SUPPLEMENTAL MATERIALS

Figure S1. Prediction of Olig2 *cis*-regulatory elements and experimental design for functional verification. (**A**) Sequence alignment reveals the conserved regions of the Olig2 gene locus from human, mouse, and other species. For simplicity, only the human to mouse alignment is shown. Twelve highly conserved noncoding regions were predicted as Olig2 *cis*-regulatory elements (CR1 – CR12). Pink regions represent conserved noncoding sequences; blue regions represent exons. CR5 (Chr. 16:91082197-91082573) contains 377 bp and located ~32kbp upstream of Olig2 transcription start site (highlighted in a blue box). (**B**) Schematic diagram showing the design of plasmid reporter constructs for experimental construct and various control constructs, i.e., the negative controls and transfection controls. The experimental construct contains a predicted *cis*-element upstream of a minimal β-globin promoter and a reporter GFP. Negative control constructs contain the minimal β-globin promoter and the reporter GFP without a *cis*-element or with a random sequence of comparable size. The transfection control contains a strong ubiquitous CAG promoter (chicken β-actin promoter with CMV enhancer) and a reporter, GFP or DsRed. (**C**) Schematic representation of the P0 mouse brain at a sagittal plane. DNA construct was injected using a Hamilton microsyringe to target the neural progenitors in the SVZ followed by electroporation. VZ, ventricular zone; SVZ, subventricular zone; RMS, rostral migratory stream; IVE, in vivo electroporation.

Figure S2. Method of cell counting for immunofluorescently stained cells. (**A**) A P7 mouse forebrain section after *in vivo* electroporation at P0 was immunostained with anti-GFP (green) antibody and a type-C neural progenitor cell marker Mash1 (red). Nuclei were stained by Dapi (blue). The number of GFP+ cells were manually counted as indicated by arrowheads and arrows (**A,B).** Dapi staining was used to determine the number of GFP+ cells when GFP signal was too strong to count (oval, **A, B, D**). For cells that are GFP+ but not stained with Dapi (arrows, **A, B, D**), they were also counted as GFP+ cells with the assumption that Dapi failed to stain or the nuclei were in different focal planes. GFP+ cells that express a cell marker Mash1 (rectangular in **A** and **C**) were manually counted to determine the number of co-stained cells. The percentage of co-stained cells over the total number of GFP+ cells was then calculated. Boxed area in the diagram showed where the images were taken. Cx, cortex; cc, corpus callosum. Scale bar=50μm.

Figure S3. CR5-GFP+ cells express glial lineage markers. Sagittal sections of the P7 mouse forebrain after *in vivo* electroporation at P0 were immunostained with anti-GFP (green) antibody and specific cell markers (red). Boxed area in the diagram of the Sagittal plane showed where the images were taken. GFP+ cells were examined for the expression of glial markers GFAP (**A, B**), S100ß (**C, D**), and neuronal marker NeuN (**E, F**). The double labeled cells were indicated by arrowheads. Cells only express GFP but not stained with a cell marker were indicated by arrows. No CR5-GFP+ cells were co-labeled with NeuN. Scale bar=50μm.

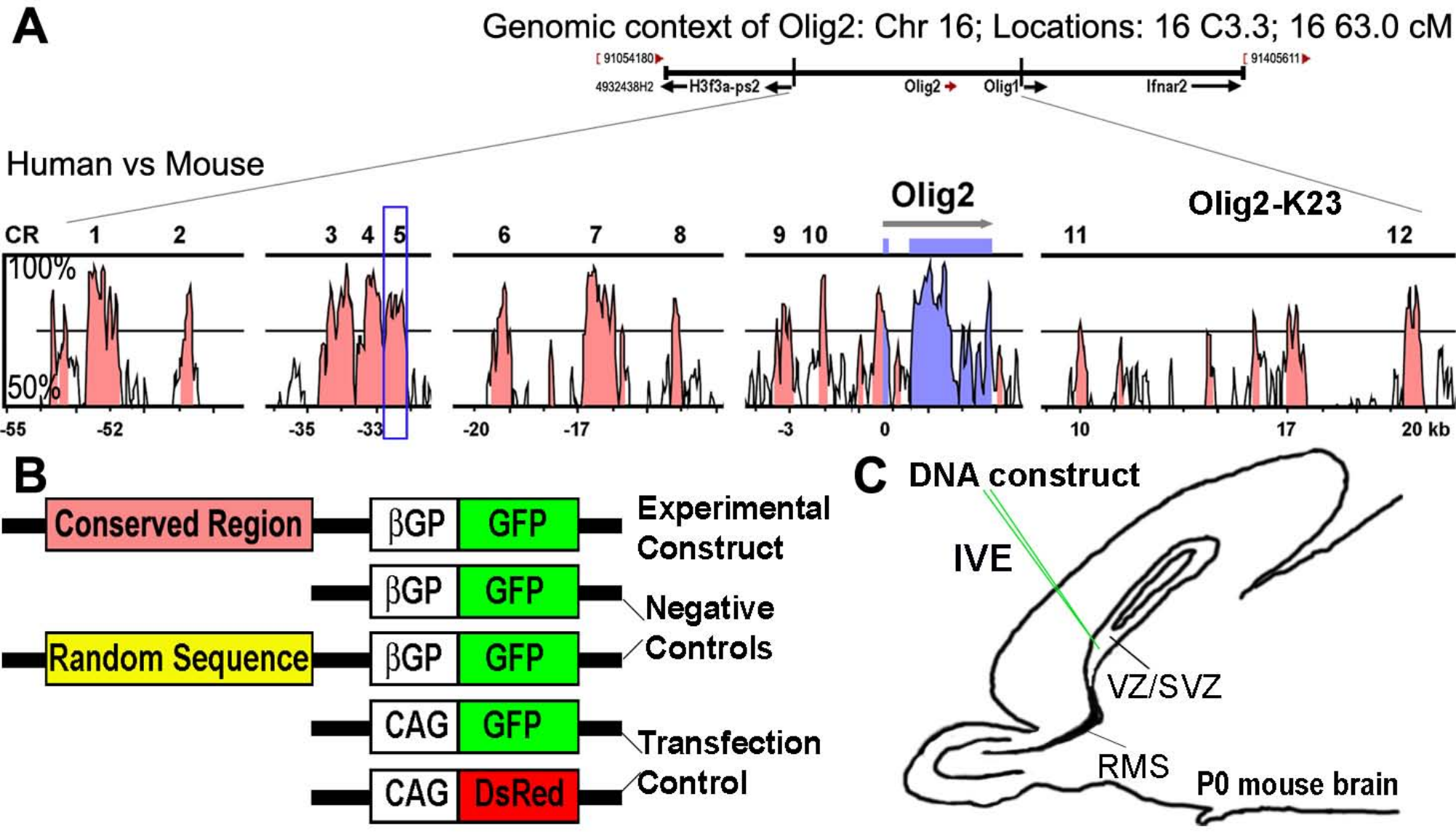
Figure S4. **Analysis of CR5-GFP+ cells in the ventral brain of P0 transgenic mouse.** CR5-GFP+ cells were immunostained with specific cell marker and cell counting was performed to determine the percentage of co-labeled cells. We found that CR5-GFP+ cells were negative for intermediate progenitor/ postmitotic neuron markers including Tbr1 (**A**), Tbr2 (**B**), Pax2 (**C**), and Pax6 (**D**); radial glia/astrocyte cell markers including S100β (**E**), GFAP (**F**), and BLBL (**G**); and oligodendrocyte progenitor marker Olig2 (**H**) and PDGFRα (**I**). Boxed area in the diagram of the Sagittal plane showed where the images were taken. Scale bar=50 μm.

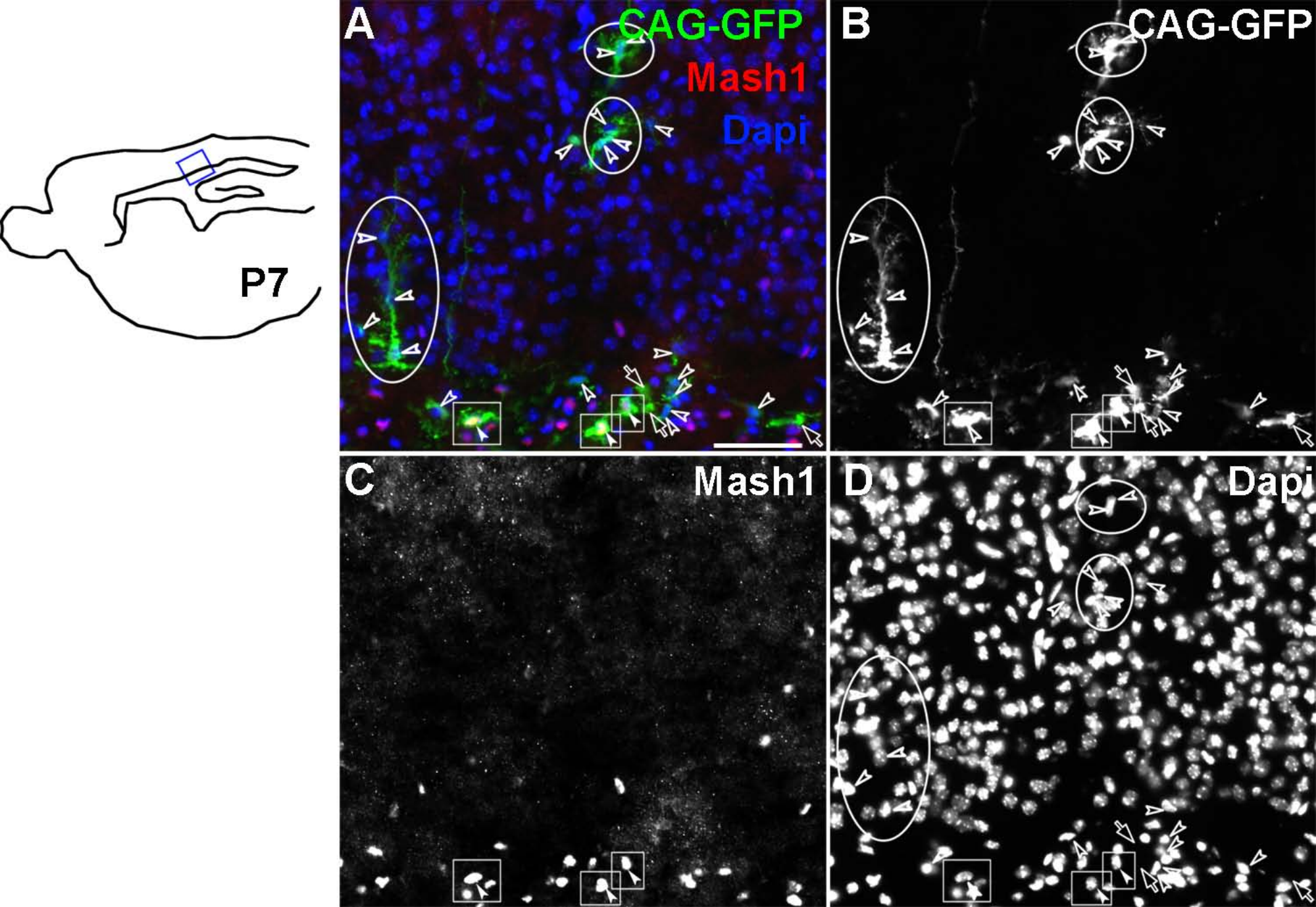
Table S1. Summary of assays by *in vivo* electroporation.

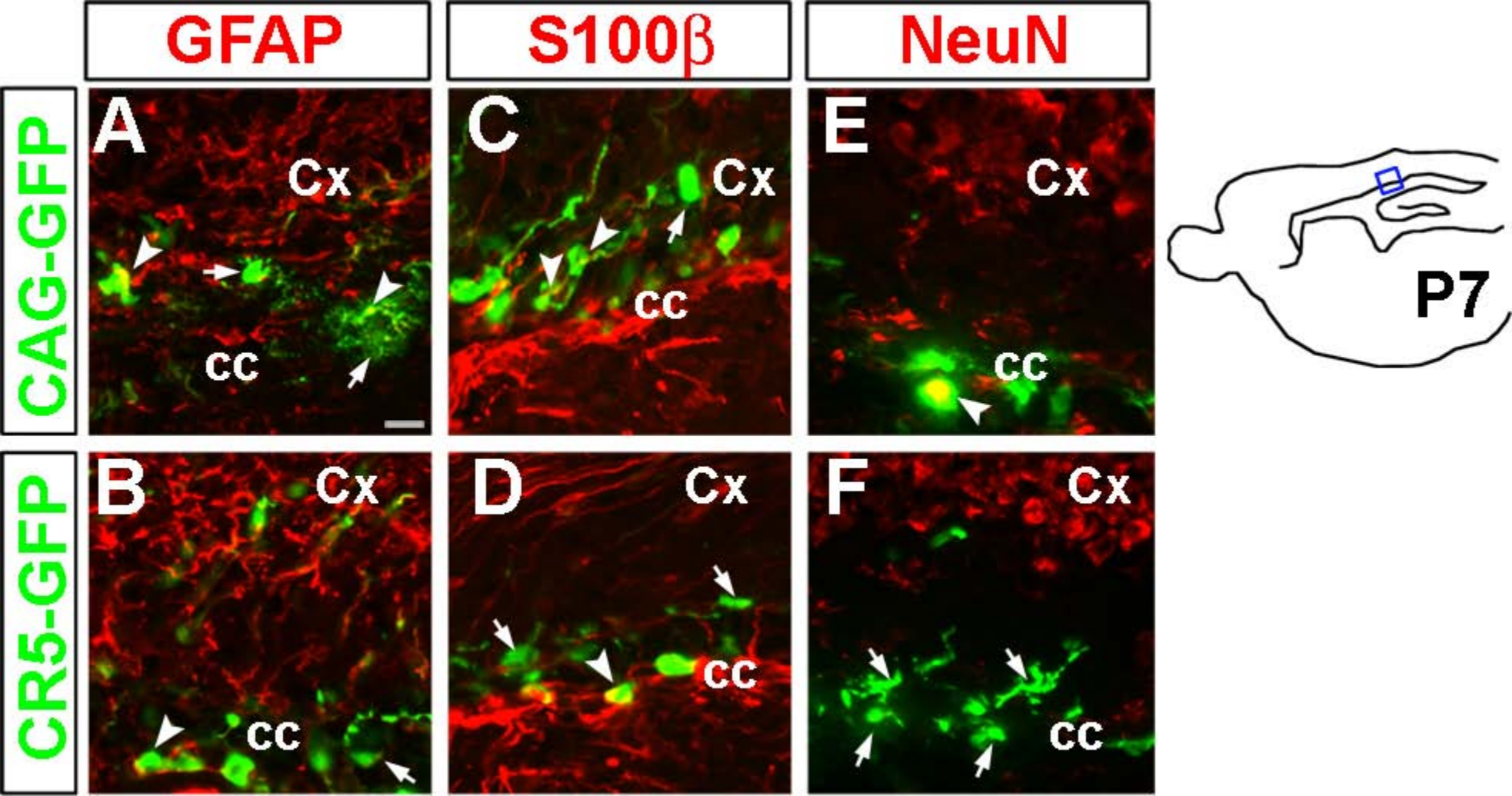
	Control		**CR5**		p-value
	% of co-labeled cells		% of co-labeled cells		
Marker	Mean	SD	Mean	SD	
Sox5	23.1	3.2	52.6	2.8	< .0001
NG2	27.3	3.6	68.9	5.2	< .0001
NeuN	8.5	7.2	0	0	< .0001
Olig2	27.1	2.9	36.6	2.3	< .0001
S100β	36	6.8	39	6.5	0.04749
GFAP	41	3.3	37.8	3.6	0.04207
BLBP	37.9	2.9	35.2	3	0.04983
Mash1	17.9	5.5	22.6	5.6	0.05359

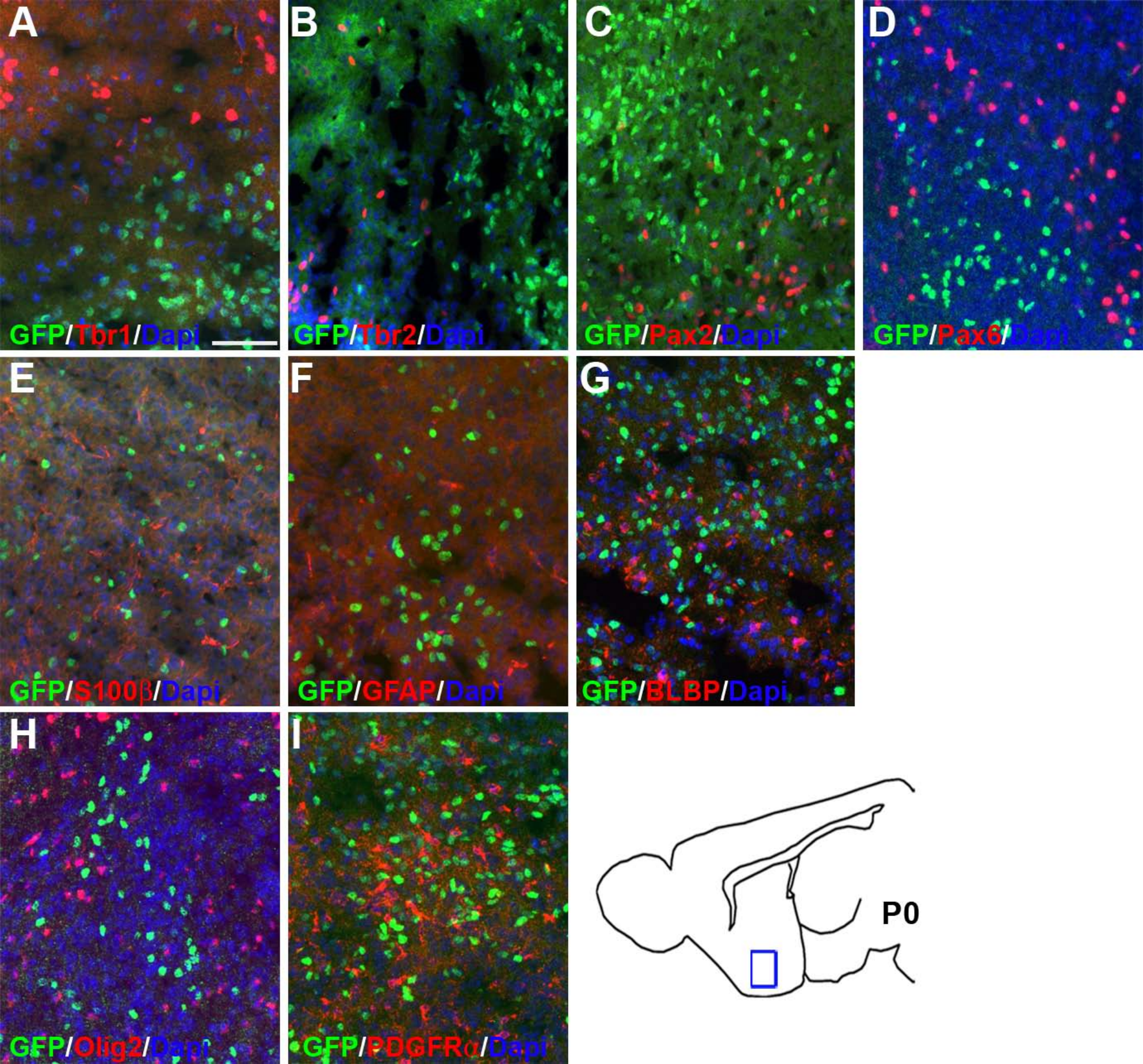
Table S2. Summary of assays by site-directed mutagenesis and *in vivo* electroporation.

	CR5		**CR5$^{\Delta Sox5}$**		p-value
	% of co-labeled cells		% of co-labeled cells		
Marker	Mean	SD	Mean	SD	
Sox5	23.1	3.2	16.98	4.5	< .0001
NG2	27.3	3.6	23.98	2.5	< .0001
Olig2	36.6	2.3	19.98	3.1	< .0001
BLBP	35.2	3	58.58	2.9	< .0001


A
Genomic context of Olig2: Chr 16; Locations: 16 C3.3; 16 63.0 cM
91054180
91405611
4932438H2
H3f3a-ps2
Olig2
Olig1
Ifnar2
Human vs Mouse
Olig2
Olig2-K23
CR
1
2
3
4
5
6
7
8
9
10
11
12
100%
50%
-55
-52
-35
-33
-20
-17
-3
0
10
17
20 kb
B
Conserved Region
βGP
GFP
Experimental Construct
βGP
GFP
Negative Controls
Random Sequence
βGP
GFP
CAG
GFP
Transfection Control
CAG
DsRed
C
DNA construct
IVE
VZ/SVZ
RMS
P0 mouse brain


P7
A
CAG-GFP
Mash1
Dapi
B
CAG-GFP
C
Mash1
D
Dapi


GFAP
S100β
NeuN
CAG-GFP
CR5-GFP
A
B
C
D
E
F
Cx
cc
P7


A
GFP/Tbr1/Dapi
B
GFP/Tbr2/Dapi
C
GFP/Pax2/Dapi
D
GFP/Pax6/Dapi
E
GFP/S100β/Dapi
F
GFP/GFAP/Dapi
G
GFP/BLBP/Dapi
H
GFP/Olig2/Dapi
I
GFP/PDGFRα/Dapi
P0