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Supplemental Information

Adult Neural Stem Cells and Multiciliated Ependymal Cells Share a Common Lineage Regulated by the Geminin Family Members

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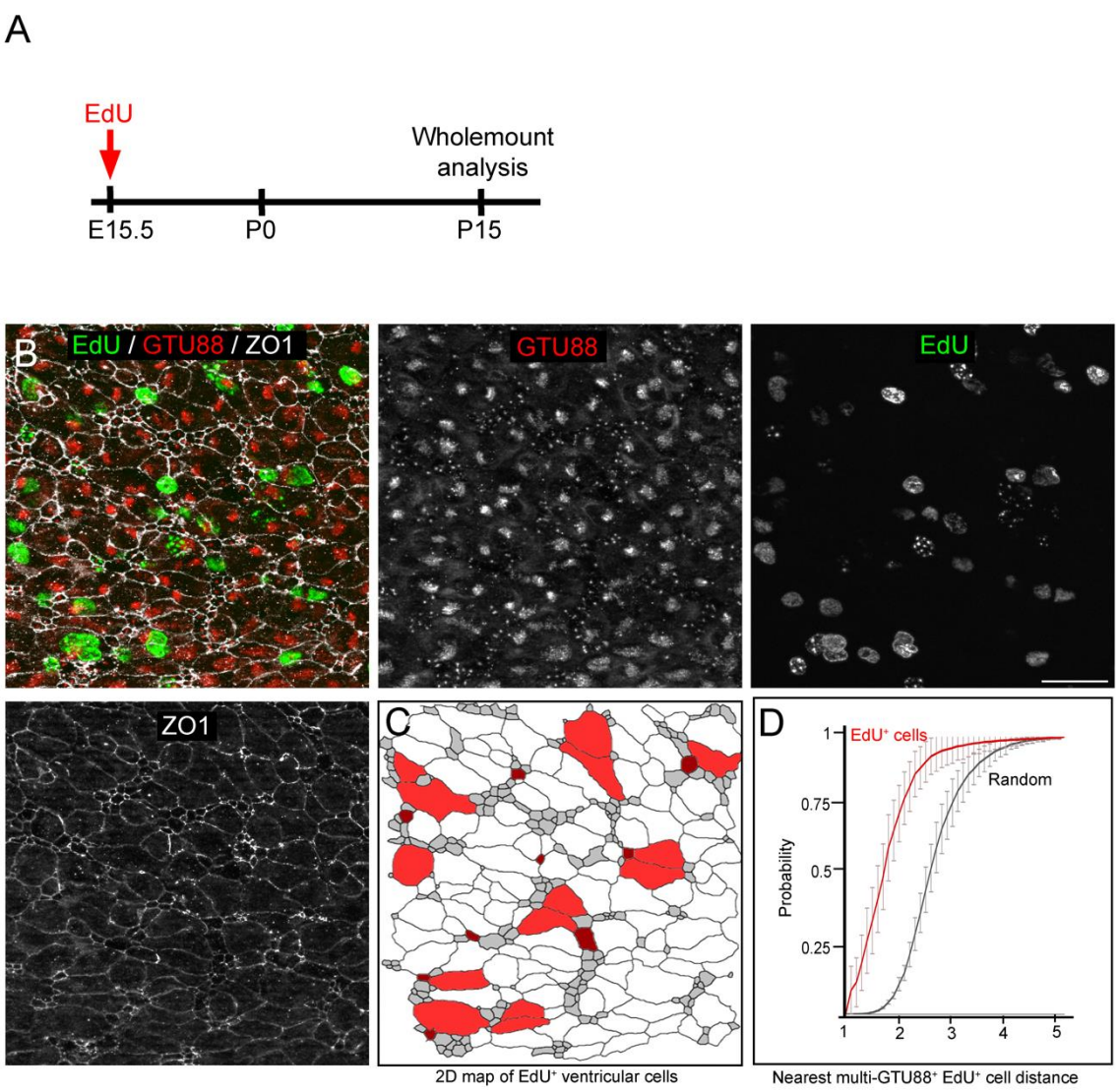
Supplementary Figure 1



Supplementary Figure 1: Ependymal cells derived from E15.5 progenitors are closer than random at P15 (related to Figure 1)

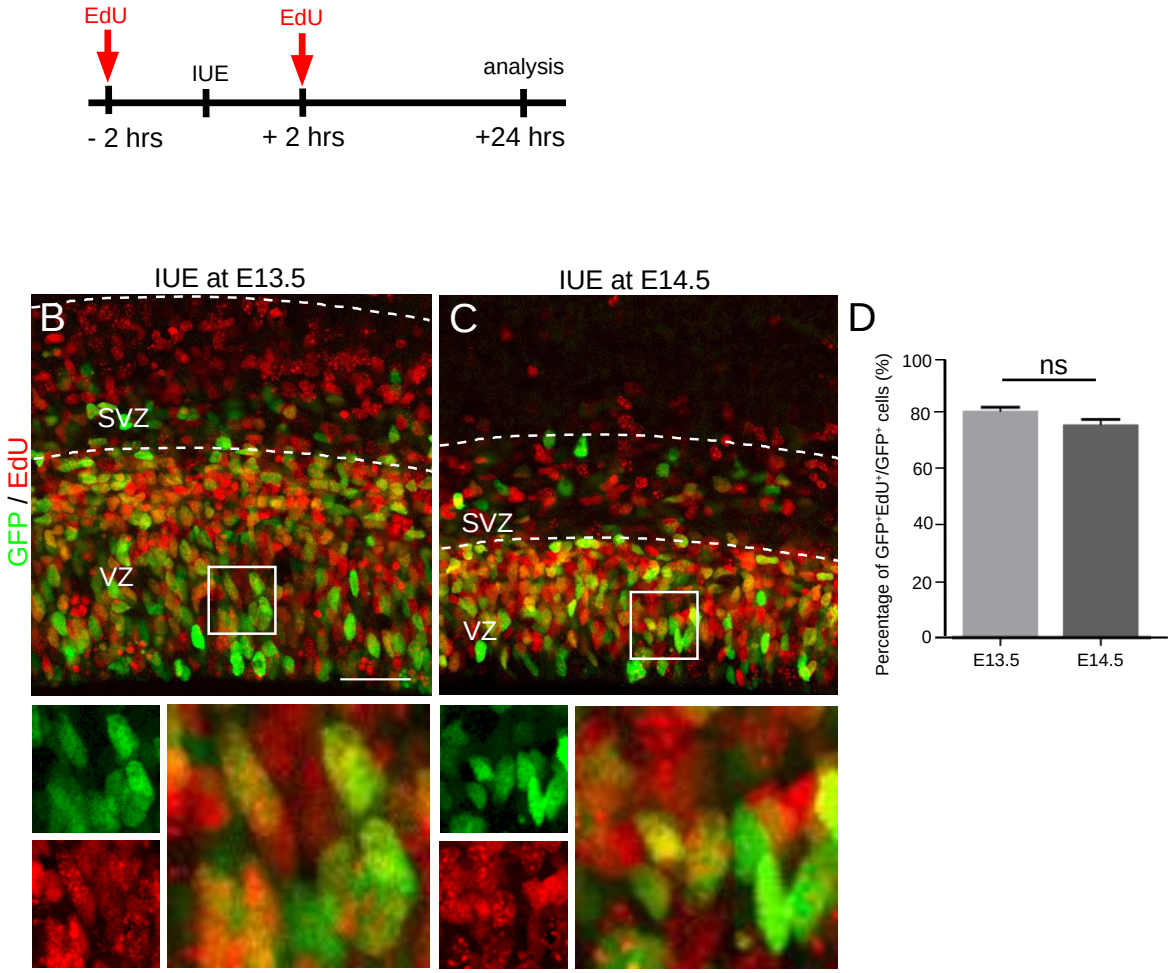
(A) Experimental schema for (B): Timed-pregnant female mice received one injection of EdU at E15.5 and wholemounts of the V-SVZ of the offspring were analyzed at P15.

(B) Triple immunolabeling with ZO1 (white), EdU (green) and gamma tubulin (clone GTU88, basal body marker, red).

(C) 2D map of EdU^+ ependymal and B1 cells. SME projection was used to extract a 2D image of the surface in the vicinity of the apical layer of the 3D stack. Watershed segmentation was then performed on 2D image, using local maxima of the adaptive gaussian-smoothed input image as seeds. The segmented cells were then classified in ependymal multiple-dotted $GTU88^+$ EdU^+ (red), B1 double-dotted $GTU88^+$ EdU^+ (brown) or EdU^- (white) cells with a rule-based classifier applied to texture features computed from each single segmented cell.

(D) Nearest neighbor distance analysis of EdU^+ ependymal cells (with multiple $GTU88^+$ basal bodies) in the P15 V-SVZ injected with EdU at E15.5. According to the average amount and proportion of EdU^+ cells observed in 24 images obtained from 5 different mouse brains, 500 artificial images were generated, each containing a regular hexagonal grid of 345 cells with a 0.065 probability of being randomly EdU^+. From there, a distribution of the distance of the closest EdU^+ cell from each EdU^+ cell was obtained with the distance defined as the number of cells between two EdU^+ cells. A mean of the 500 cumulative distributions are represented by the black curve. The red curve represents the same computed results made on the real dataset of 25 images. Error bars represent the SD. The p-value was determined with the non-parametric Kolmogorov-Smirnov test for 2 samples; ***$p \leq 0.001$. The scale bar represents 25 µm.

Supplementary Figure 2



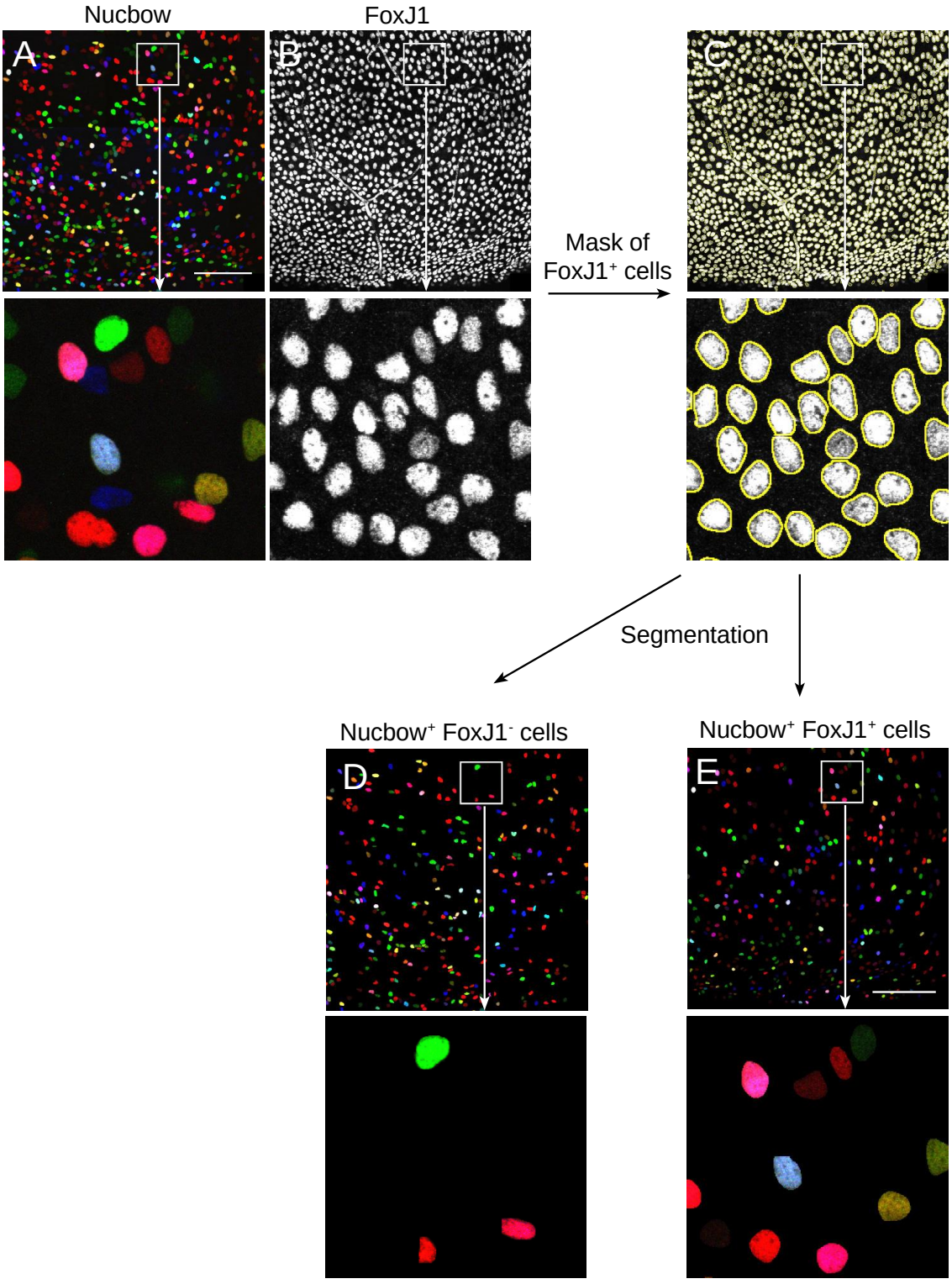
Supplementary Figure 2: *In utero* electroporation targets proliferating radial glia progenitors (related to Figure 2)

(A) Experimental schema for (B): Timed-pregnant female mice received a single injection of EdU 2 hours before and after *in utero* electroporation of H2B-GFP at E13.5 (B) or E14.5 (C) and coronal sections of the forebrain were analyzed 24 hours later.

(B-C) EdU labeling on coronal sections of the H2B-GFP$^+$ brains, 24 hours after the electroporation.

(D) Mean percentage of GFP$^+$EdU$^+$ among all GFP$^+$ cells one day after the electroporation at E13.5 or E14.5. Data are presented as the mean ± SEM. The p-value was determined by the Mann-Whitney test; ns, p>0.05, n=3 experiments. VZ, ventricular zone; SVZ, subventricular zone. The scale bar represents 75 µm.

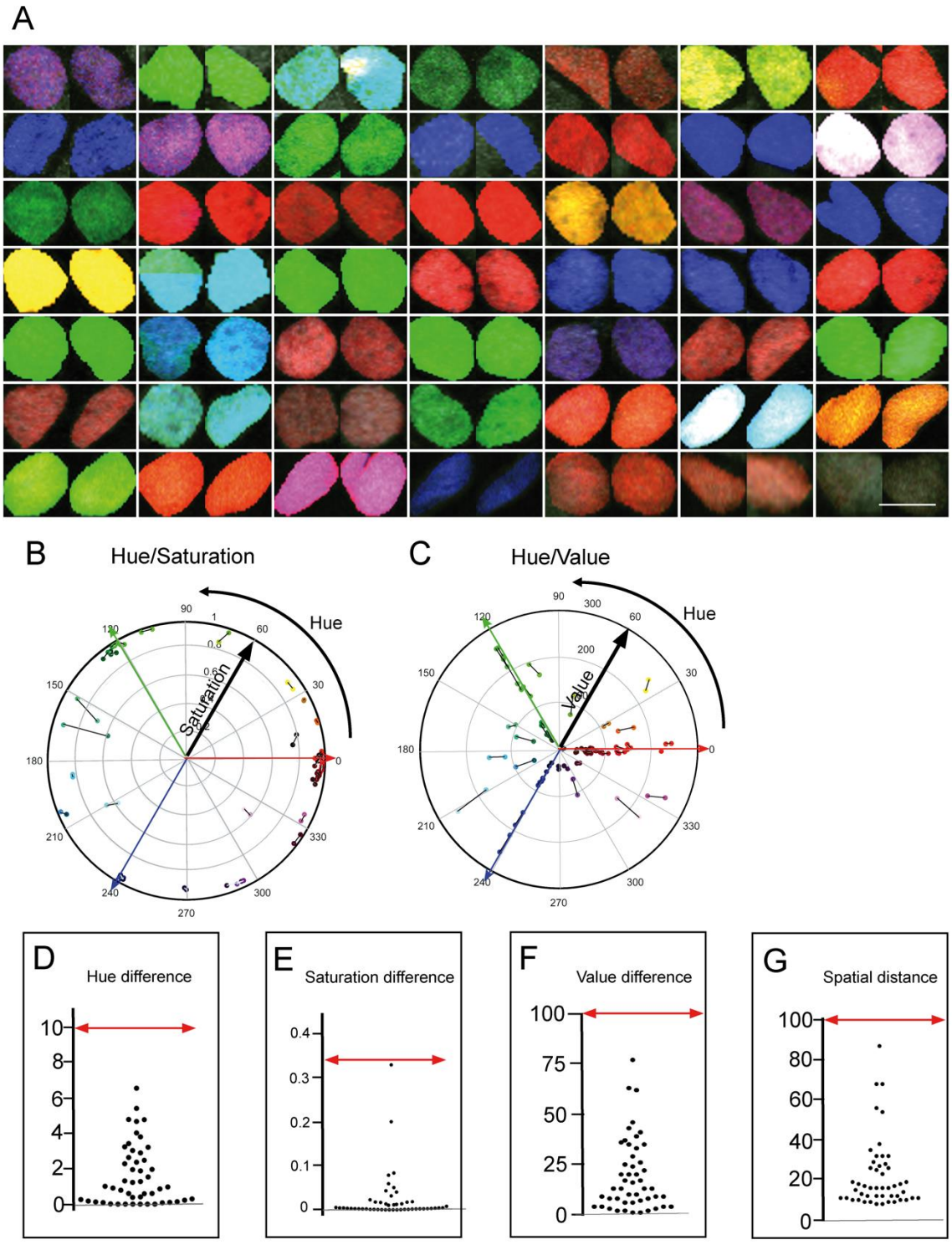
Supplementary Figure 3



Supplementary Figure 3: Methodology for the detection of FoxJ1^{+} and FoxJ1^{-} Nucbow^{+} cells (related to Figure 3)

(A-B) Representative raw images of an *en-face* view of the V-SVZ electroporated at E14.5 with ^{PB}*CAG-Nucbow* along with the PiggyBac transposase and the self-excising Cre recombinase (A) and immunostained at P15 with FoxJ1 antibody (B).
(C) 25 µm 3D-segmentation of FoxJ1^{+} cells outlined in yellow using Gaussian smoothing, Log3D filtering and 3D watershed segmentation implemented as a Fiji macro (see methods).
(D-E) Segmented images of Nucbow^{+}FoxJ1^{-} and Nucbow^{+}FoxJ1^{+} cells, respectively. The scale bar represents 300 µm.

Supplementary Figure 4



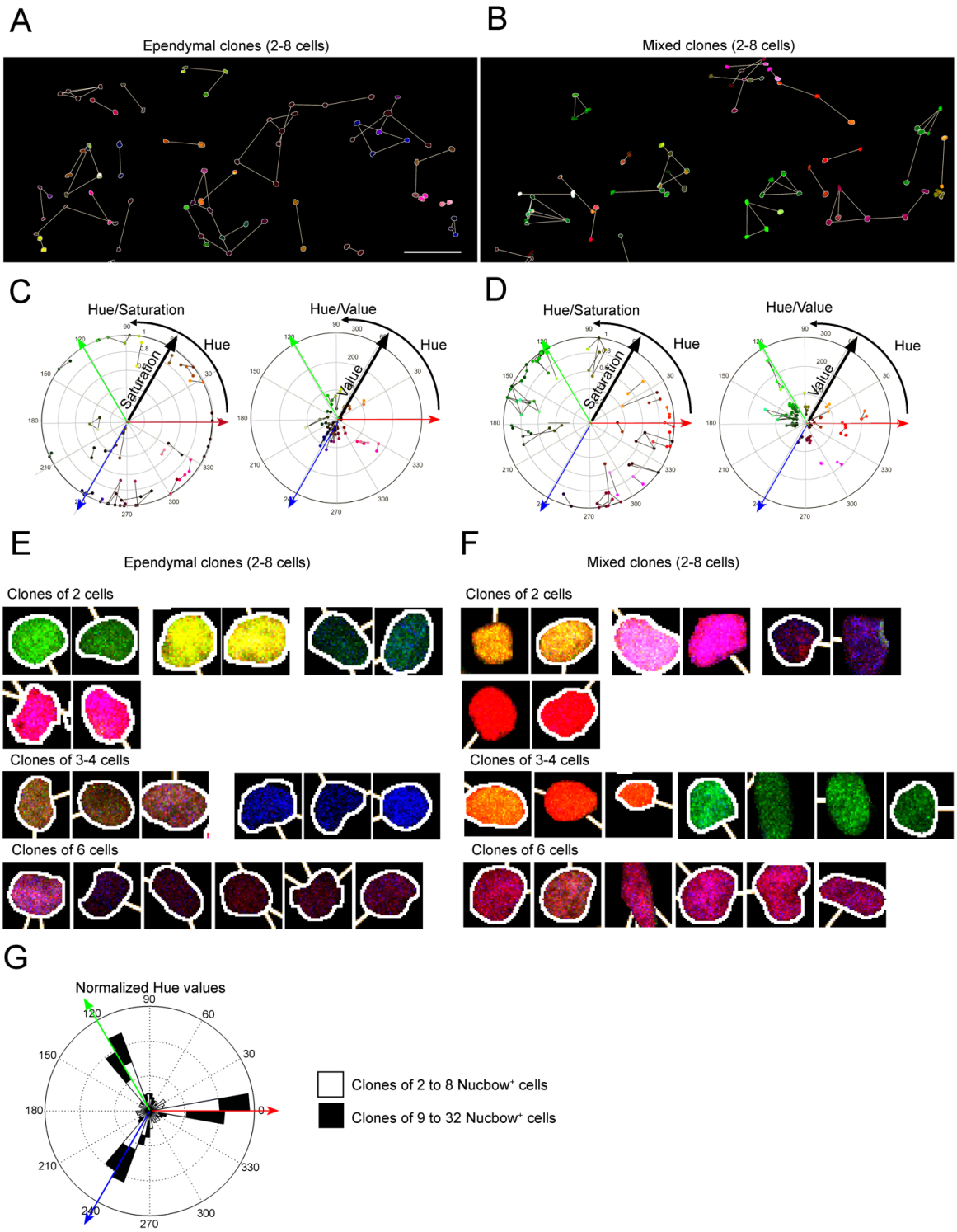
Supplementary Figure 4: Color analysis of manually selected Nucbow^{+} clones (related to Figure 3)

(A) 49 couples of cells were manually selected by 2 independent researchers from 4 different electroporated brains with ^{PB}*CAG-Nucbow* along with the PiggyBac transposase and the self-excising Cre recombinase at E14.5.

(B-C) Circular Hue-Saturation (B) and Hue-Value (C) plots of manually selected Nucbow^{+} cells shown in (A).

(D-G) Hue, Saturation, Value differences and spatial distance between each cell of the manually selected Nucbow^{+} clones shown in (A). The red arrows indicate the thresholds chosen for the automatic analysis. The scale bar represents 15 µm.

Supplementary Figure 5



Supplementary Figure 5: Color analysis of Nucbow$^+$ clones (related to Figure 3)

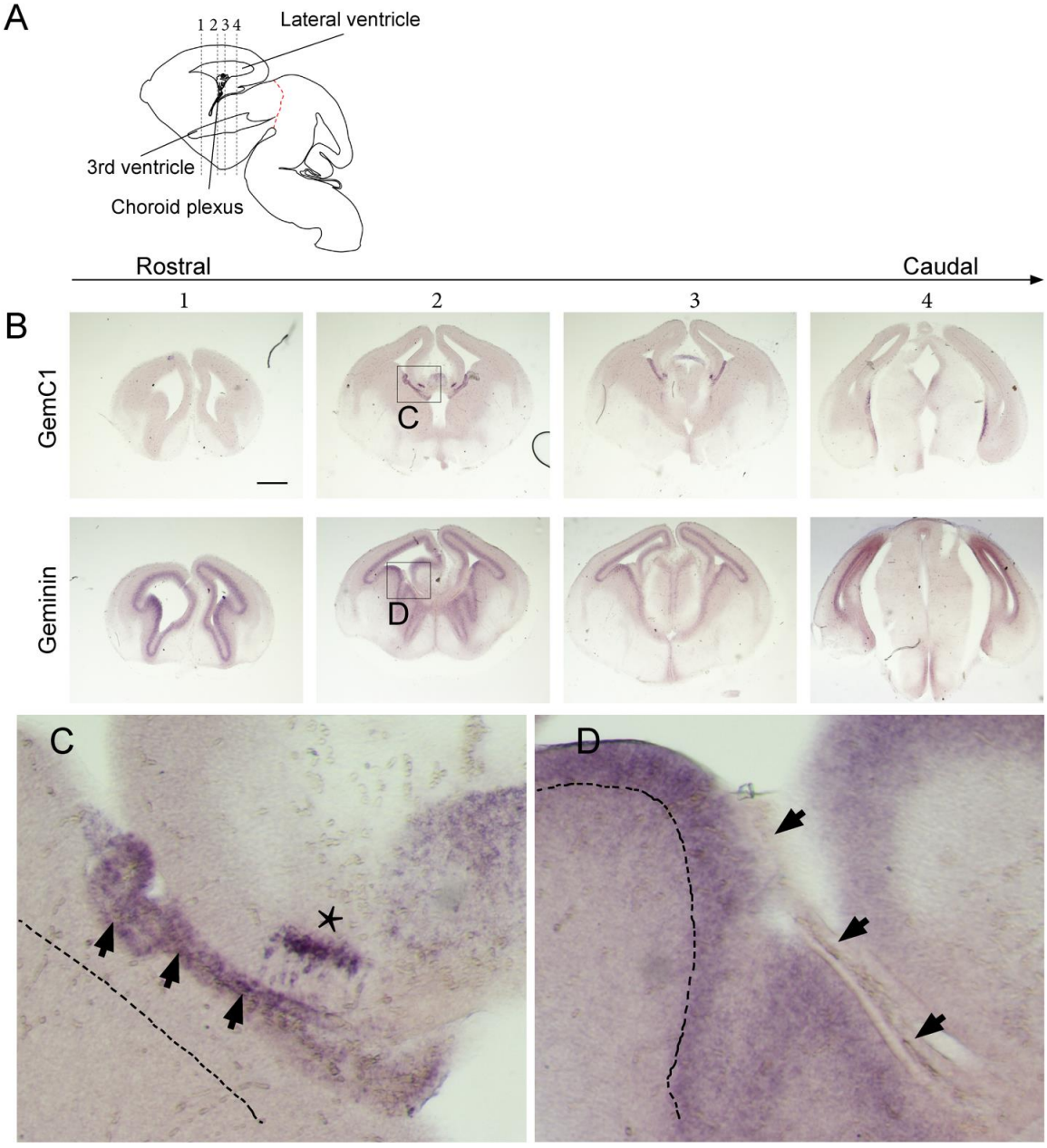
(A-B) Map of Nucbow$^+$ clones containing 2 to 8 ependymal cells (A) or a mixed population of FoxJ1$^+$ and FoxJ1$^-$ cells (B).

(C-D) Circular Hue-Saturation and Hue-Value plots of all depicted Nucbow$^+$ cells from (A-B), respectively.

(E-F) Examples of ependymal cell clones formed by 2, 3 or 6 FoxJ1$^+$ cells (E) and clones containing at least one FoxJ1$^+$ cell formed by 2, 3, 4 or 6 cells. In all maps, FoxJ1$^+$Nucbow$^+$ cells are outlined in white.

(G) Normalized circular histogram of Hue values of cells contained in clones of 2 to 8 cells (small clones, white) or clones of 9 to 32 cells (big clones, black). Cells from big clones are more frequent around primary colors compared to cells from small clones (Kolmogorov-Smirnov test, p=0.001). The scale bar represents 100 µm (A-B) and 10 µm (E, F).

Supplementary Figure 6



Supplementary Figure 6: Pattern of expression of GemC1 and Geminin in the E14.5 forebrain (related to Figure 5-6)

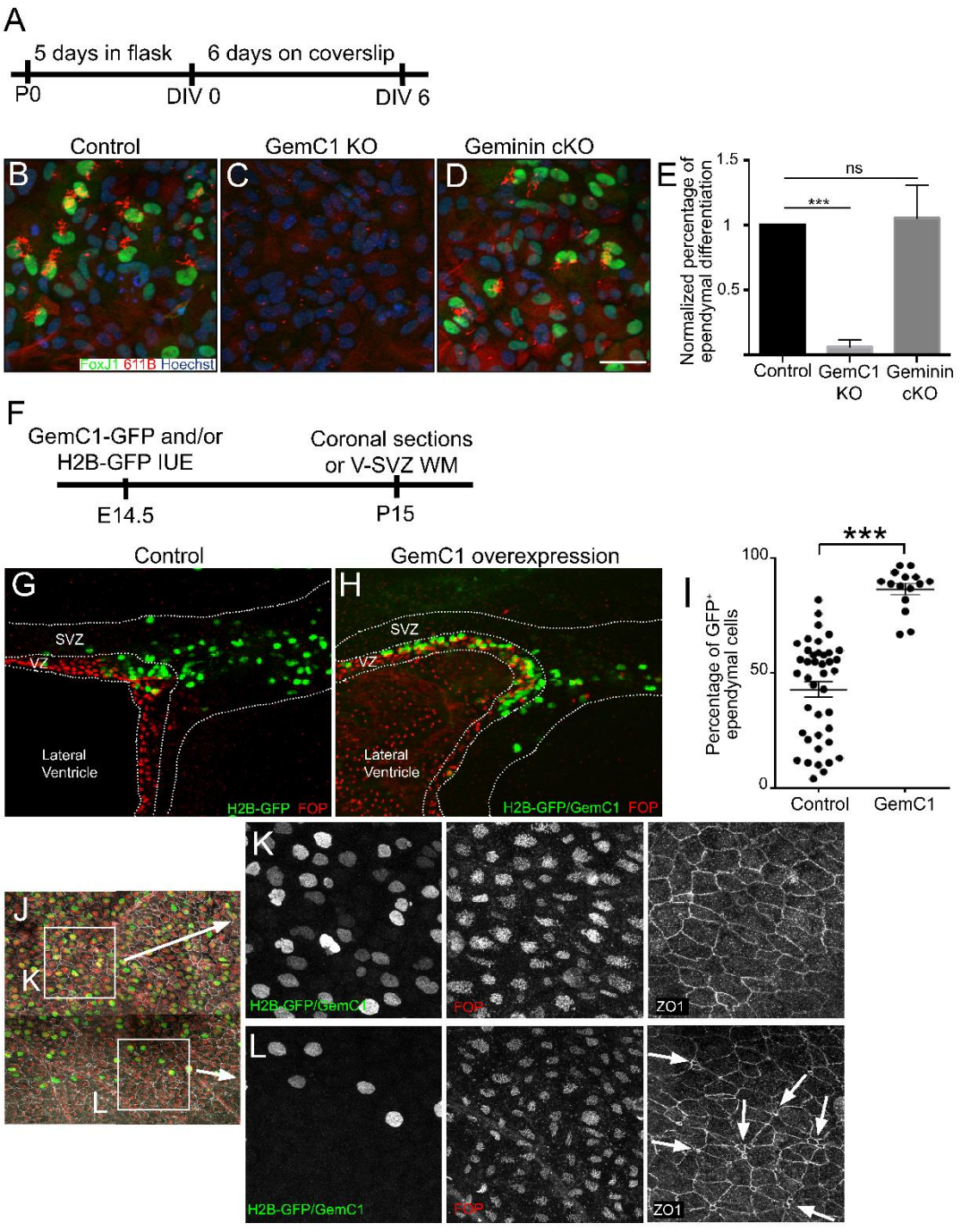
(A) Schematic representation of an E14.5 developing brain and the coronal planes (1 to 4, dashed lines) along the rostrocaudal axis of the lateral ventricles that are represented in B-C. Note that planes 2 and 3 pass along the choroid plexus. The red dashed line indicates the separation between the forebrain and the midbrain.

(B) Stereo microscope images of the i*n situ* hybridization of GemC1 and Geminin in four sections along the rostrocaudal axis.

(C-D) High magnification images of the pictures in (B) for GemC1 (C) and Geminin (D). The dashed line indicates the area of the germinal zone. Arrowheads point at the choroid plexus. The asterisk points at the fimbria.

The scale bar represents 500 µm (B) and 36 µm (C-D).

Supplementary Figure 7



Supplementary Figure 7: GemC1 is necessary and sufficient for ependymal differentiation (related to Figure 5-6)

(A) Experimental schema for (B-E): Primary cultures of RGCs from WT, GemC1$^{KO/KO}$ or Geminin$^{flox/ko}$;NestinCre$^{\pm}$ animals were seeded in flasks containing serum-rich medium for 5 days. Ependymal progenitors were then seeded at high confluency on coverslips in serum-deprived medium and they were left for 6 days to differentiate.

(B-D) Double immunolabeling with FoxJ1 (green) and acetylated tubulin (clone 611B, cilia marker, red) for WT (B), GemC1$^{KO/KO}$ (C) or Geminin$^{flox/ko}$;NestinCre$^{\pm}$ cultures (D, Geminin cKO).

(E) Mean assessment of ependymal differentiation normalized to the percentage of differentiation of the controls (WT animal cultures). Error bars represent the SEM; p-values were calculated via the Mann-Whitney test; ns, $p>0.05$, ***$p\leq0.001$.

(F) Experimental schema: GemC1 and/or H2B-GFP plasmids were electroporated *in utero* at E14.5 and V-SVZ WM or coronal sections were analyzed at P15.

(G-H) Confocal image of coronal sections of the forebrain at P15 immunostained with GFP and FOP antibodies (red), previously electroporated with H2B-GFP (G) or GemC1 and H2B-GFP (H). Ependymal cells are identified by the co-localisation with the basal body marker FOP. VZ: ventricular zone; SVZ: Subventricular zone.

(I) Mean percentage of GFP+FOP+ ependymal cells among all GFP+ cells. Data are presented as the mean ± SEM. The p-value was determined by the Mann-Whitney test; ***$p\leq0.001$, n=3.

(J-L) Confocal images of the V-SVZ WM at P15 immunostained with GFP (green), the basal body marker FOP (red) and ZO1 (white) antibodies. (K-L) High magnification images of the insets in (J) showing that pinwheel structures are absent when most cells express GemC1 (K) whereas pinwheels (arrows in L) are present in regions weakly electroporated with GemC1 (L). The scale bar represents 20 µm (B-D), 40 µm (G-H) and 150 µm (J).

Table S1

E13.5 Cre

Clone ID	Clone Size	Composition of clones		Clone type
		Green cell	Red cell	
1B-3	1	B		B
1C-3	1	B		B
1C-1	1	E		E
1A-5	2	E	B	M
3A-2	2	E	B	M
1C-6	2	B	E	M
1A-7	3	B	EE	M
1B-4	3	E	BB	M
1A-3	3	EE	B	M
1B-7	3	B	EE	M
1B-8	3	EB	E	M
3A-1	3	EB	E	M
1A-6	3	E	EE	E
1B-9	3	E	EE	E
5B-1	4	EE	BB	M
1A-11	4	E	EBB	M
1C-4	4	B	EEB	M
1C-5	4	EB	BB	M
3A-3	4	EBB	E	M
1B-2	4	EEB	B	M
3A-4	4	E	BBB	M
1A-8	4	EE	EE	E
1B-5	4	EEE	E	E
1A-4	5	EEEB	E	M
1A-12	5	EEEB	E	M
1C-2	5	EEB	EE	M
3B-3	5	EB	EEB	M
1B-6	5	BBB	EE	M
3B-2	6	EEBB	EE	M
1A-10	6	EEEEB	E	M
1A-9	7	EEEEB	EE	M
1B-1	7	EEB	EBBB	M

E14.5 Cre

Clone ID	Clone Size	Composition of clones		Clone type
		Green cell	Red cell	
19B-1	1		E	E
23B-4	1		E	E
15C-1	1		E	E
6B-1	2	E	B	M
14A-2	2	B	E	M
10B-3	2	B	E	M
13A-2	2	E	B	M
13B-1	2	E	B	M
15B-1	2	E	B	M
15C-2	2	E	B	M
15C-3	2	B	E	M
15C-4	2	E	B	M
22C-1	2	B	E	M
23B-2	2	B	E	M
9B-1	2	E	E	E
9B-3	2	E	E	E
12B-1	2	E	E	E
13A-1	2	E	E	E
14A-1	2	E	E	E
14A-4	2	E	E	E
14A-5	2	E	E	E
14A-6	2	E	E	E
19A-2	2	E	E	E
23B-1	2	E	E	E
8B-4	3	B	BE	M
12A-1	3	B	BE	M
12C-1	3	EB	E	M
14A-7	3	EE	B	M
19A-1	3	B	EE	M
23B-3	3	B	EE	M
15B-2	3	BB	E	M
15B-8	3	EB	E	M
19B-2	3	EB	E	M
14A-3	3	E	EE	E
15B-4	3	E	EE	E
10B-1	4	BB	EB	M
10B-2	4	BB	EE	M
15B-5	4	EB	EE	M
12C-2	4	EE	EE	E
12C-4	4	EE	EE	E
15B-7	5	EBB	EB	M
9B-2	5	EEE	EE	E
15B-6	5	EEE	EE	E
6B-2	6	EEE	EEB	M
12C-3	6	EEEE	EE	E
15B-9	6	EEEE	EE	E
6B-3	6	EEE	EEE	E

Supplementary Table 1: MADM lineage tracing after Cre expression at E13.5 or E14.5 (related to Figure 4)

Cre activity was induced through IUE in MADM embryos at E13.5 or E14.5 and red-green clones were analyzed on V-SVZ at P15-P20. B=Type B1 astrocytes; E=Ependymal cells; M=Mixed clones; E= Ependymal clones.

Table S2

E13.5 Cre GemC1

Clone ID	Clone Size	Composition of clones		Clone type
		Green cell	Red cell	
21B-5	1		E	E
21B-6	1	E		E
21B-7	1	E		E
21B-2	2	E	B	M
21B-3	2	B	E	M
21C-5	2	B	E	M
22A-2	2	B	E	M
21A-4	2	E	E	E
22A-1	2	E	E	E
21B-4	2	E	E	E
21C-2	2	E	E	E
21C-6	2	E	E	E
22A-3	2	E	E	E
21C-1	3	E	EB	M
21A-1	3	E	EE	E
21A-5	3	EE	E	E
21C-3	3	EE	E	E
21C-4	3	E	EE	E
21A-2	4	EEB	E	M
21B-1	4	EB	EB	M
21A-6	4	EEE	E	E
21A-7	4	EE	EE	E
21A-3	5	EE	EEEE	E

E14.5 Cre GemC1

Clone ID	Clone Size	Composition of clones		Clone type
		Green cell	Red cell	
25C-1	1		E	E
25C-2	1		E	E
25A-3	1	E		E
25A-6	1	E		E
25C-3	2	B	E	M
25A-10	2	E	B	M
28A-7	2	E	B	M
25A-2	2	E	E	E
25A-5	2	E	E	E
25A-7	2	E	E	E
25A-8	2	E	E	E
25A-9	2	E	E	E
26B-2	2	E	E	E
26B-3	2	E	E	E
26B-3'	2	E	E	E
27A-1	2	E	E	E
28A-1	2	E	E	E
28A-2	2	E	E	E
28A-3	2	E	E	E
28A-4	2	E	E	E
28B-1	2	E	E	E
29A-1	2	E	E	E
29A-5	2	E	E	E
29B-1	2	E	E	E
29B-3	2	E	E	E
29B-5	2	E	E	E
25C-4	3	EB	B	M
26B-3"	3	E	EB	M
26A-3	3	B	EE	M
26B-4	5	EEEB	E	M
25A-4	3	EE	E	E
25A-6	3	EE	E	E
26A-1	3	E	EE	E
28A-6	3	E	EE	E
29A-3	3	E	EE	E
29A-4	3	EE	E	E
29A-6	3	E	EE	E
29B-4	3	EE	E	E
29A-2	3	E	EE	E
27A-2	4	EE	EE	E
28A-5	4	EEE	E	E
29A-7	4	EE	EE	E
29B-2	4	EEE	E	E
26A-2	4	EE	EE	E
26B-1	5	EE	EEE	E

Supplementary Table 2: MADM lineage tracing after Cre and GemC1 overexpression at E13.5 or E14.5 (related to Figure 5)

GemC1 overexpression together with Cre activity was induced through IUE in MADM embryos at E13.5 or E14.5 and red-green clones were analyzed on V-SVZ at P15-P20. B=Type B1 astrocytes; E=Ependymal cells; M=Mixed clones; E= Ependymal clones.

Table S3

E13.5 Cre Geminin

Clone ID	Clone Size	Composition of clones		Clone type
		Green cell	Red cell	
400-3-12c	1	B		B
404-1-3	1		B	B
404-1-5	1		B	B
394-1-2	2	B	B	A
394-1-6b	2	B	B	A
400-1-1	2	B	E	M
400-1-2	2	B	E	M
400-1-3	2	E	E	E
400-3-10	2	E	E	E
404-1-1	2	B	E	M
404-1-2	2	B	E	M
404-1-6	2	B	B	B
392-2-2	3	EB	B	M
392-2-4	3	EE	E	E
400-1-5	3	BB	E	M
400-1-9	3	E	EE	E
400-2-4	3		EBB	M
400-3-1	3	EE	B	M
400-3-3	3	B	EE	M
400-3-8	3	EB	B	M
400-3-12b	3		EEE	E
400-4-3	3	EB	E	M
400-4-3b	3	E	EE	E
404-1-7	3	EB	B	M
404-2-1	3		EEE	E
404-2-5	3	EB	B	M
394-1-4	3	EE	E	E
392-2-4b	4	BBB	B	B
392-2-5	4	EB	BB	M
394-1-1	4	EB	EB	M
394-1-5	4	EEB	B	M
394-1-5c	4	EEE	E	E
394-1-6	4	EE	EE	E
394-1-8	4	EE	EE	E
400-1-4	4	EEB	B	M
400-1-7	4		EEEE	E
400-1-8	4		EEEE	E
400-3-2	4	EE	EB	M
400-3-3b	4	EE	EB	M
400-3-4	4	EE	EB	M
400-3-5	4	EB	EB	M
400-3-6	4	EE	EE	E
400-3-9	4		EEEE	E
400-3-10b	4	EE	EB	M
400-3-12	4	EEEB		M
400-3-13	4	EBB	E	M
400-3-14	4	E	EEE	E
400-4-4	4	EB	BB	M
404-1-4	4	BB	EE	M
404-2-2	4	EBB	E	M
404-2-4	4	B	EEE	M
392-2-6	5	EBBB	B	M
392-2-9	5	B	EEBB	M
394-1-3	5	EB	EBB	M
400-4-1	5	B	EEEB	M
392-2-3	5	EEBB	B	M

E14.5 Cre Geminin

Clone ID	Clone Size	Composition of clones		Clone type
		Green cell	Red cell	
341-1-2	1		A	A
360-1-2	1	A		A
362-5-17	1	E		E
362-5-17'	1		E	E
362-5-15	2		E	E
376-4-21	1		A	A
378-4-3	1		A	A
378-4-7	1	E		E
378-4-9	1	E		E
378-5-3	1		E	E
378-3-9	1		E	E
378-3-10	1		E	E
378-3-11	1		E	E
378-3-12	1		E	E
378-3-13	1		E	E
378-3-14	1		E	E
378-3-15	1		E	E
378-3-16	1		A	A
378-3-17	1		A	A
378-3-18	1		A	A
378-3-19	1	A		A
378-3-20	1	A		A
378-3-21	1	A		A
378-3-22	1	A		A
378-3-23	1	A		A
378-3-24	1	A		A
335-1-2	2	A	A	A
376-4-19	2	A	A	A
376-4-25	2	A	A	A
376-5-8	2	A	A	A
376-5-12	2	A	A	A
376-6-4	2	A	A	A
378-3-8	2	A	A	A
378-4-2	2	A	A	A
378-4-8	2	A	A	A
378-4-26	2	A	A	A
360-1-1	2	E	E	E
376-4-4	2	E	E	E
376-4-8	2	E	E	E
376-4-9	2	E	E	E
376-4-11	2	E	E	E
376-4-12	2	E	E	E
376-5-2	2	E	E	E
376-5-10	2	E	E	E
378-3-1	2	E	E	E
378-3-7	2	E	E	E
378-4-5	2	E	E	E
378-4-11	2	E	E	E
378-4-13	2	E	E	E
341-3-2	2	A	E	M
359-2-2	2	E	A	M
359-1-1	2	A	E	M
360-1-3	2	E	A	M
362-5-1	2	A	E	M
362-5-3	2	E	A	M
362-5-10	2	A	E	M

392-2-1	6	EEEEB	B	M
392-2-8	6	EEEEBB		M
392-2-8b	6	EEBB	EB	M
400-2-1	6	EEBB	EB	M
400-2-2	6	EB	EEBB	M
400-3-7	6	EE	EEEB	M
400-4-2	6	EEB	EEB	M
394-1-4b	6	EEBB	EE	M
392-2-12	7	EEBB	EEB	M
392-2-14	7	EBB	EEBB	M
394-1-5b	7	EEBB	EEE	M
394-1-7	7	EEEB	EEA	M
400-2-3	7	EB	EEEAA	M
400-3-11	7	EBB	EEEE	M
404-2-3	7	EEE	EEBB	M
392-2-7	7	EEBBB	EE	M
392-2-11	7	EBB	EEAA	M
392-2-13	7	EEEE	EEE	E
392-2-3b	7	EEEB	EEA	M
392-2-10	8	EB	EEEEBB	M

376-4-13	2	A	E	M
376-4-23	2	A	E	M
376-4-24	2	E	A	M
376-4-28	2	E	A	M
376-5-3	2	A	E	M
376-5-4	2	E	A	M
376-5-9	2	A	E	M
376-6-2	2	E	A	M
378-3-4	2	E	A	M
378-3-6	2	E	A	M
378-4-1	2	E	A	M
378-4-12	2	E	A	M
378-4-16	2	E	A	M
378-4-21	2	E	A	M
378-4-24	2	E	A	M
378-5-2	2	E	A	M
378-5-4	2	A	E	M
359-2-1	3	E	EE	E
362-5-2	3	E	EE	E
360-1-4	3	E	EE	E
376-4-6	3	EE	E	E
376-4-16	3	EE	E	E
376-4-22	3	E	EE	E
376-5-14	3	EE	E	E
376-6-5	3	EE	E	E
378-4-6	3	EE	E	E
362-5-5	3	E	EA	M
335-1-1	3	EE	A	M
376-4-2	3	EE	A	M
376-4-15	3	A	EA	M
376-4-17	3	E	EA	M
376-4-20	3	EA	A	M
376-4-26	3	EA	A	M
376-5-13	3	AA	E	M
376-6-8	3	A	EE	M
378-3-2	3	EA	A	M
378-4-4	3	E	EA	M
378-4-15	3	EE	A	M
376-4-5	4	EE	EE	E
376-4-10	4	EE	EE	E
376-6-6	4	EE	EE	E
376-6-7	4	E	EEE	E
378-4-18	4	EE	EE	E
378-4-25	4	EE	EE	E
362-5-14	4	EE	EE	E
376-5-1	4	EA	EE	M
376-5-5	4	AEE	A	M
376-5-11	4	E	EEA	M
376-4-3	4	E	EEA	M
376-6-1	4	EA	EA	M
378-3-3	4	EA	EA	M
378-3-5	4	EE	AA	M
378-4-14	4	EE	EA	M
378-4-17	4	AA	EE	M
378-4-19	4	EA	EE	M
378-4-22	4	EEA	E	M
378-5-1	4	EE	EA	M
361-6-1	4	EE	EA	M
360-1-5	4	EEA	A	M
362-5-4	4	EE	AA	M
362-5-6	4	AA	AE	M
362-5-7	4	A	EEE	M
362-5-9	4	EAA	A	M
362-5-8	5	EE	EEE	E
362-5-18	5	EEEE	E	E
376-6-3	5	EEE	EE	E
362-5-11	5	EEE	EA	M
362-5-12	5	EEA	EA	M
376-5-6	5	AA	EEE	M
376-5-7	5	AAE	AA	M
376-4-1	5	EA	EAA	M

378-4-20	6	EEE	EEE	E
378-4-10	6	EEA	EEE	M
378-4-23	6	EEA	EAA	M
376-4-18	6	EEEAA	A	M
376-4-18bis	7	EEA	EEEE	M
376-4-14	7	EEEEA	EE	M

Supplementary Table 3: MADM lineage tracing after Cre and Geminin overexpression at E13.5 or E14.5 (related to Figure 6)

Geminin overexpression together with Cre activity was induced through IUE in MADM embryos at E13.5 or E14.5 and red-green clones were analyzed on V-SVZ at P15-P20. B=Type B1 astrocytes; E=Ependymal cells; M=Mixed clones; E=Ependymal clones.