse antibodies anti-5-bromo-2′-deoxyuridine (BrdU) (1:50; DSHB, Iowa City, USA), NeuN (1:300; Millipore, Billerica, USA), and Cux1 (1:300; Santa Cruz Biotechnology, Santa Cruz, USA).

Statistical analysis

At least three brains and at least three sections from each brain were collected for antibody labeling, cell counting, and statistical analysis. Positive cells were counted in the same-width electroporated cortical region. The percentage of double-positive cells for cell type markers and green fluorescent protein (GFP) in total GFP-positive cells were presented in this study. Statistical analysis was performed by unpaired Student's *t*-test, and data were presented as mean ± standard deviation (SD).

Results

Sox11 is highly expressed in post-mitotic neurons

In situ hybridization analysis was performed to investigate the expression pattern of Sox11 during neocortical development. Results showed that at E12.5, Sox11 was highly expressed in the early-born neurons in the cortex and ganglionic eminence, while weakly expressed in the proliferating regions VZ and SVZ [**Fig. 1(A,A′)**]. At E15.5, Sox11 is highly expressed in two layers among cortex—early-born neurons, which localized near the IZ, and late born neurons, which localized near to the top of the CP. Among the VZ and SVZ, Sox11 was weakly expressed in these neurogenic regions [**Fig. 1(B,B′)**].

Sox11 siRNA construct cocktail efficiently knockdowns Sox11 expression in the developing cortex

Based on Sox11 mRNA coding region, two Sox11 siRNA oligos were designed according to Ambion pSilencer^{TM} 1.0-U6 siRNA Expression Vector protocol [**Fig. 2(A)**] and cloned into pSilencer 1.0 vector. To test knockdown effect, two Sox11 siRNA constructs were mixed as cocktail, and electroporated into E13.5 murine cortex. The empty pSilencer vector was electroporated as control. Four days after electroporation, the electroporated brain tissues (green regions under a fluorescence microscope) were harvested and proteins were extracted for western blot analysis [**Fig. 2(B)**]. Western blot results showed that Sox11 siRNA cocktail largely inhibited the Sox11 expression in the embryonic cortex after electroporation [**Fig. 2(C)**].

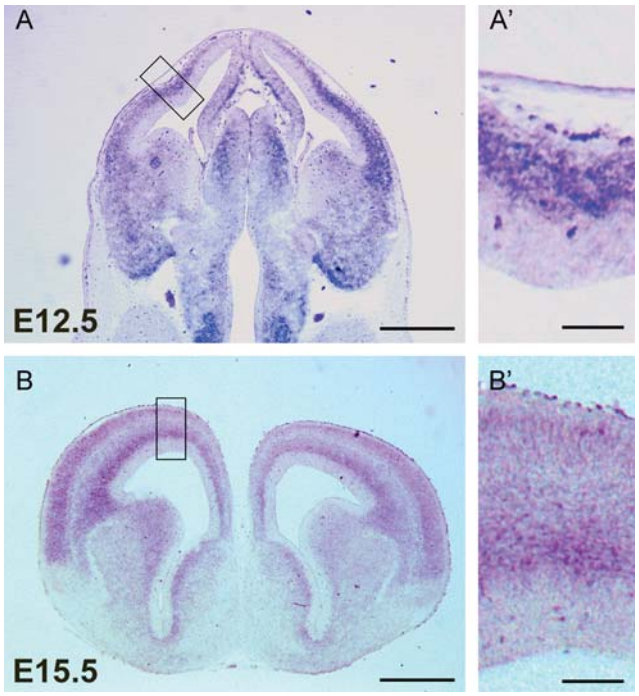

Figure 1 Expression pattern of Sox11 during the mouse cortical development *In situ* hybridization analysis of Sox11 expression was performed in embryonic day 12.5 (E12.5) murine cortex (A and A′), E15.5 murine cortex (B and B′). Scale bar: 500 μm in A and B; 100 μm in A′ and B′.

Knockdown of Sox11 enhances the proliferation of intermediate progenitors

The effect of Sox11-knockdown on neural progenitor proliferation is tested by electroporating Sox11 siRNA cocktail into E13.5 mouse embryos, and brains were collected 24 h after electroporation. One hour before sacrifice of mice, one dose of BrdU was i.p. injected into mice to label the dividing progenitors [**Fig. 3(A)**]. Antibody against GFP was applied to label the electroporated cells; antibodies against BrdU (to labels the S phase of cell cycle) and Ki67 (to labels the all cell cycle except G0) were applied to immunostain proliferating cells; antibody recognizing Tbr2 was applied to mark IP cells.

Results showed that compared with the brain tissues electroporated with empty vector, those electroporated with Sox11 siRNA have significant higher percentage of both $BrdU^+/GFP^+$ cells (38.0% ± 4.0% versus 30.6% ± 1.8%; $n = 23$) [**Fig. 3(B)**] and $Ki67^+/GFP^+$ cells (44.3% ± 3.2% versus 32.6% ± 2.8%; $n = 23$) [**Fig. 3(C)**] in total GFP^+ cells, and the percentage of $Tbr2^+/GFP^+$ double-positive cells in total GFP^+ cells in Sox11-knockdown group was

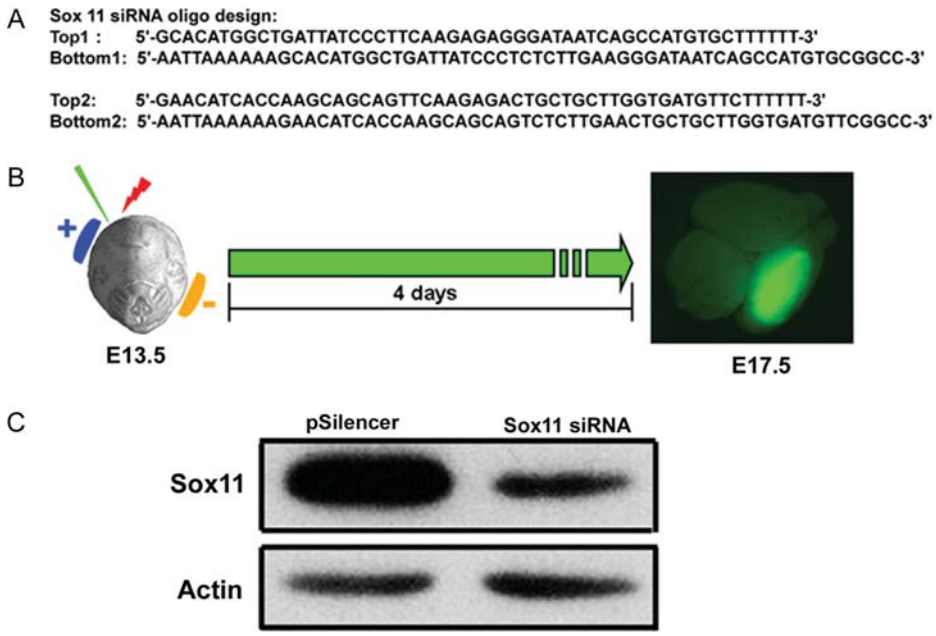

Figure 2 Sox11 siRNA constructs knockdown the expression of Sox11 24 h after *in utero* electroporation in E13.5 murine brains (A) Sox11 siRNA oligo sequences. (B) The schematic description of *in utero* electroporation. (C) Western blot analysis of Sox11 expression in brain tissues electroporated with Sox11 siRNA constructs in comparison with the brain tissues electroporated with empty vector pSilencer. Antibody against actin was used to demonstrate equal protein loading.

also higher than empty vector group (45.8% ± 2.9% versus 35.2% ± 2.9%; $n = 23$) [**Fig. 3(D)**], suggesting that knockdown of Sox11 results in an increased proliferation of IP. Since knockdown of Sox11 caused enhanced neural progenitor proliferation, it will be very interesting to investigate whether knockdown of Sox11 could also affect neuronal differentiation.

Knockdown of Sox11 impairs neuronal differentiation by keeping neural precursors in cell cycle

By electroporating Sox11 siRNA construct cocktail into E13.5 mouse brains, the effect of loss of function of Sox11 on neuronal differentiation was analyzed 4 days after electroporation [**Fig. 4(A)**]. Antibody against GFP was applied to label electroporated cells. Antibodies against different neuronal markers NeuN (to label total mature neurons), Tbr1 (to label early-born neurons in layer VI), Ctip2 (to label neurons in layer V), and Cux1 (to label late-born pyramidal neurons of the upper layers II–IV) were applied to label mature neurons at different CP layers. The percentages of $Ki67^+/GFP^+$, and $Tbr2^+/GFP^+$ double-labeled cells in the total electroporated GFP^+ cells were also analyzed to determine what percentage of electroporated cells still stays in progenitor status 4 days after electroporation.

Results showed that 4 days after electroporation, murine brains electroporated with Sox11 siRNA construct cocktail have remarkable less $NeuN^+/GFP^+$ (17.7% ± 3.2% versus 28.3% ± 5.4%; $n = 12$) [**Fig. 4(B)**], $Ctip2^+/GFP^+$ (11.3% ± 1.1% versus 14.3% ± 2.5%; $n = 12$) [**Fig. 4(C)**], $Tbr1^+/GFP^+$ (16.9% ± 1.5% versus 22.8% ± 2.8%; $n = 12$) [**Fig. 4(D)**], and $Cux1^+/GFP^+$ (44.3% ± 1.9% versus 48.1% ± 2.9%; $n = 12$) [**Fig. 4(E)**] double-positive cells in total GFP^+ cells compared with the control brains electroporated with empty vector. At the meantime, the percentages of $Ki67^+/GFP^+$ (29.3% ± 3.4% versus 24.0% ± 2.4%; $n = 15$) [**Fig. 5(A,B)**] and $Tbr2^+/GFP^+$ (26.8% ± 1.9% versus 22.5% ± 1.8%; $n = 14$) [**Fig. 5(C,D)**] double-positive cells in total GFP^+ cells from the Sox11 siRNA-electroporating group were significantly higher than those in the control group. Interestingly, both in SVZ (25.2% ± 2.0% versus 22.0% ± 1.4%; $n = 14$) [**Fig. 5(E)**] and IZ (1.7% ± 1.7% versus 0.4% ± 1.0%; $n = 14$) [**Fig. 5(E)**], the percentages of $Tbr2^+/GFP^+$ cells in total GFP^+ cells from Sox11-knockdown group were higher than those in the control group. All these observations suggest that knockdown of Sox11 results in an impaired neuronal differentiation, but increased neural progenitor pool.

Discussion

Sox proteins are a family of transcription factors with high-mobility group-type DNA-binding domain, and have been subdivided into eight subfamilies A–H [10,20]. Among all Sox proteins, SoxB1 proteins, including Sox1, Sox2, and Sox3, have been shown to participate in maintenance of undifferentiation status of embryonic and adult neural stem cells (NSCs) [21]. SoxE proteins, including Sox8, Sox9, and Sox10, are expressed and play their roles in neural

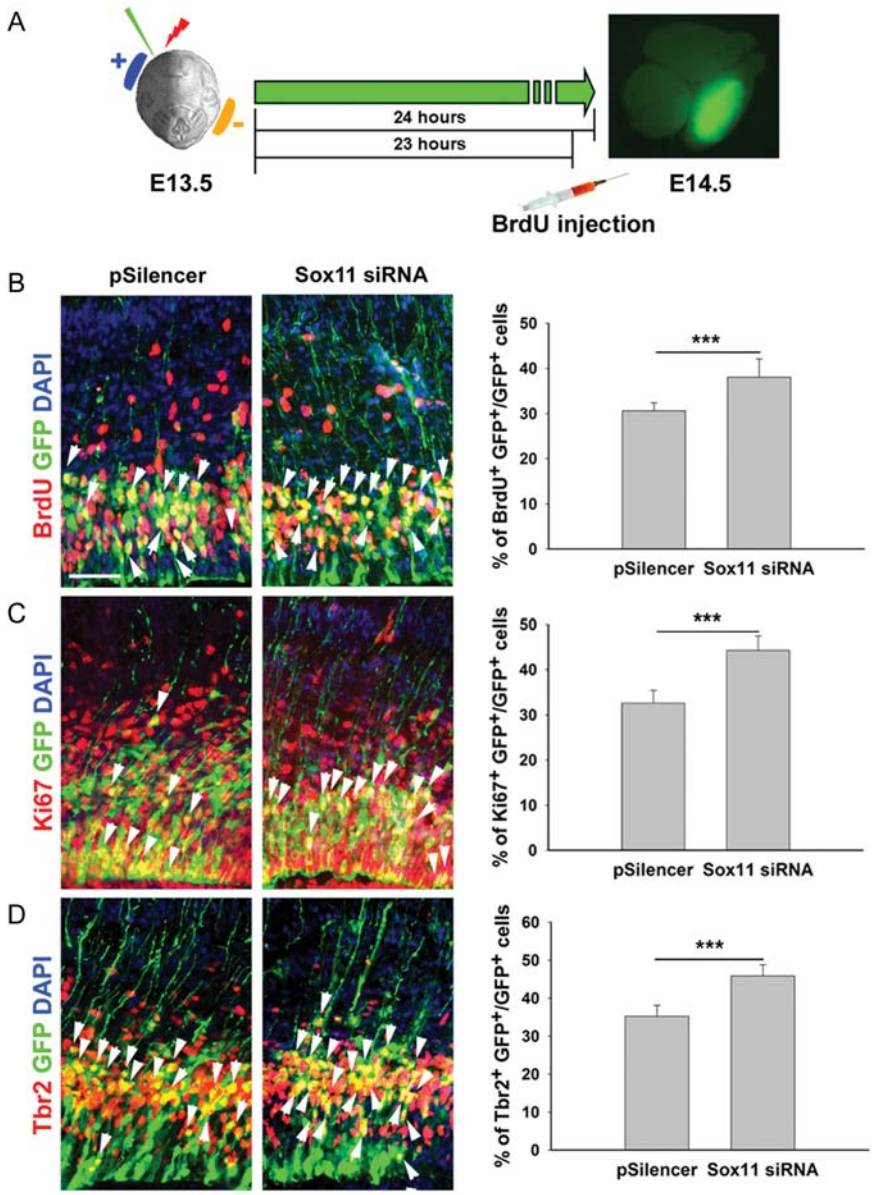

Figure 3 Knockdown of Sox11 results in an enhanced proliferation of intermediate procursors (A) The schematic description of *in utero* electroporation for proliferation assay. E13.5 mouse embryonic brains were electroporated and collected 24 h later at E14.5. One hour before sacrifice, one dose of BrdU was i.p. injected to label the proliferating cells. (B–D) Photomicrographs and quantification of percentage of $BrdU^+/GFP^+$ (yellow) double-positive cells (B), $Ki67^+/GFP^+$ (yellow) double-positive cells (C), and $Tbr2^+/GFP^+$ (yellow) double-positive cells (D) in total GFP^+ (green) cells. Statistical significance was assessed by Student's *t*-test. $^{***}P < 0.001$. Scale bar: 50 μm.

crest cells [22,23]. SoxC proteins, including Sox4, Sox11, and Sox12, are expressed in both central nervous system (CNS) and peripheral nervous system, and play roles in the establishment of neuronal properties [14,24–26].

High-throughput RNA sequencing analysis of transcriptome profiling of embryonic and neonatal murine cortex revealed that SoxC proteins, Sox4 and Sox11, were highly expressed in the embryonic stage, a neurogenic period, rather than postnatal stage [27]. Immunohistochemical analysis of SoxC proteins revealed that Sox11 as well as Sox4 were co-expressed in the neurogenic region in the embryonic and adult murine brain, such as the SVZ of the lateral ventricle, the subgranular zone of the hippocampal dentate gyrus, and the rostral migratory stream [15,16]. Further investigation showed that Sox11 was expressed in DCX-expressing precursor cells and immature neurons in the adult neurogenic niches [15,16]. In this study, to investigate functions of Sox11 in the neocortical development, the expression pattern of Sox11 was analyzed during the embryonic neocortical development using *in situ* hybridization technique. At E12.5, the starting time point of cortical neurogenesis, Sox11 was highly expressed in the upper layer of cerebral cortex, which is formed by the early-born immature neurons and some NPCs. At E15.5, the peak of

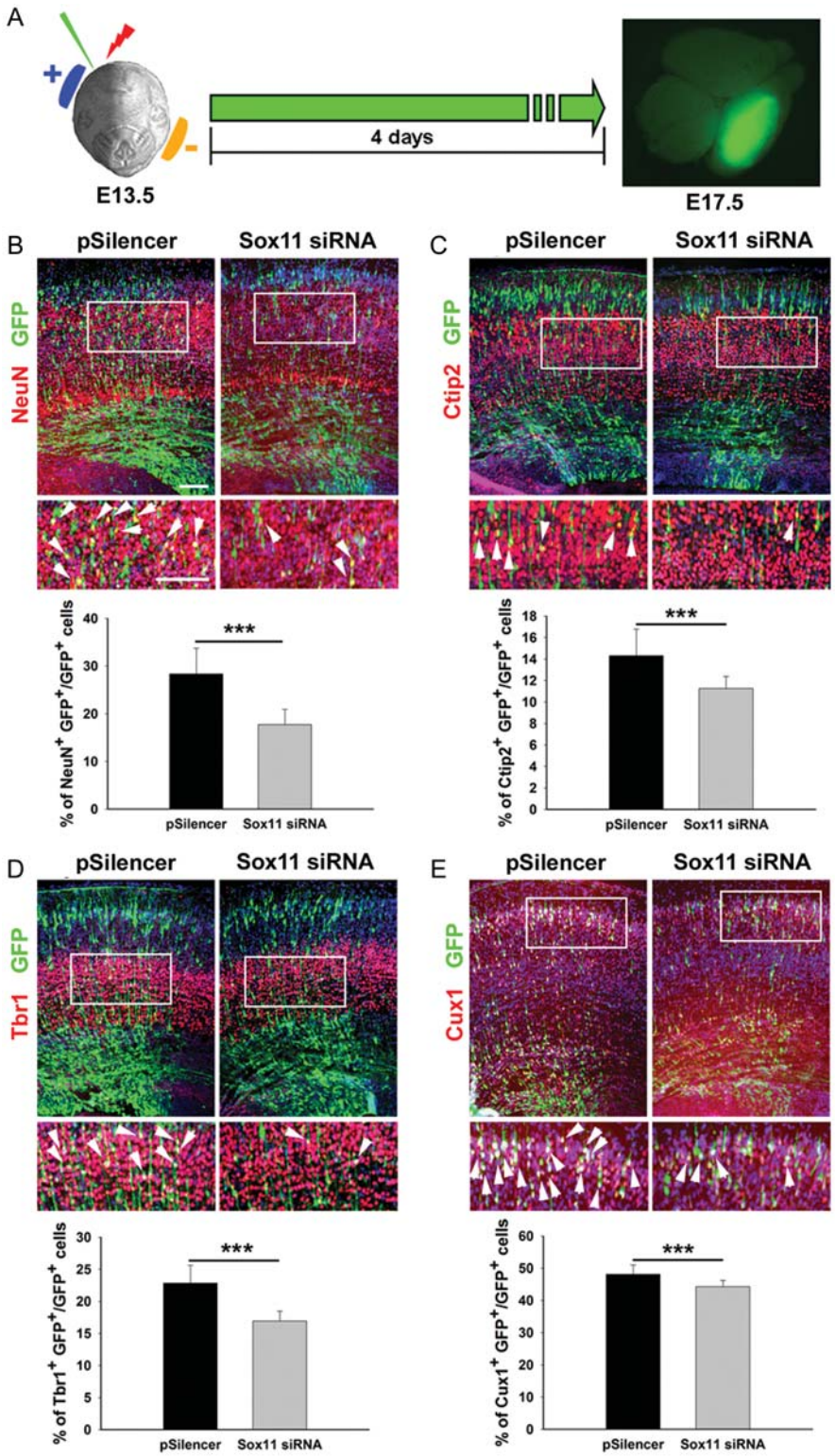

Figure 4 Knockdown of Sox11 results in an impaired neuronal differentiation (A) The schematic description of *in utero* electroporation for neuronal differentiation assay. E13.5 mouse embryonic brains were electroporated and collected 4 days later at E17.5. (B–E) Photomicrographs and quantification of percentage of $NeuN^+/GFP^+$ (yellow) double-positive cells (B), $Tbr1^+/GFP^+$ (yellow) double-positive cells (C), $Ctip2^+/GFP^+$ (yellow) double-positive cells (D), and $Cux1^+/GFP^+$ (yellow) double-positive cells (E) in total GFP^+ (green) cells. Statistical significance was assessed by Student's *t*-test. $^{***}P < 0.001$. Scale bar: 100 μm.

cortical neurogenesis, Sox11 was highly expressed in the top of the CP, formed by late-born neurons, lower layer of CP, formed by early-born neurons, as well as IP, formed by immature neurons and IP cells. All these observations indicated that Sox11 might be involved in cortical neurogensis.

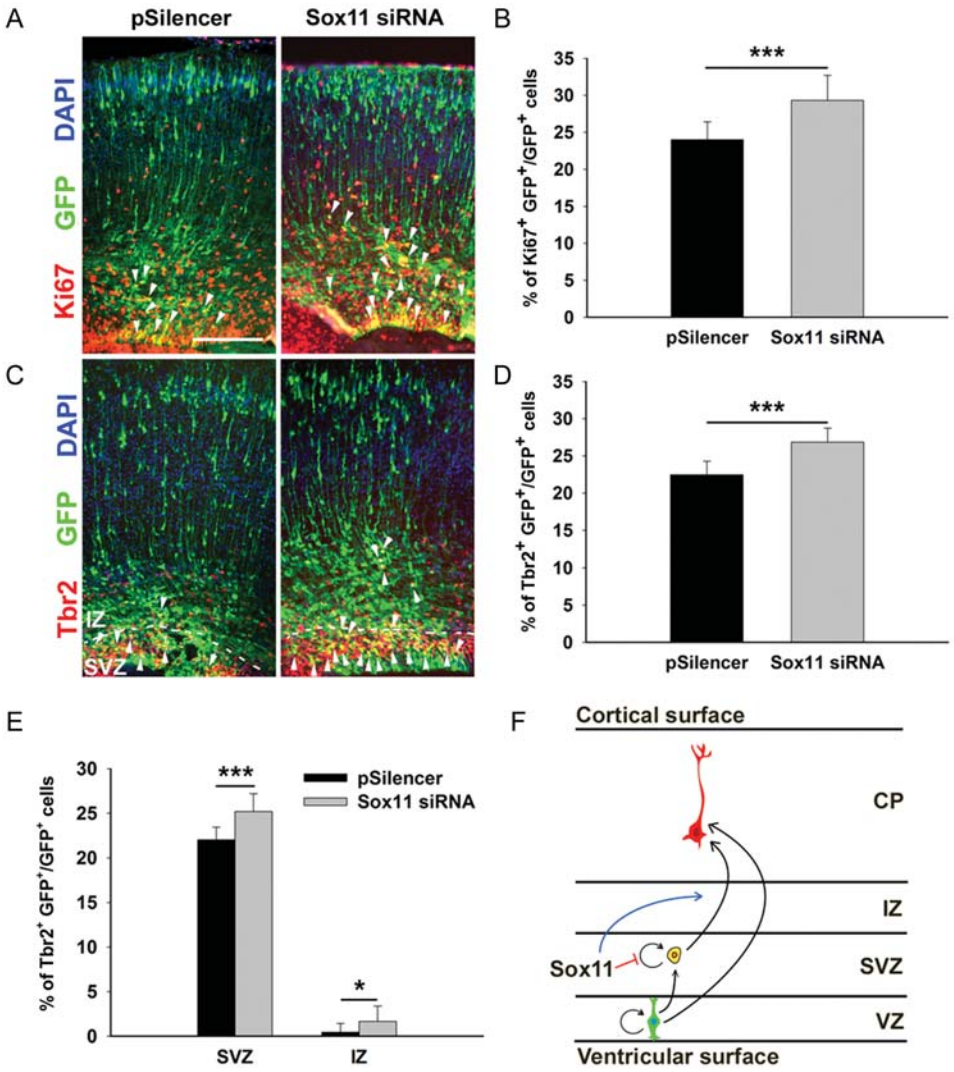

Figure 5 Knockdown of Sox11 keeps neural progenitors stay in proliferation status during neurogenesis. (A,B) Photomicrographs and quantification of percentage of Ki67$^+$/GFP$^+$ (yellow) double-positive cells in total GFP$^+$ (green) cells. (C,D) Photomicrographs and quantification of percentage of Tbr2$^+$/GFP$^+$ (yellow) double-positive cells in total GFP$^+$ (green) cells. (E) Quantification of percentage of Tbr2$^+$/GFP$^+$ (yellow) double-positive cells in total GFP$^+$ (green) cells at the IZ and the SVZ. (F) Summary of Sox11 functions during the embryonic cortical development. Radial glial cell was shown as green; IP cell was shown as yellow; neuron was shown as red. Statistical significance was assessed by Student's *t*-test. *$P < 0.05$; ***$P < 0.001$. Scale bar: 100 μm.

Previous studies showed that as a transcription factor induced by proneural protein basic helix-loop-helix as NPCs develop into immature neurons, Sox11 plays important role in embryonic and adult neurogenesis [14]. Overexpressing Sox11 in the VZ of chicken spinal cord resulted in a strong ectopic expression of neuronal markers Tuj1 and MAP2, while knocking down the expression of Sox11 showed opposite effects, leading to a suppression of endogenous expression of multiple neuronal markers [14]. All these indicated the essential roles of Sox11 in the neurogenesis. In another study, overexpressing Sox11 in *in vitro* cultured adult NSCs by retrovirus resulted in an increased generation of DCX$^+$ immature neurons, and promoted neurogenesis of adult NSCs [15]. Furthermore, using transgenic mouse model as well as retrovirus system, Lie's group has demonstrated the roles of SoxC, including Sox4 and Sox11, in the neurogenesis in adult hippocampus [16]. Deletion of Sox4/Sox11 inhibited the neurogenesis of adult hippocampal NSCs both i*n vitro* and *in vivo*, whereas ectopic expression of Sox4 and Sox11 promoted neurogenesis of cultured adult hippocampal precursors *in vitro* [16]. Lie's group observed that under *in vitro* culture condition, deletion of Sox4 and Sox11 led to a decreased proliferation of differentiating adult NSCs, while *in vivo* in adult hippocampus, many Sox4/Sox11-deficient cells also expressed stem/precursor marker Sox2, but not proliferation marker Ki67, IP marker Tbr2, nor astroglial marker glial fibrillary acidic protein (GFAP) [16]. They suggested that Sox4/Sox11-deleted cells remained a non-proliferative, GFAP$^-$, but Sox2-expressing status, but cell fate of these cells remained unclear.

Although the function of SoxC proteins, including Sox11, in neurogenesis has been demonstrated in different species and different neural regions, there is no report about roles of Sox11 in neocortical development. In this study, loss of function of Sox11 has been performed using siRNA and *in utero* electroporation technique to investigate roles of Sox11 in neurogenesis during corticogenesis. Similar to the previous reports, knockdown of Sox11 caused a diminished neuronal differentiation: 4 days after knockdown of Sox11, the percentages of different neuronal markers, including NeuN, Tbr1, Ctip2, and Cux1, positive cells in total electroporated cells were significantly decreased compared with the control group. Interestingly, more $Ki67^+$ and $Tbr2^+$ cells were observed in Sox11-knockdown cells 4 days after electroporation. Analysis of the percentage of $Tbr2^+/GFP^+$ cells from the SVZ and the IZ separately showed that not only in SVZ, but also in the IZ, Sox11-knockdown cells had more $Tbr2^+$ co-expressed cells than empty vector-electroporated cells. Proliferation analysis was performed 1 day after electroporation. $Ki67^+$, $Tbr2^+$, as well as S-phase marker $BrdU^+$ cells were counted, which showed knockdown of Sox11 led to an enhanced proliferation of IP cells in Sox11-ablated cells in the developing cortex. All these observations indicated that during neocortical formation, suppressing the expression of Sox11 could keep Sox11-abalated cells stay in the proliferating and NPC status [**Fig. 5(F)**]. Our observation of the function of Sox11 on neurogenesis is consistent with the results of previous studies from different groups. But regarding the effects of Sox11 on proliferation and cell fate, our results were not completely identical with theirs, which might be due to different cell origins, different developing stages, and detection methods. It has been shown that SoxC proteins, Sox4, Sox11, and Sox12, are expressed complementary to stem/precursor markers SoxB1 proteins, Sox1, Sox2, and Sox3: all three SoxB1 proteins are expressed in most neural progenitors in developing and adult CNS, and maintain them in a precursor state. SoxC proteins are mostly expressed in post-mitotic differentiating neurons, and induce expression of different neuronal markers Tuj1 and MAP2 [14,28]. Further investigation using chromatin immunoprecipitation technique revealed that SoxC proteins share a high number of target genes with SoxB1 proteins, suggesting that SoxC proteins and SoxB1 proteins modulate cell fate of NPCs via a common sets of target genes involved in CNS development, including Sox proteins, Notch signaling molecules, Wnt protein, transforming growth factor-β family members, fibroblast growth factors and Pax transcription factors [28]. Further real-time PCR analysis revealed that among these shared target genes, ectopic expression of SoxB1 protein upregulated the expression of NPC genes, while forced expression of SoxC resulted in an increased expression of neuronal genes, and competitive binding activities of SoxB1 and SoxC balance the transition between NPCs and post-mitotic neurons [28]. Together with the findings from Lie's group—many Sox4/Sox11-deficient cells also expressed stem/precursor marker Sox2—knockdown of Sox11 maintain NPCs in undifferentiated state. Further investigation of spatiotemporal expression and functions of Sox11 at different neural regions and different developing periods is expected to allow insights into Sox11-regulating neural development and neural disorders.

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