Figure S1 Ohtsuka et al.

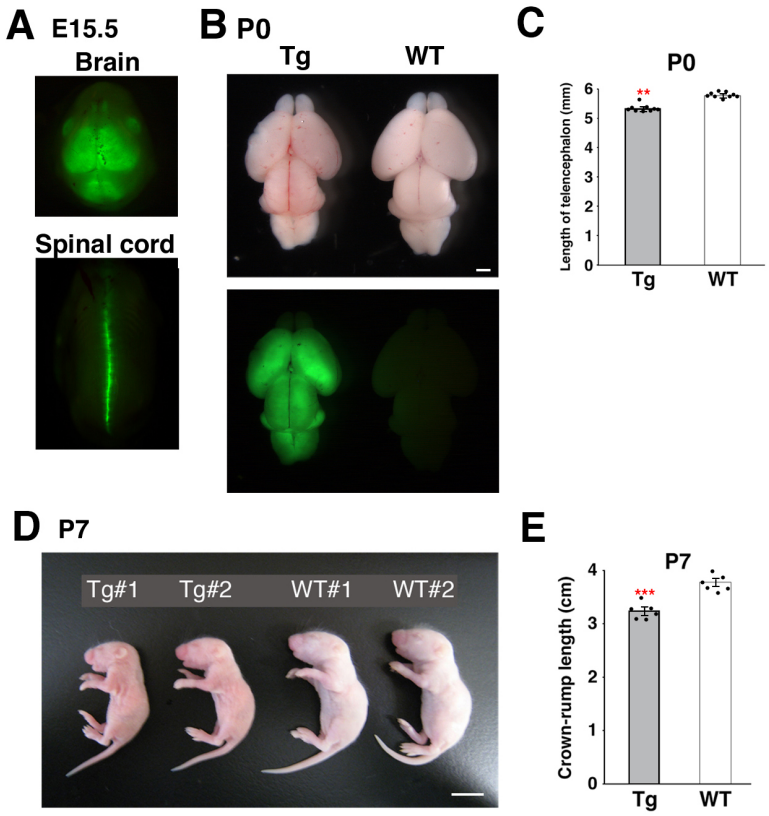


Fig. S1. Appearance of Hes1-overexpressing Tg mice and GFP expression in the central nervous system. (**A**) GFP expression in the brain and spinal cord of Tg mice at E15.5. (**B**) Appearance of Tg and WT brains and GFP expression at P0. (**C**) Comparison of the length of telencephalon of Tg vs WT pups at P0. (**D**) Appearance of the whole body of Tg and WT mice at P7. Note that the Tg pups exhibit smaller body size. (**E**) Comparison of body size (crown-rump length) of Tg vs WT pups at P7. Data are presented as mean±s.e.m. (n=9 for C; n=6 for E); **P<0.01, ***P<0.001 (Student's t-test). Scale bars: 1 mm in B; 10 mm in D.

Figure S2 Ohtsuka et al.

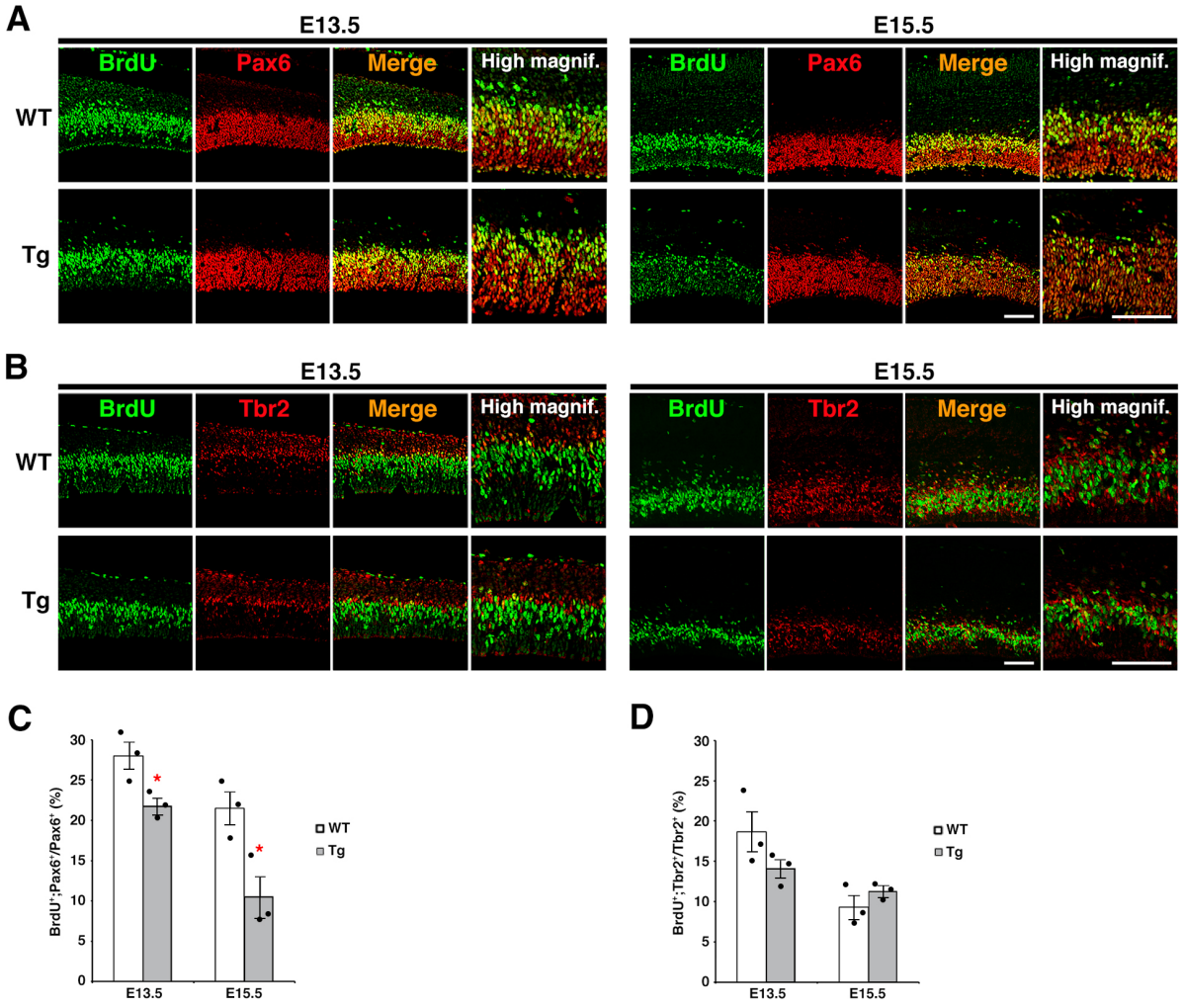


Fig. S2. Cell proliferation rate in NSCs and IPCs. (**A,B**) Double-labeling with anti-BrdU (green) and anti-Pax6 (red) (A) or anti-Tbr2 (red) (B) antibodies on coronal sections of neocortical regions of WT and Tg mice at E13.5 and E15.5. BrdU was injected intraperitoneally into pregnant mice 30 minutes before sacrifice. Higher magnification views are shown in the right panels. (**C,D**) Graphs showing the proportions of $BrdU^+$ cells among $Pax6^+$ cells (C) or $Tbr2^+$ cells (D). Data are presented as mean±s.e.m. (n=3); *P<0.05 (Student's t-test). Scale bars: 100 µm.

Figure S3 Ohtsuka et al.

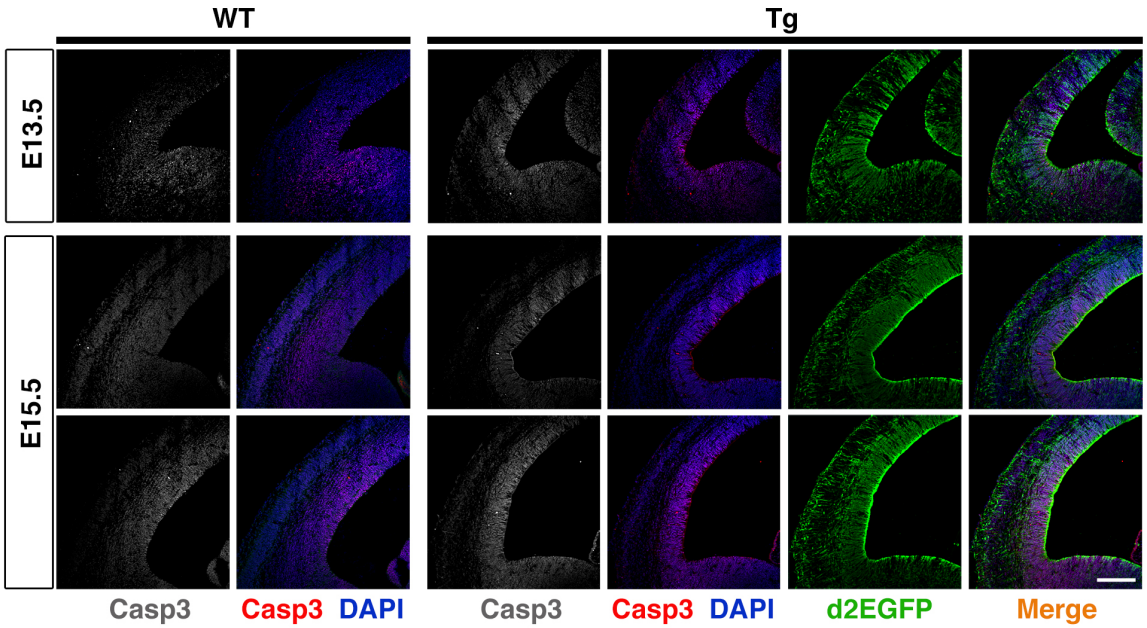


Fig. S3. Estimation of cell death in embryonic neocortical regions. Coronal sections of neocortical regions of WT and Tg embryos at E13.5 and E15.5 were immunostained with anti-cleaved caspase-3 (Casp3) (red) and anti-GFP (green) antibodies. Casp3 signals are shown in white on the left panels (grey scale). DAPI (blue) represents nuclear staining. Scale bars: 200 μm.

Figure S4 Ohtsuka et al.

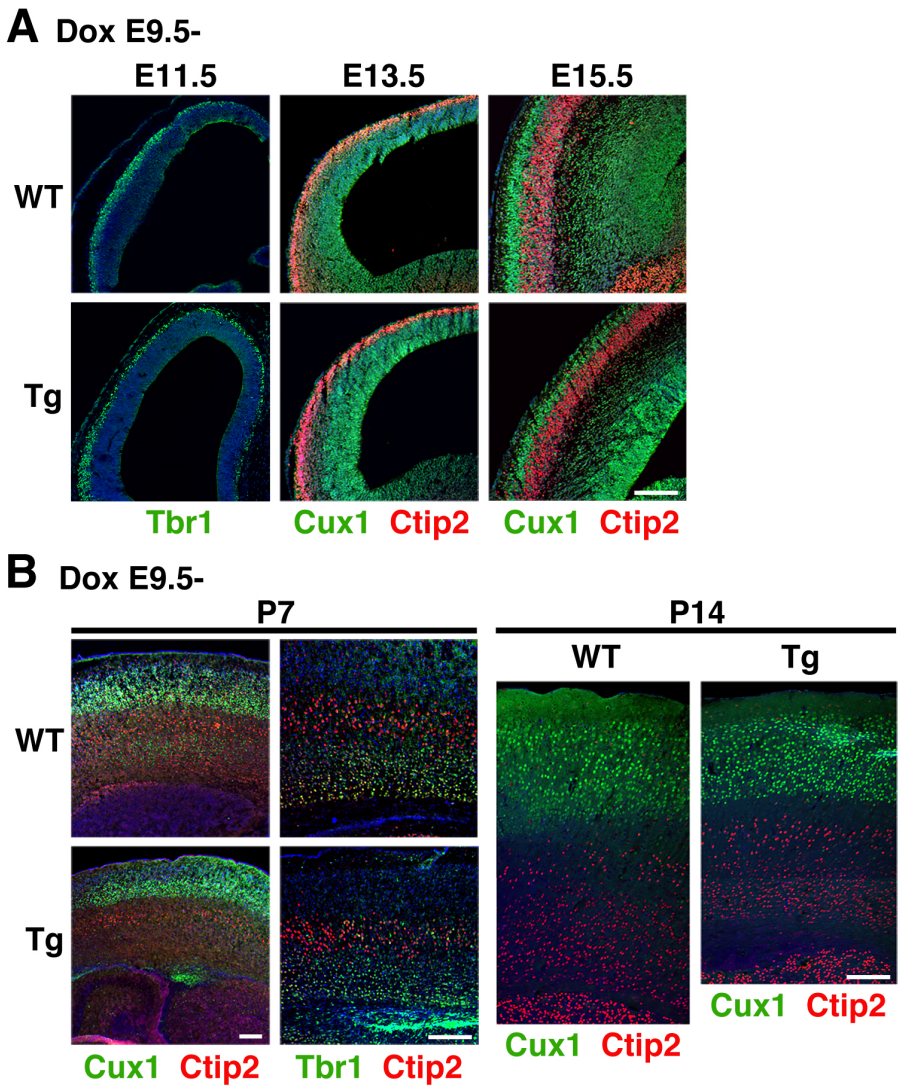


Fig. S4. Neurogenesis at early to mid-embryonic stages and layer structure of the postnatal cerebral cortex. (**A**) Immunohistochemistry on coronal sections of the dorsolateral telencephalon of WT and Tg embryos; immunostaining with an anti-Tbr1 (green) antibody at E11.5 and double-labeling with anti-Cux1 (green) and anti-Ctip2 (red) antibodies at E13.5 and E15.5. (**B**) Immunohistochemistry on coronal sections of WT and Tg cortices with anti-Cux1 (green), anti-Ctip2 (red), and anti-Tbr1 (green) antibodies at P7; and with anti-Cux1 (green) and anti-Ctip2 (red) antibodies at P14. Scale bars: 200 μm.

Figure S5 Ohtsuka et al.

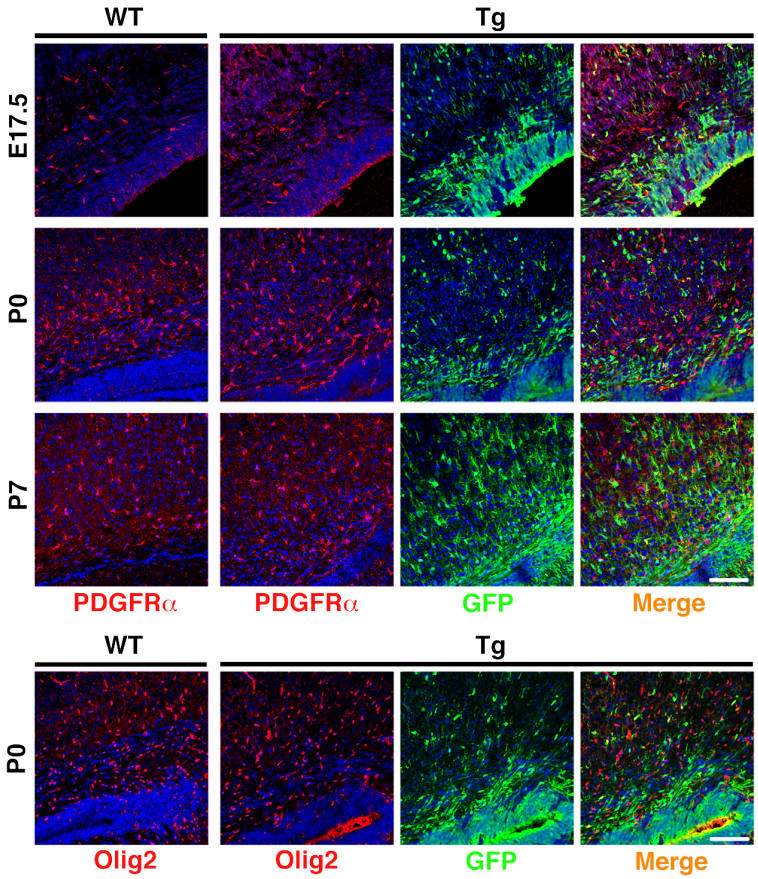


Fig. S5. Analysis of generation of oligodendrocyte lineage. Immunohistochemistry on coronal sections of neocortical regions of WT and Tg brains; immunostaining with anti-GFP (green) and anti-PDGFRα (red) antibodies at E17.5, P0, and P7; and with anti-GFP (green) and anti-Olig2 (red) antibodies at P0. DAPI (blue) represents nuclear staining. Scale bars: 100 μm.

Figure S6 Ohtsuka et al.

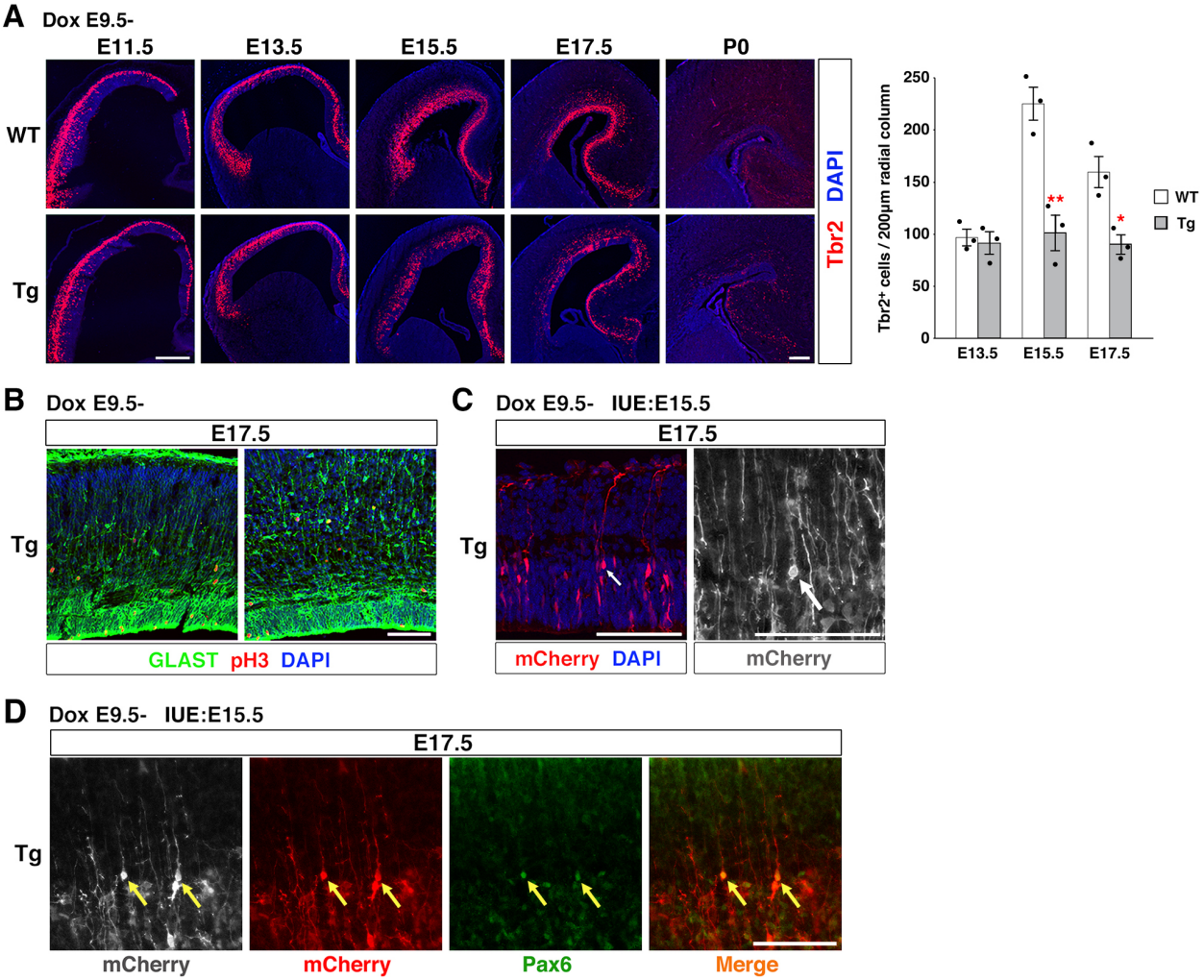


Fig. S6. Decrease in Tbr2$^+$ IPCs and increase in bRGC-like cells. (**A**) Immunostaining with anti-Tbr2 (red) antibody on coronal sections of the dorsal telencephalon of WT and Tg mice at various developmental stages. (**B**) Double-labeling with anti-GLAST (green) and anti-pH3 (red) antibodies on coronal sections of neocortical regions of E17.5 Tg embryos. (**C,D**) *pEF-mCherry* expression vectors were transfected into the VZ cells of Tg embryos by *in utero* electroporation (IUE) at E15.5. The morphology of transfected cells was analyzed by immunohistochemistry on coronal sections of neocortical regions at E17.5. (C) Single labeling with anti-mCherry antibody (red) with DAPI staining (blue). mCherry signals are shown in white on the right panel (grey scale). (D) Double labeling with anti-mCherry (red) and anti-Pax6 (green) antibodies. mCherry signals are shown in white on the left panel (grey scale). The arrows indicate mCherry$^+$ cells with bRGC-like morphology that retain only basal radial processes but lack apical processes and are located in the SVZ. The yellow arrows in (D) indicate mCherry$^+$;Pax6$^+$ cells in the SVZ. DAPI (blue) represents nuclear staining. Data are presented as mean±s.e.m. (n=3); *P<0.05, **P<0.01 (Student's t-test). Scale bars: 200 μm in A; 100 μm in B, C, D.

Figure S7 Ohtsuka et al.

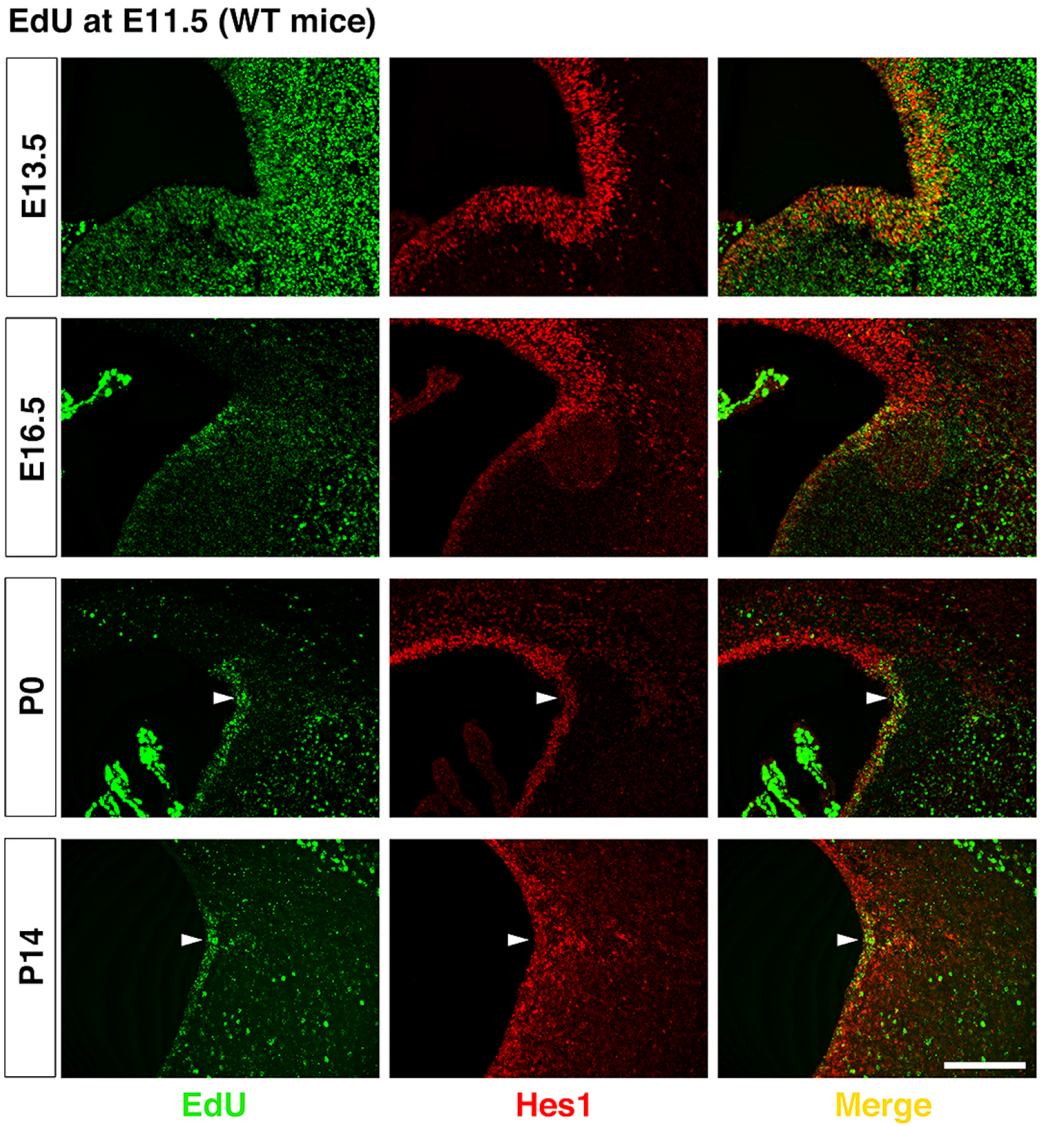


Fig. S7. Estimation of Hes1 expression levels in EdU label-retaining cells in the SVZ. EdU was injected intraperitoneally into WT pregnant mice at E11.5 and detected by Click reaction at E13.5, E16.5, P0 and P14 using Alexa Fluor 488 Azide (green). In addition, immunostaining with anti-Hes1 antibody (red) was performed on coronal brain sections. EdU label-retaining cells were observed in the SVZ around the dorso-ventral boundary and the ventrolateral wall of lateral ventricles. Note that Hes1 expression levels in these EdU label-retaining cells (white arrowheads) were equivalent to or rather lower than those in surrounding EdU^- cells in the SVZ at postnatal stages. Scale bar: 200 μm.

Table S1. Primary antibodies

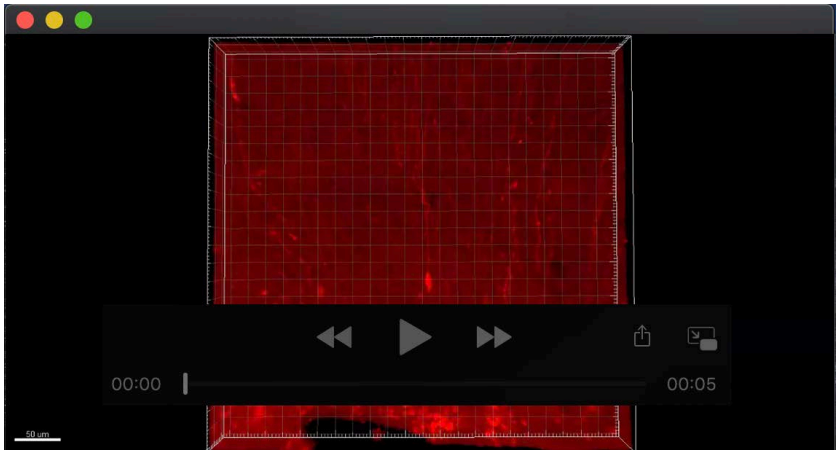
Antigen	Host	Manufacturer	Catalogue No.	Dilution
GFP	rabbit	Molecular Probes	A11122	1:500
GFP	chicken	Abcam	Ab13970	1:500
Hes1	rabbit	Kobayashi et al., 2009	----------	1:1000
Tuj1	mouse	Covance	MMS-435P	1:1000
Pax6	rabbit	Covance	PRB-278P	1:200
Brn-1	goat	Santa Cruz	sc-6028	1:100
Neurog2	goat	Santa Cruz	sc-19223	1:1000
BrdU	mouse	Becton Dickinson	347580	1:100
BrdU	rat	Serotec	MCA2060	1:500
pH3	mouse	Sigma-Aldrich	H6409	1:500
Ki67	mouse	BD Pharmingen	556003	1:100
Ccnd1	rabbit	Thermo Scientific	RM-9104-SO	1:100
Cux1	rabbit	Santa Cruz	sc-13024	1:100
Ctip2	rat	Abcam	ab18465	1:500
GFAP	rabbit	DAKO	Z0334	1:500
NeuN	mouse	Merck Millipore	MAB337	1:500
Tbr2	rabbit	Abcam	ab23345	1:500
Tbr2	rat	eBioscience	14-4875-82	1:500
pVim	mouse	MBL International	D076-3S	1:500
mCherry	rat	Life technologies	M11217	1:500
Dcx	goat	Santa Cruz	sc-8066	1:200
Cleaved Caspase-3	rabbit	Cell Signaling	#9661S	1:500
Tbr1	rabbit	Abcam	Ab31940	1:400
PDGFRα	rat	BD Pharmingen	558774	1:500
GLAST	guinea pig	Merck Millipore	AB1783	1:100

Reference:

Kobayashi, T., Mizuno, H., Imayoshi, I., Furusawa, C., Shirahige, K. and Kageyama, R. (2009). The cyclic gene Hes1 contributes to diverse differentiation responses of embryonic stem cells. *Genes Dev*. 23, 1870-1875.

Table S2. Primers for quantitative real-time RT-PCR

Gene	Primer	Sequence
Hes1	Fw (Forward)	5′-GTGAAGCACCTCCGGAACCTGCAGC-3′
	Rv (Reverse)	5′-GCGGGTCACCTCGTTCATGCACTCG-3′
Ccnd1	Fw	5′-TTGACTGCCGAGAAGTTGTGC-3′
	Rv	5′-TTGTTCTCATCCGCCTCTGGC-3′
Gapdh	Fw	5′-TGGGTGTGAACCACGA-3′
	Rv	5′-AAGTTGTCATGGATGACCTT-3′



Movie 1. 3D image of bRGC-like cell

pEF-mCherry expression vectors were transfected into the VZ cells of Tg embryos by *in utero* electroporation at E15.5. Fixed and cryoprotected brain was cryosectioned at 70 µm and the morphology of transfected cells was analyzed by immunohistochemistry with anti-mCherry (red) antibody on a coronal section of neocortical region at E17.5. Note that a cell shown near the center of this movie exhibits a bRGC-like morphology that retains only basal radial processes but lacks apical processes and is located in the SVZ.