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Supplemental Information

Progenitor Cell Dynamics in the Newt Telencephalon during Homeostasis and Neuronal Regeneration

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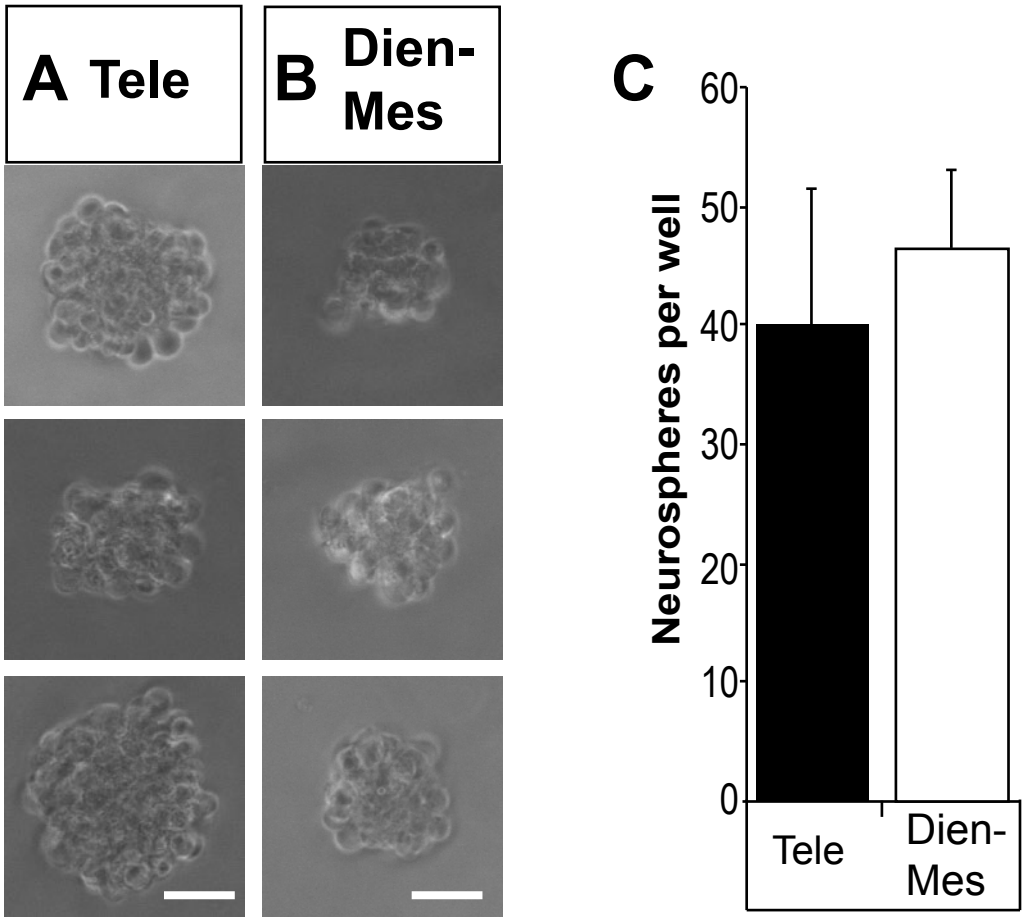
Supplemental Information Inventory

SFig. 1



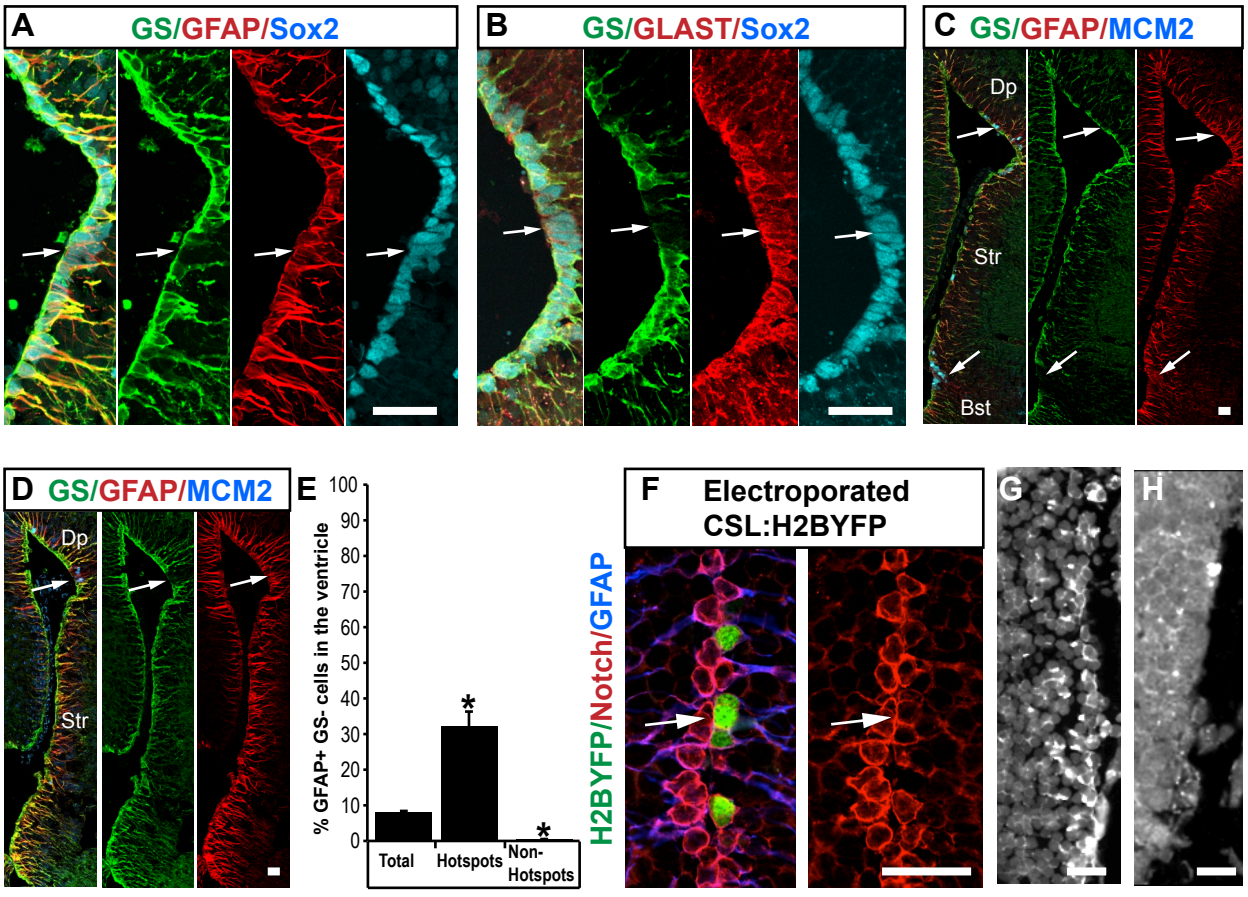
Supplementary Fig: 1 Neurospheres can be generated from all parts of the brain (related to Fig. 1)

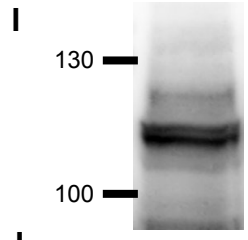
(A) Example of spheres generated from the telencephalon (Tele).

(B) Example of spheres generated from the dien- and mesencephalon (Dien-Mes).

(C) Quantification of sphere-formation. Data represented as mean ± SEM. $P<0.05$ n=4.

SFig. 2





J

	% Identical AA
H.sapiens	76%
M.musculus	76%
X.laevis	78%

K

H.sapiens	LPQESPALPTSLPSSLVPPVTAAQFLTPPSQHSYS	2491
M.musculus	LPQESQALPTSLPSSMVPPMTTTQFLTPPSQHSYS	2466
X.laevis	MPQDTQIFAASLPSNLTQSMTTAQFLTPPSQHSYS	2460
N.viridescens	LPQDTQLLTSSLPSSLTQSLATTQFLTPPSQHSYS	2461
H.sapiens	S-PVDNTPSHQLQVPEHPFLTPSPESPDQWSSSSP	2525
M.musculus	SSPVDNTPSHQLQVPEHPFLTPSPESPDQWSSSSP	2501
X.laevis	S-PMDNTPSHQLQVPDHPFLTPSPESPDQWSSSSP	2494
N.viridescens	S-PMDNTPSHQLQVSDHPFLTPSPESPDQWSSSSP	2495
H.sapiens	HSNVSDWSEGVSSPPTSMQSQIARIPEAFK	2555
M.musculus	HSNISDWSEGISSPPTTMPSQITHIPEAFK	2531
X.laevis	HSNMSDWSEGISSPPTSMQPQRTHIPEAF	2523
N.viridescens	HSNISDWSEGISSPPTSMQAQIAHIPEAFKXMR	2528

Supplementary Fig. 2: Characterization of GS+ ependomyglia cells and Notch signaling in the newt brain (related to Fig. 2)

(A) Sox2 labels all type-2 ependymolglia (GS-/GFAP+). Arrow points to GS- cells.

(B) GLAST labels all type-2 ependymolglia (GS-/Sox2+). Arrow points to GS- cells.

(C-D) Over views of the ventricular wall showing that the vast majority of the ependymoglia cells are non-proliferating type-1 (GFAP+/GS+). Apart from the proliferating hotspot regions (arrows) such as the dorsal pallium (Dp), and the bed nucleus of the stria terminalis (Bst) there are no clusters of type 2 cells present in the ventricular wall. Striatum: (Str).

(E) Quantification of type-2 ependymoglia cells (GFAP+/GS-) in the ventricle wall of the telencephalon. Note that type-2 ependymoglia cells are essential absent in non-hotspots. Data represented as mean ± SEM. $P<0.05$ n=4.

(F) Type-1 (GFAP+/Notch1+) ependymoglia cells in the lateral wall of the lateral ventricle (non-hotspot) are capable of driving H2BYFP expression from a mammalian 12xCSL promoter (arrow).

(G-H) In situ hybridization of anti-sense probe against Notch1 labeled ependymoglia cells in non-hotspot region (G), compared to the sense probe against Notch1 (H).

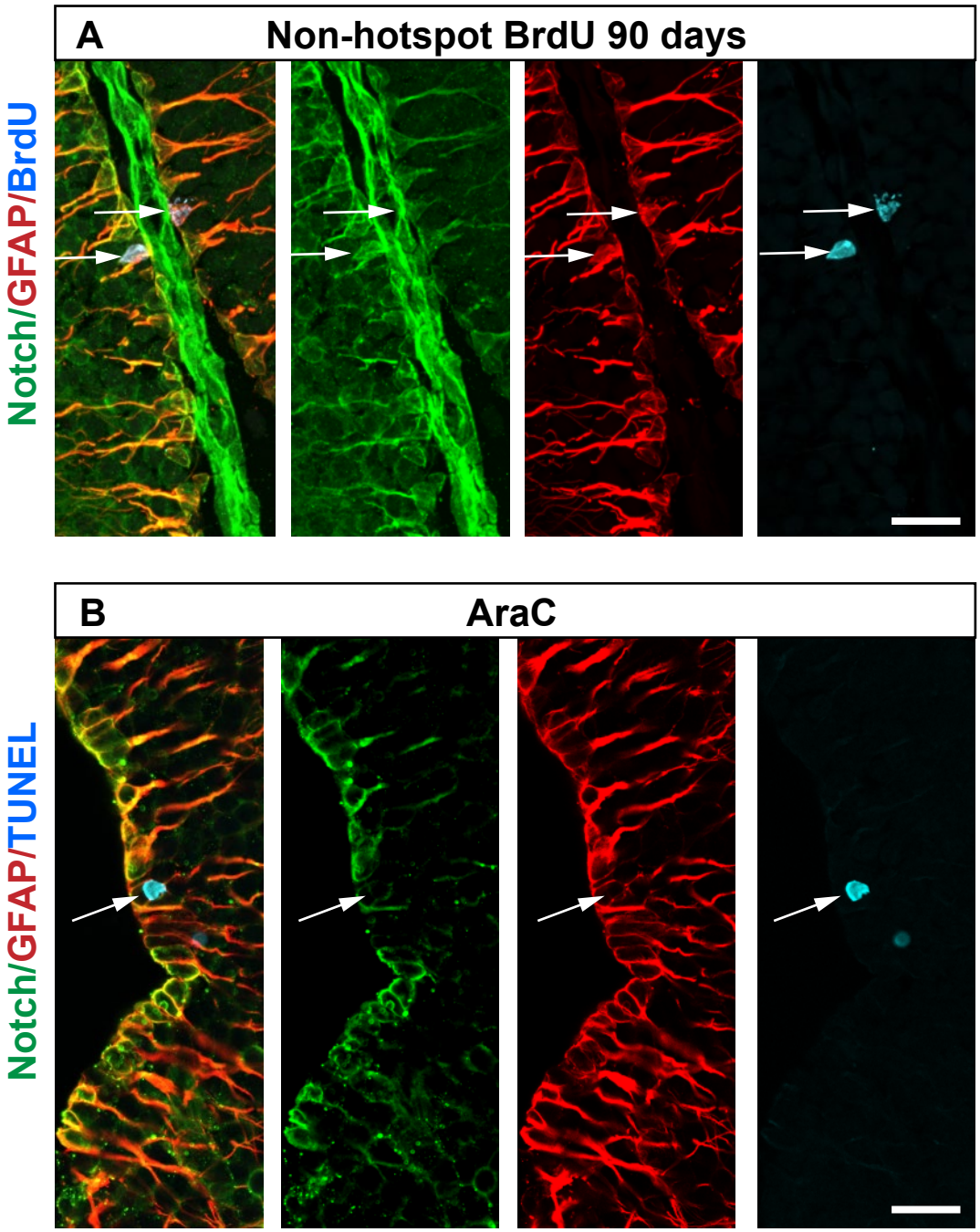
(I) Whole brain lysate contains the cleaved form of the Notch1, detected by Western blot.

(J) N.viridescens Notch1 is highly conserved compared to other species.

(K) Alignment of N.viridescens Notch1 terminal sequence to other mammalian and amphibian species. Amino acids highlighted in black are identical across all species analyzed. Anti-Notch1 antibody was raised to a sequence that mapped to a region with in amino acids 2500 to 2550 (red box) of the H.sapiens Notch1.

Scale bars 50μm

SFig. 3



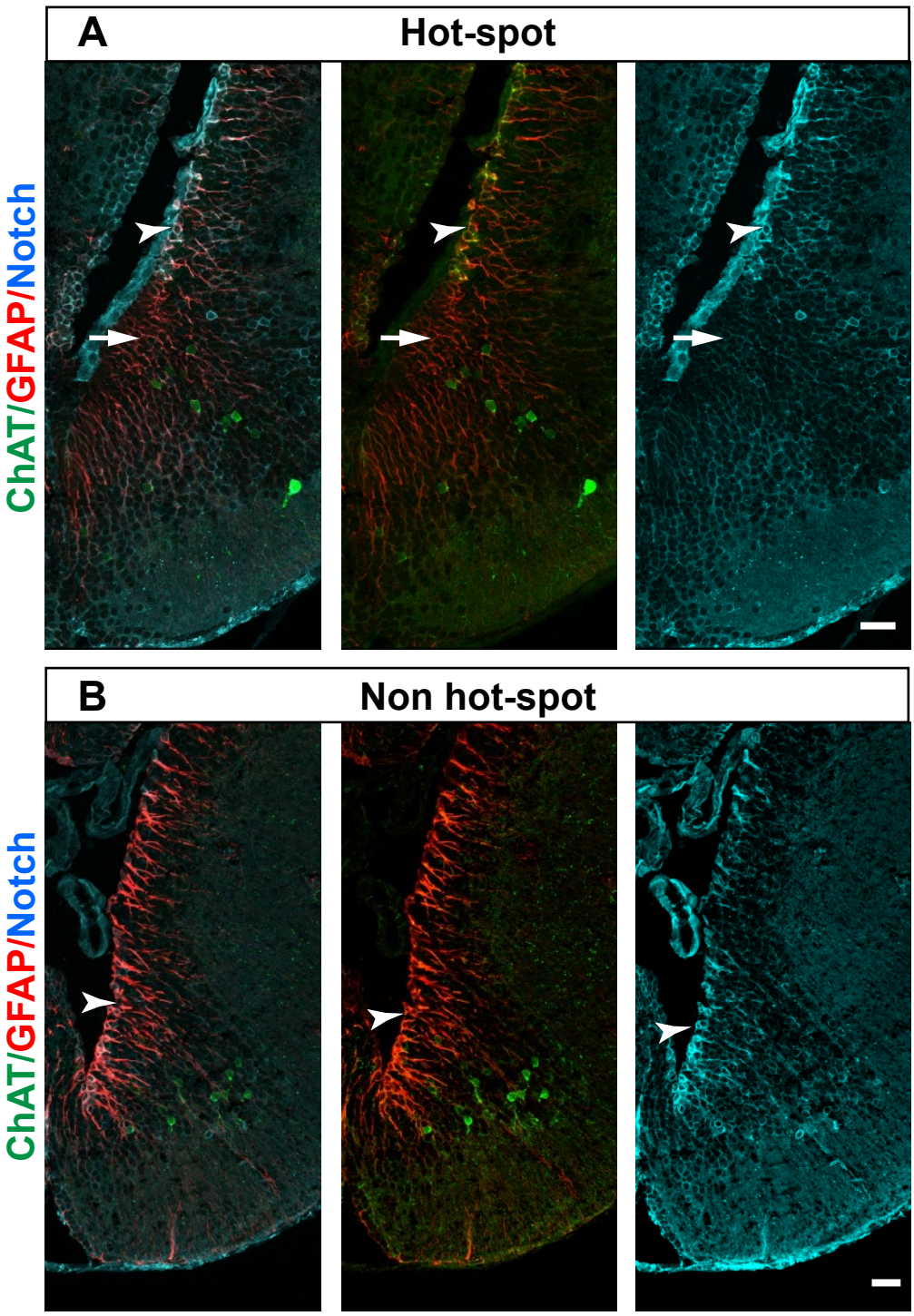
Supplementary Fig. 3: BrdU chased for 90 days labels type-1 cells and AraC treatment kills type-2 cells (related to Fig. 3).

(A) A pulse of BrdU was chased for 90 days. Arrows point to non-hotspot type-1 ependymoglia cells retaining BrdU.

(B) AraC treatment kills type-2 cells. Arrows point to TUNEL staining in hotspots.

Scale bars 50µm

SFig. 4



Supplementary Fig. 4: ChAT-expressing neurons in the parenchyma of the ventral telencephalon (related to Fig. 4)

(A-B) ChAT+ neurons are present in the parenchyma of the bed nucleus of the stria terminalis adjacent to the ventral hotspot (A). The ventral hotspot is identified by the presents of type-2 ependymoglia cells (arrow) Arrowheads point to type-1 ependymoglia outside of the ventral hotspot. The ChAT+ cells form a continuum starting caudally from the bed nucleus of the stria terminalis to the preoptic recess. (B) Ependymoglia cells of the ventral non-hotspots are type-1cells (arrowheads).

Scale bars 50µm.