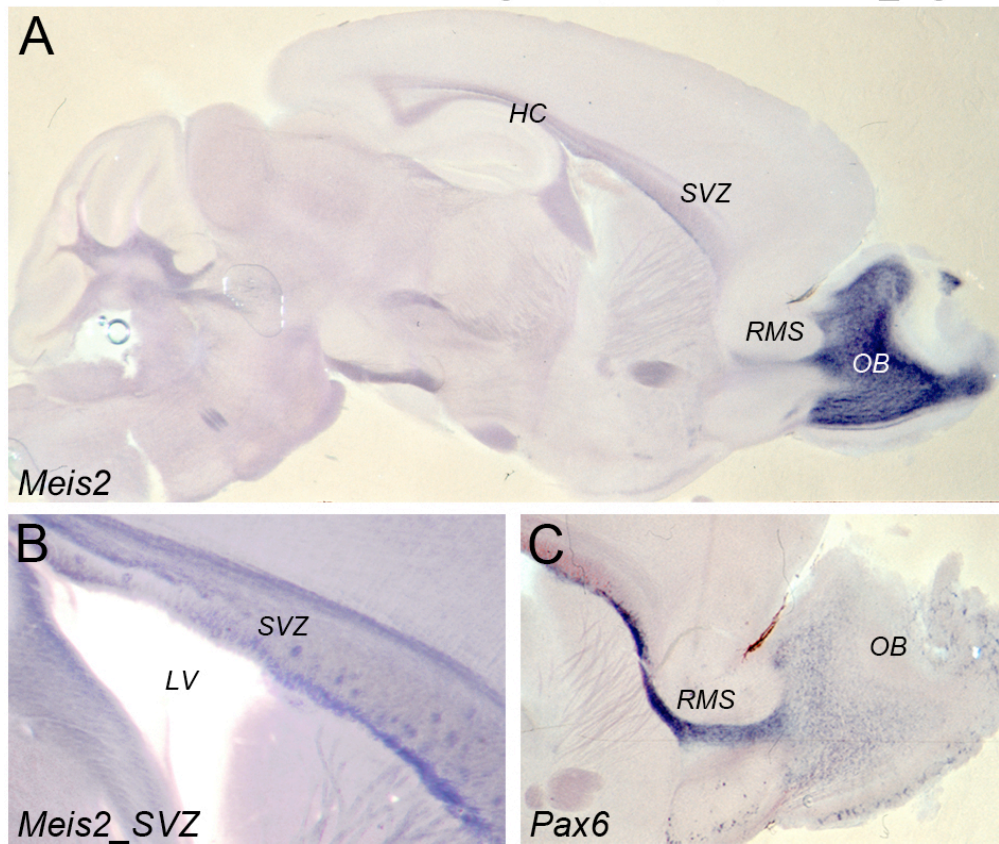
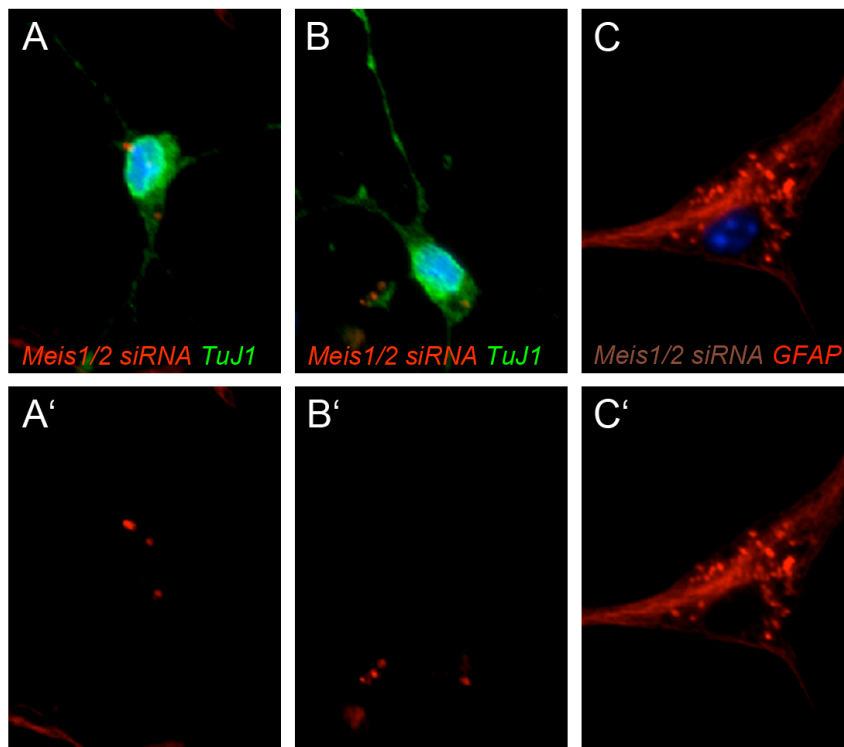


Agoston, Heine, Brill et al._Fig.S1



Supporting Figure 1: *Meis2* and *Pax6* transcripts in the adult murine brain detected by in situ hybridization on vibratome sections. (A) *Meis2*-specific transcripts on a sagittal section through the brain of a 7 week old mouse; strong expression is detectable in the SVZ-OB neurogenic system. (B) *Meis2*-mRNA in the SVZ. (C) *Pax6* specific transcripts in the SVZ, RMS and OB. Cells in the glomerular layer and neuroblasts that migrate from the RMS laterally into the outer olfactory bulb express *Pax6*. [HC: hippocampus; LV: lateral ventricle; OB: olfactory bulb; RMS: rostral migratory stream; SVZ: subventricular zone]

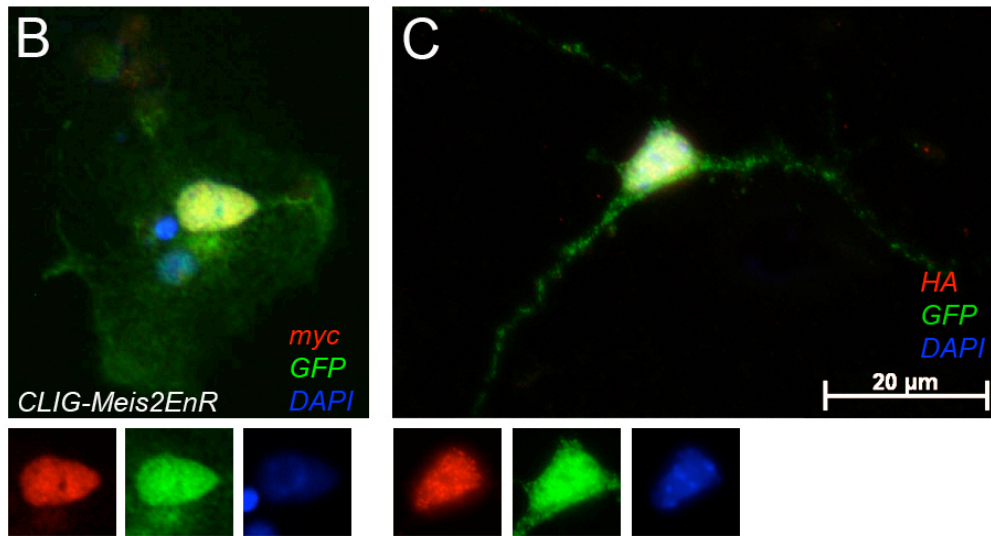
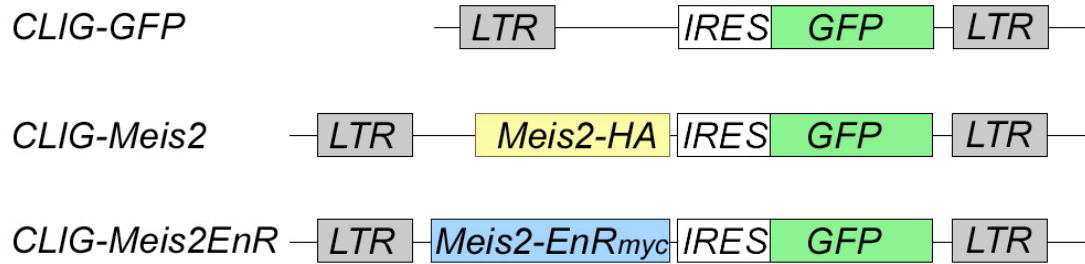
Agoston, Heine, Brill et al., Fig. S2



Supporting Figure 2. Representative examples of neurosphere cells that were transfected with siRNAs specific for *Meis1* and *Meis2* and allowed to differentiate in vitro for two days. (A, B): cells stained for TuJ1 (green, detected with an Alexa488-coupled secondary antibody); (C) cell stained for GFAP (red, detected with an Alexa594-coupled secondary antibody). The rare TuJ1-expressing neurons generated from *Meis1/2 siRNA* transfected cells exhibited markedly fewer red-fluorescent speckles (indicative of lower uptake of the Cy5-labeled siRNA molecules) than astrocytes generated from *Meis1/2 siRNA* transfected cells. In (A'), (B') and (C') only the red channel is shown for clarity. The fluorescent signals of GFAP and *Meis1/2 siRNA* overlap in (C, C').

Agoston, Heine, Brill et al._Fig.S3

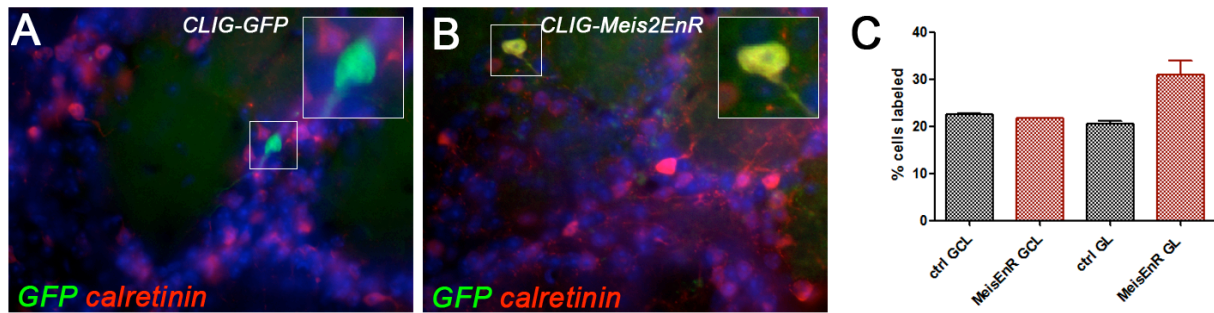
A



Supporting Figure 3. Schematic representation of the retroviral construct used in this study.

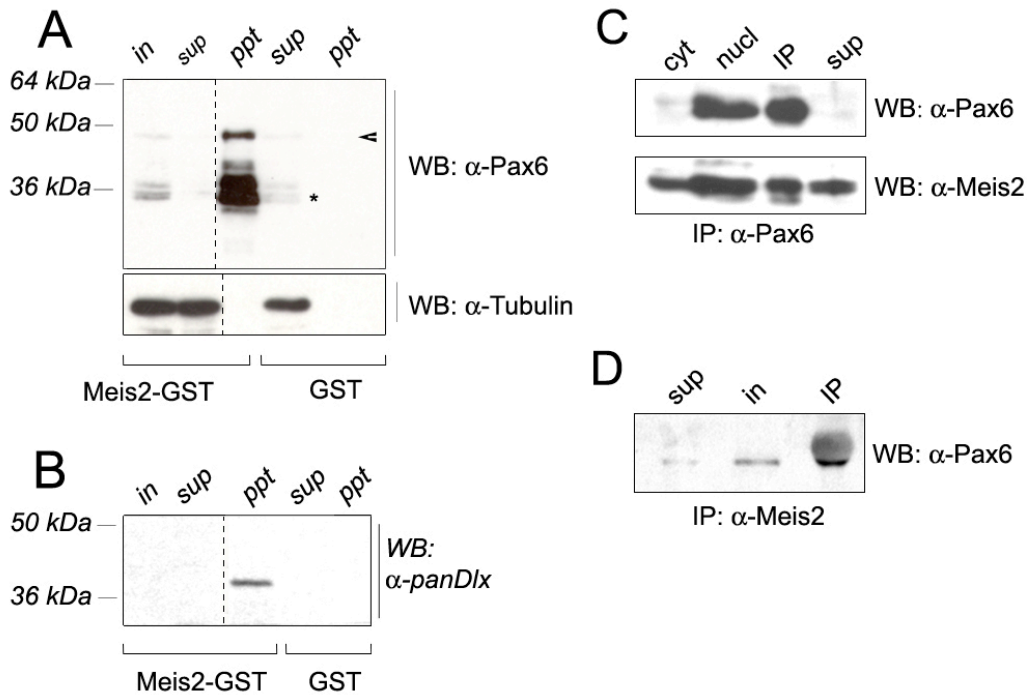
(A) All CLIG-derived retroviral vectors express GFP from an internal ribosome entry site (IRES). CLIG-Meis2 contains *Meis2a* C-terminally fused to a triple HA-epitope, CLIG-Meis2EnR contains *Meis2a* fused to the EnR domain of *D. melanogaster* and a single C-terminal myc-epitope. (B, C) Co-expression of GFP with the transgenes in CLIG-Meis2 and CLIG-Meis2EnR. (B) An in vitro differentiated, neurosphere derived cell infected with Meis2EnR exhibits GFP- and nuclear myc staining. (C) An in vitro differentiated, neurosphere derived cell infected with Meis2HA exhibits GFP- and nuclear HA staining.

Agoston, Heine, Brill et al._Fig. S4



Supporting Figure 4. Some Meis2EnR transduced periglomerular neurons acquire a calretinin-positive PGN fate. Images were taken 21 days after the CLIG-GFP control virus or CLIG-Meis2EnR were injected into the RMS, (A) Representative example of a GFP transduced PGN (green) not immunoreactive for calretinin (red); (B) Representative example of a Meis2EnR transduced PGN (green) expressing calretinin (green). (C) Quantification of the results.

Agoston, Heine, Brill et al.,_Fig.S5



Supporting Figure 5. Meis2-Dlx2-Pax6 containing protein complexes exist in the embryonic chick eye. (A) GST pull-down with Meis2-GST or GST alone and protein extracts from HH (Hamburger Hamilton stage) 15-17 embryonic chick eyes. Top panel: Western blot with a Pax6 specific antibody; bottom panel: Western blot with α -tubulin as control. (B) Same blot as in (A) but re-probed with an antibody with broad specificity for Dlx proteins. Dlx can be enriched by Meis2-GST but not GST alone. The dashed lines indicate that the blot was cut to remove the marker lane. (C) Immunoprecipitation from chick eye extracts with a mouse monoclonal antibody directed against Pax6. Top panel: Western blot with a rabbit polyclonal Pax6-antibody; bottom panel: the same blot as above but re-probed with a rabbit polyclonal antibody specific for Meis2. Meis2 can be successfully co-precipitated with the Pax6. (D) Immunoprecipitation from chick eye extracts with a rabbit polyclonal antibody directed against Meis2. Western blot with a mouse monoclonal Pax6-antibody reveals association of Pax6 with Meis2. [*in*: input; *sup*: supernatant; *ppt*: precipitate of the pull-down; *IP*: immunoprecipitate]

Agoston, Heine, Brill et al. Fig. S6

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CCATATGAAAGAAAGAAAATAAATCCTTTTGTCTCTCTCAGCCTCGGATACTTCACTCAGTATAT
 CATTTCGGTTCCACACAATTTCTT GCAAATCTCATAATTT CACATTTTACTGATGGGTGATTAA
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 GGAGCTCAGACAACAAAGGCATAGAGAGATTGGTTTTCTTCTCTCAGCATCTCCACCAACCAG
//..... GGTTCACCAAAAT ⁺¹ ATG

Supporting Figure 6. Sequence of genomic region upstream of the DCX translational start, which was analyzed in the present study; putative transcription factor binding sites are highlighted: Meis/Pbx (red), Pax4/6 (blue), Dlx (green); + and - indicate location of the consensus binding site on the coding or non coding strand; putative CAAT- and TATAA-boxes are underlined; the location of the primers used for ChIP-qPCR are italicized and marked by arrows.

Table S1: Antibodies

Primary antibodies for immunohistochemistry and Western Blot			
name	species	source	dilution
anti-calbindin	mouse	Sigma Immunochemicals, Germany, C9848	1:1000
anti-calretinin	mouse	BD Biosciences, Franklin Lakes, NJ, 610908	1:2000
anti-pan-Dlx	rabbit	J. Kohtz, Children's Memorial Hospital and Feinberg School of Medicine, Northwestern University, Chicago, IL	1:750
anti-doublecortin (DCX)	guinea pig	Millipore Bioscience Research Reagents, Billerica, MA, AB2253	1:2000
anti-doublecortin (DCX)	rabbit	Abcam, Cambridge, MA, ab18723	1:2000
anti-GFAP	mouse	Sigma Immunochemicals, Germany, G 9269	1:500
anti-GFP	rabbit	Molecular Probes, OR	1:5000
anti-glutamate decarboxylase 67kDa (GAD67)	mouse	Millipore, MAB5406	1:1000
anti-HA	rat	Roche Diagnostics, Germany, 3F10	1:1000
anti-Ascl1 (Mash1)	mouse	D. Anderson, California Institute of Technology, Pasadena, CA	1:200
anti-Meis2	rabbit	A. Buchberg, Kimmel Cancer Centre, University of Philadelphia Medical School	1:5000
anti-Meis2	mouse	Sigma Immunochemicals, clone 1H4	1:1000
anti-myc	mouse	Abcam, Cambridge, MA, ab11917	1:100
anti-Olig2	rabbit	Millipore Bioscience Research Reagents, Billerica, MA, AB9610	1:1000
anti-O4	mouse	hybridoma supernatant; M. Schachner, Center for Molecular Neurobiology, Hamburg, Germany	1:5
anti-Pax6	mouse	Developmental Studies Hybridoma Bank, IA	Purified IgGs: 1:5000 or hybridoma supernatant 1:10
anti-Pax6	mouse	clone AB2.38 of (Engelkamp et al., 1999, Development 126(16):3585-96.	Purified IgGs: 1:5000 for Western Blots or supernatant

			1:10
anti- Pax6	rabbit	Covance, Princeton, NJ, PRB-278P	1:1000 for Western Blots
anti-PSA-NCAM ,	mouse	Millipore Bioscience Research Reagents, Billerica, MA, MAB5324	1:1000
anti-tubulin, isotype III, (TuJ1)	mouse	Covance, Princeton, NJ, MMS435P	1:1000
anti-tyrosine hydroxylase (TH)	mouse	Millipore Bioscience Research Reagents, MAB5280	1:500
Antibodies for ChIP			
name	species	source	
anti-Meis2 N-17X	goat	Santa Cruz Biotechnology, Santa Cruz, CA, sc-10600	
anti-Pax6	mouse	purified IgGs, Developmental Studies Hybridoma Bank, IA	
anti-pan-Dlx	rabbit	J. Kohtz, Northwestern University, Chicago, IL	
anti-RNA Pol II	mouse	Merck Millipore, Germany, clone CTD4H8	
Normal IgG control	mouse	Merck Millipore / Upstate, # 12-371B from EZ-ChIP Kit # 17-371	

Secondary antibodies for immunohistochemistry were Alexa 594-, Alexa 488-, Cy2 or Cy3 conjugated (Molecular Probes, OR, Invitrogen, Karlsruhe, Germany or Dianova, Hamburg, Germany). Some sections were counterstained with 4'-6-Diamidino-2-phenylindole (DAPI) to visualize cell nuclei. Stainings were analyzed with either a LSM5 confocal microscope (Zeiss, Germany), an Axioplan2 with deconvolution software, or an Olympus FV1000 laser-scanning confocal microscope with optical sections of maximum 1–2 μm intervals.

Table S2: Primer Sequences for ChIP

name	sequence	amplicon
DCXI for	5'- CTCGGATACTTCACTCAGTATATC	
DCXI rev	5'- GCATATCTGTGTTTATGGCTGC	178bp
DCXII for	5'- AAACCTTTCTAGCTGTTAATGCAGG	
DCXII rev	5'- CTCCAAGCAAGAAATTCCTGCCAGGGTG	174bp
TH1 for	5'- CCTCTTTAGTTTCCTGATGTCCTGG	
TH1 rev	5'- GCCTGTGGAGCAGGCAACAGAAGG	192bp
TH2 for	5'- GTCTCCTGTCCCAGAACACCAGCC	
TH2 rev	5'- TAAAGGCCAGGCTGACGTCAAAGC	245bp
GAPDH	#PP1045500, Diagenode, Liège, Belgium	
Myogenin for	5'- CAAATTACAGCCGACGGCCTC	
Myogenin rev	5-' GAAAAGGCTTGTTTCCTGCCACTG	284bp

Table S3: Total number of Meis2-immunoreactive cells in the SVZ, RMS and OB analyzed

antigen	% of cells co-labeled with Meis2	total number of cell counted
SVZ/RMS		
DCX	98.7%	1678 cells, 3 animals
TuJ1	95.1%	870 cells, 3 animals
PSA-NCAM	99.3%	772 cells, 3 animals
OB		
tyrosine hydroxylase, TH	94.4% (+/- 3.1%)	1913 cells, 3 animals
calbindin	62.9% (+/- 2.1%)	2571 cells, 4 animals
calretinin	28.3% (+/- 1.4%)	2121 cells, 3 animals
Pax6	83.5% (+/- 2.5%)	1010 cells, 3 animals
GAD67:GFP	86.8% (+/- 3.7%)	3287 cells, 3 animals
BrdU (3w pulse, 3w chase)	GCL: 87.5% GL: 57.3%	GCL: 512 cells, 1 animal GL: 128 cells, 1 animal

Table S4: Total number of number of cells analyzed following differentiation of neurosphere cells in vitro**A: in vitro differentiation of neurosphere cells following siRNA mediated knock-down**

experimental design	number of independent experiments	total number of cell counted
ctrl. siRNA	3	1820
Meis1/2 siRNA	3	1645

B: in vitro differentiation of neurosphere cells following retroviral transduction

experimental design: viral vector / marker / differentiation duration	number of independent experiments	% cells generated	total number of cell counted
CLIG-GFP/ TuJ1 / 3d differentiation	6	11.48 (+/- 0.297 s.e.m.)	3185
CLIG-GFP/ GFAP / 3d differentiation	6	48.44 (+/- 6.28 s.e.m.)	3030
CLIG-Meis2HA/ TuJ1 / 3d differentiation	3	11.39 (+/- 0.695 s.e.m.)	3475
CLIG-Meis2HA/ GFAP / 3d differentiation	3	32.96 (+/- 0.1 s.e.m)	3521
CLIG-Meis2EnR/ TuJ1 / 3d differentiation	3	6.51 (+/- 0.178 s.e.m.)	3219
CLIG-Meis2EnR/ GFAP / 3d differentiation	3	52.19 (+/- 8.50 s.e.m.)	2780
CLIG-GFP/ TuJ1 / 7d differentiation	7	32.49 (+/- 4.37 s.e.m.)	1944
CLIG-GFP/ GFAP / 7d differentiation	3	49.37 (+/- 0.61 s.e.m.)	2126
CLIG-GFP/ O4 / 7d differentiation	3	4.38 (+/- 0.53 s.e.m.)	1391
CLIG-Meis2HA/ TuJ1 / 7d differentiation	3	26.7 (+/- 10.37 s.e.m.)	1807
CLIG-Meis2HA/ GFAP / 7d differentiation	3	53.03 (+/- 1.92 s.e.m.)	1716
CLIG-Meis2HA/ O4 / 7d differentiation	3	7.98 (+/- 0.695 s.e.m.)	1082
CLIG-Meis2EnR/ TuJ1 / 7d differentiation	5	10.99 (+/- 2.53 s.e.m.)	1853
CLIG-Meis2EnR/ GFAP / 7d differentiation	3	64.54 (+/- 1.9 s.e.m.)	1535
CLIG-Meis2EnR/ O4 / 7d differentiation	3	5.485 (+/- 0.665 s.e.m.)	1034

Table S5: Total number of cells counted following in vivo transduction

experimental design: viral vector / injection site / days post injection / marker	number of injected brain hemispheres	total number of cell counted
CLIG-GFP, SVZ, 3d, PSA-NCAM	4	177
CLIG-Meis2, SVZ, 3d, PSA-NCAM	4	104
CLIG-Meis2EnR, SVZ, 3d, PSA-NCAM	6	132
CLIG-GFP, SVZ, 4d, GFAP	4	963
CLIG-Meis2EnR, SVZ 4d GFAP	6	336
CLIG-GFP, SVZ, 10d, GFAP	2	113
CLIG-Meis2EnR, SVZ 10d GFAP	4	30
CLIG-GFP, RMS, 21d, Pax6	4	571
CLIG-Meis2EnR, RMS, 21d, Pax6	4	98
CLIG-GFP, RMS, 21d, TH	4	462
CLIG-Meis2, RMS, 21d, TH	4	109
CLIG-Meis2EnR, RMS, 21d, TH	7	246
CLIG-GFP, RMS, 60d, TH	4	312
CLIG-Meis2, RMS, 60d, TH	5	153
CLIG-Meis2EnR, RMS, 60d, TH	5	88
CLIG-Meis2EnR, RMS, 21d, Nestin	4	105
CLIG-Meis2EnR, RMS, 21d, TuJ1	4	44
CLIG-GFP, RMS, 21d, GAD67	4	225
CLIG-Meis2EnR, RMS, 21d, GAD67	4	172
CLIG-GFP, RMS, 21d, calretinin	2	168
CLIG-Meis2EnR, RMS, 21d, calretinin	2	128
CLIG-GFP, RMS, 21d, calbindin	2	107
CLIG-Meis2EnR, RMS, 21d, calbindin	2	85