Stem Cell Reports, Volume *9*

Supplemental Information

Lineage Reprogramming of Astroglial Cells from Different Origins into Distinct Neuronal Subtypes

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Supplemental information to the manuscript *"Lineage reprogramming of astroglial cells from different origins into distinct neuronal subtypes"* by Chouchane et al.

Supplemental Experimental Procedures

Animals

We used C57BL/6, actin-GFP (Okabe et al., 1997), GAD67-GFP (Tamamaki et al., 2003), PV-Cre (Tanahira et al., 2009), GLAST-CreERT2 (Mori et al., 2006) and CAG-CAT-GFP mice (Nakamura et al., 2006). All procedures were done in accordance with national and international laws and were approved by the local ethical committee (CEUA/UFRN, license # 008/2014).

Isolation and expansion of astroglial cells

Cerebellum and cerebral cortex tissues were mechanically triturated and plated in T75 culture flasks containing Astromedium, composed of DMEM/F12 (Gibco), 3.5 mM glucose (Sigma), 10% fetal bovine serum (Gibco), 5% horse serum (Gibco), 100U/ml penicillin/streptomycin (Gibco), 2 % B27 (Gibco), 10 ng/mL epidermal growth factor (EGF, Gibco) and 10ng/mL fibroblast growth factor 2 (FGF2, Gibco). Cultures were incubated at 5% CO_2 and 37°C without moving. After 3-4 days, cultures were washed vigorously with PBS in order to remove unattached and weakly attached cells and medium was replaced with fresh Astromedium. After 7-10 days, astroglial cells reached up to 90% of confluence and were used for transfection.

Astroglial cell passage and transfection

Astroglial cells were chemically detached from T75 culture flasks using trypsin/EDTA (Gibco) 0,5% for 10 minutes at 37^{o}C. In order to stop trypsin action, an equal amount of medium with

10% FBS was added to the detaching cells. Cells were then centrifuged at 1500rpm, 4ºC for 5 min. To generate subsequent passages, 10^6 cells were plated in T75 culture flasks containing Astromedium. Between each passage, the total number of cells was counted, allowing the calculation of the growth rate. For the neurosphere assay, $5x10^5$ were plated in T25 culture flasks containing 5 ml neurosphere medium, composed of DMEM/F12 (Gibco), 3.5 mM glucose (Sigma), 100U/ml penicillin/streptomycin (Gibco), 2% B27 (Gibco), 10 ng/mL epidermal growth factor (EGF, Gibco) and 10ng/mL fibroblast growth factor 2 (FGF2, Gibco). After 7 days, neurospheres were dissociated using trypsin/EDTA (Gibco) 0,5% for 10 minutes at 37^{o}C. Dissociated cells were then plated onto PDL-coated glass coverslips in neurosphere medium without growth factors. For nucleofection, cells were resuspended at 5 x 10^4 cells/µl in the P3 solution (Lonza) containing 1-2 µg of plasmid DNA. Cell/DNA suspension was dropped in the nucleofection well to receive an electrical shock with the program EM110 for mammalian glial cells (Lonza). Astroglial cells were nucleofected with either *pCAG-Neurog2-IRES-DsRed*, *pCAG-Ascl1-IRES-DsRed* or the control plasmid *pCAG-IRES-DsRed*. Next, cells were plated at a density of 70.000 to 100.000 cells/well in poly-D-lysine coated 24-well tissue plates containing Astromedium. 24 hours after nucleofection, medium was replaced with differentiation medium composed of DMEM/F12, 3.5 mM glucose, penicillin/streptomycin and 2% B27. Brain-derived neurotrophic factor (BDNF, Sigma) was added at 20ng/mL every fifth day during the differentiation process.

Co-culture of lineage-reprogrammed astroglial cells with hippocampal neurons

Due to the decreasing survival rate of iNs starting from 20 days post transfection, for analyses performed after this period we co-cultured transfected cells 5 days after nucleofection with hippocampal neurons isolated from P0 pups. Briefly, hippocampal tissues were dissected and

dissociated in trypsin/ EDTA for 15 min. Cells were then centrifuged (1000 rpm, 4ºC) and suspended in a serum-containing medium to stop trypsin activity. Next, cells were centrifuged again, suspended in serum-free medium and added at a density of 50.000 to 70.000 cells/well.

Time-Lapse video microscopy

Time-lapse video microscopy of astroglia cell cultures was performed with a Cell Observer Microscope (Zeiss) at constant conditions of 37°C and 5% CO_2. Phase contrast images were acquired every 10 minutes and fluorescence images every 3h up to 5 days using a 10x phase contrast objective (Zeiss). Acquisition and analysis were preformed using TAT and TTT software, respectively (Hilsenbeck et al., 2016).

Electrophysiology

Cell cultures with induced neurons were transferred to a recording chamber mounted on the stage of a microscope (Zeiss Examiner.A1), equipped with a water immersion x40 objective (Nikon, NA 1.0) and perfused at room temperature, oxygenated with external solution (1 – 1.25 ml/min). Data were acquired using a patch-clamp amplifier Axopatch 200B (Molecular Devices, USA) in current clamp mode, a 16-bit data acquisition card (National Instruments), and WinWCP software implemented by Dr. John Dempster (University of Strathclyde, UK). Patch-pipettes of borosilicate glass capillaries (GC150F-10 Harvard Apparatus) were pulled on a vertical puller (Narishige, Japan) with resistances around 7 MΩ. Pipettes were filled with internal solution (~290 Osm) containing (in mM) 130 K+-gluconate, 7 NaCl , 0.1EGTA, 0.3MgCl2, 0.8 CaCl2, 2 Mg-ATP, 0.5 NaGTP, 10 HEPES, and 2 EGTA (pH 7.2 adjusted with KOH 1M). The external solution (~300 Osm) contained (in mM) 120 NaCl, 3 KCl, 1.2 MgCl2, 2.5 CaCl2, 23 NaHCO3, 5 HEPES, and 11 Glucose (pH 7.4 adjusted with NaOH 1M).

Tissue preparation and histology

Cell cultures were fixed with 4% PFA for 10 minutes at room temperature and stored in PBS. For anti-glutamate staining, we also added 0.3% glutaraldehyde to the fixative solution. Primary antibodies were prepared in a solution of 0.5% Triton X-100, 10% normal goat serum (NGS) and 2% bovine serum albumin (BSA). Samples were incubated with primary antibody solution at 4ºC overnight. The following primary antibodies were used: polyclonal anti-green fluorescent protein (GFP, chicken, 1:500, Aves Labs, GFP-1020), polyclonal anti-Glial Fibrillary Acidic Protein (GFAP, rabbit, 1:4000, DakoCytomation, Z0334), polyclonal anti-Red Fluorescent Protein (RFP, rabbit, Rockland, 1:1000, 600-401-379), monoclonal anti-Microtubule Associated Protein 2 (MAP2, mouse IgG1, 1:200, Sigma-Aldrich, M4403), monoclonal anti-NEUN (mouse, 1:500, Millipore, MAB377), monoclonal anti-SYNAPSIN 1 (mouse IgG2, 1:2000, Synaptic Systems, 106001), polyclonal anti-TBR1 (rabbit, 1:500, Abcam, ab51502), monoclonal anti-BIII TUBULIN (mouse IgG2b, 1:500, Sigma, T5076), monoclonal anti-CALBINDIN 28K (mouse IgG1, 1:2000, Swant), monoclonal anti-PARVALBUMIN (mouse IgG1, 1:2000, Sigma, p3088), monoclonal anti-CUX1 (mouse IgG1, 1:500, ABCAM). For some nuclear staining, TO-PRO-3 (1:2000, Invitrogen) was incubated together with secondary antibody solution. After washing with PBS cells were incubated with appropriate species secondary AlexaFluor (Invitrogen) antibodies for 2 hours at room temperature. After 3 washes in PBS, cell nuclei were stained with DAPI and coverslips were mounted on glass slides with an anti-fading mounting medium (Aqua Poly/Mount).

Tissue fixation was performed 20 to 30 days post transplantation. For that, animals were deeply anesthetized with THIOPENTAX (Cristalia) and transcardially perfused using a ventricular catheter with 0.9% saline solution for 15 min and 4% phosphate-buffered saline-buffered

paraformaldehyde (PFA) for another 15 min. Brains were removed and kept in phosphate-buffered saline (PBS) overnight. The next day, brains were kept in 30% sucrose solution for cryoprotection before freezing procedure. Brains were cut in slices going from 40 to 50µm of thickness using a cryostat (Leica). Subsequently, slices were mounted on gelatin-coated slides and stored at -20ºC until immunohistochemistry (see previous paragraph).

Quantifications

For cerebellar astroglia cell culture characterization we counted 2254 cells for GFAP and BIII-TUBULIN immunoreactivity through 3 independent experiments. A similar number of cells were also checked for Sox2 and O4 immunoreactivity. For the in vitro study, cells were quantified for colocalization of DSRED and BIII-TUBULIN immunoreactivity at 7 days post nucleofection (dpn) (CerebAstro-ASCL1: 1320 cells; CerebAstro-NEUROG2: 1234 cells; CerebAstro-DSRED: 1000 cells). A similar number of cells were quantified for CtxAstro condition. Colocalization of DSRED, MAP2 and NEUN was performed 15 dpn (CerebAstro-ASCL1: 686 cells; CerebAstro-NEUROG2: 446 cells). CALBINDIN (CerebAstro-ASCL1: 170 cells; CerebAstro-NEUROG2: 118 cells) and PARVALBUMIN (CerebAstro-ASCL1: 120 cells; cerebAstro-NEUROG2: 143 cells) expression were analyzed at 20 dpn whereas expression of GABA (CerebAstro-ASCL1: 68 cells; CerebAstro-NEUROG2: 58 cells), GLUTAMATE (CerebAstro-ASCL1: 52 cells; Cereb-NEUROG2: 53 cells) and SYNAPSIN (>40 cells for each condition) was analyzed later, at 30 dpn. Induced neurons (iNs) or RFP+ neurons terms refer to DSRED+ cells that have a clear neuronal morphology. Neuronal morphology was quantitatively analyzed using the plugin "Sholl Analysis" (version 3.4.5 June 2015) in ImageJ version 1.49 (NIH). Concentric circles were centered at the centroid of the cell body with starting radius of 40 µm and increments of 40 µm. 15 to 20 neurons were randomly sorted and analyzed in each

group. Neuronal polarity was quantified by counting the number of cell processes within 4 quadrants centered at the soma. In order to compare with CtxAstro, a similar number of cells were counted in each experiment and all results were obtained through at least 3 independent experiments.

For the in vivo experiments, we studied GFP+ cells for their morphology, hodology and neurochemical markers such as NEUN and CUX1. For each transplant, cells were quantified through the entire brain 20 or 30 days post transplantation (see Table S1 for details).

References

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Supplemental Table 1

Transplanted cells	Host animals (n)	Age	Injection site	Total of counted cells	Dispersion of cells (µm)	Presence of induced neurons
CtxAstro+DSRED	4	Postnatal	Cortex	1145	400	no
CtxAstro+NEUROG2	5	Postnatal	Cortex	2338	300	yes
CtxAstro+ASCL1	4	Postnatal	Cortex	643	250	few
CerebAstro+DSRED	3	Postnatal	Cortex	500	230	no
CerebAstro+NEUROG2	3	Postnatal	Cortex	756	210	few
CerebAstro+ASCL1	4	Postnatal	Cortex	1020	200	few
CtxAstro+DSRED	3	Postnatal	SVZ	> 100*	3000	no
CtxAstro+NEUROG2	3	Postnatal	SVZ	104*	2610	yes
CtxAstro+ASCL1	4	Postnatal	SVZ	117*	3036	yes
CerebAstro+DSRED	4	Postnatal	SVZ	106*	nd	no
CerebAstro+ASCL1	4	Postnatal	SVZ	87*	3162	yes
CtxAstro+ASCL1	2	Adult	SVZ	98*	3600	yes
CtxAstro+DSRED	2	Adult	SVZ	103*	nd	no

* Only cells in the olfactory bulb.

tx: transplanted, CtxAstro: Cortical astrocytes, CerebAstro: Cerebellar astrocytes.

Supplemental figures legends

Figure S1. ***Related to Figure1*****. Characterization of cerebral cortex and cerebellum astroglial cells cultures and neuronal reprogramming of CtxAstro.** (A-D) Images showing CerebAstro and CtxAstro cell cultures 2h after passage and immunostained for GFAP (A and C, red) and BIII-TUB (B and D, green). Quantification of GFAP+ and BIII-TUB+ cells in both CerebAstro and CtxAstro cultures (E). (F-G) Example of CerebAstro culture 7dpn with DSRED. Note that after 7dpn nucleofected cells fail to express BIII-TUB (G). (H-J) Example of CerebAstro culture 24h after nucleofection with ASCL1. Note that all RFP+ nucleofected cells (I) are GFAP+ (H and J, green). (K-P) CtxAstro cultures 7dpn with DSRED (K-L), ASCL1 (M-N) or NEUROG2 (O-P) and immunostained for BIII-TUB (L, N and P, green). Note RFP+/BIII-TUB+ cells only in cultures nucleofected with ASCL1 (N) or NEUROG2 (P). (Q) Quantification of RFP+/BIII-TUBULIN+ cells amongst all RFP+ cells in CtxAstro 7 dpn with ASCL1 (black bar), NEUROG2 (grey bar) or DSRED (white bar). (R) Scheme explaining experimental procedure for generation of passages 1-4 astrocytes, neurosphere assay and direct lineage reprogramming. (S) Image of cells differentiated from passage 3 (P3) CerebAstro neurospheres. Note that the majority of the cells are GFAP+ (green) and only a very low number of neurons are BIII-TUB+ (red, white arrowhead). (T) Example showing RFP+/BIII-TUB+ ASCL1-iNs derived from passage 4 CerebAstro culture. (U) Table summarizing the results obtained at different passages of CerebAstro and CtxAstro for neurosphere formation, proliferative capacity (measured as mean population doubling time) and reprogramming efficiency. TUB: TUBULIN, N.F: not found, ns: not significant, dpn: days post nucleofection. (Student t-test in Q, Mean±SEM). Nuclei are stained with DAPI (blue). Scale bars: 25µm. 3 independent experiments/condition.

Figure S2. *Related to Figure1*. Time lapse video microscopy shows that postmitotic cerebellum astroglial cells are converted into iNs. (A-J) Fluorescence images of nucleofected cells (arrows) taken from the time lapse series after nucleofection with either *Ascl1-DsRed* (A-D) or *Neurog2-DsRed* (G-J). Same fields can be observed in Supplementary Movies 2 and 3, respectively. (E-L) Post imaging immunohistochemistry showing that nucleofected cells (RFP+) are BIII-TUB+ (E, K). Graph showing the percentage of cell death among nucleofected cells undergoing neuronal conversion (black bar) or remaining in astroglial lineage (white bar). (M) Graph showing the percentage of transfected cells undergoing cell division (N) Time in (A-L) represents days-hours:minutes:seconds. TUB: TUBULIN.

Figure S3. *Related to Figure1*. iNs are directed from cells with astroglial origin. (A) Scheme illustrating experimental design of tamoxifen administration in double-transgenic GLAST-CreERT/CAG-CAT-GFP mice. (B-E) Example of GLAST-GFP astroglial cells culture 15dpn with DSRED. Maximal projection of Z-stacks are shown in B. Single Z-stack of dashed box in B is magnified in C-E. Note that nucleofected cell is GFP+ (C), RFP+ (D) and MAP2- (E). (F-M) Example of fluorescence pictures illustrating GLAST-GFP cells 15 dpn with Ascl1-DsRed (F-I) or Neurog2-DsRed (J-M). Maximal projection of Z-stacks are shown in F and J. Single Z-stacks of dashed boxes in F and J are magnified in G-I and K-M, respectively. Note that cells are GFP+ (G, K), RFP+ (H, L) and MAP2+ (I, M).

Figure S4. Morphological properties of iNs depend on astroglia origin and TF used. (A-D) Fluorescence images illustrating different morphologies of RFP+ (white) iNs derived from CerebAstro (A-B) and CtxAstro (C-D) following nucleofection with ASCL1 (A,C) and NEUROG2 (B, D). Example Of iN (white) plotted on concentric circles (red) for Sholl analysis

(D). Graphs of sholl analysis comparing iNs derived from CerebAstro nucleofected with ASCL1 and NEUROG2 (F), from CerebAstro and CtxAstro nucleofected with ASCL1 (H), from CerebAstro and Ctx Astro nucleofected with NEUROG2 (I) and from CtxAstro nucleofected with ASCL1 or NEUROG2 (J). Cartesian plot representing the polarity of iNs derived from CerebAstro (G) and CtxAstro (K) nucleofected with ASCL1 or NEUROG2. (Two-way ANOVA followed by Bonferroni post test, n>15 iNs/condition, Mean±SEM). Nuclei are stained with DAPI (blue). Scale bars: 100µm (A-D). 3 independent experiments/condition.

Figure S5. ***Related to Figure3*****. Expression of glutamatergic and GABAergic markers in iNs derived from lineage-converted astroglia.** (A-L) Images of CtxAstro and CerebAstro cultures 7 dpn with NEUROG2 (A-I) or ASCL1 (J-L). (A-C) Example of an iN derived from CtxAstro nucleofected with NEUROG2 co-staining for RFP (red) and BIII-TUB (green) and TBR1 (white, arrowhead). (D-F) Example of an iN derived from CerebAstro nucleofected with NEUROG2 co-staining for RFP (red) and BIII-TUB (green) but not TBR1 (E, arrowhead). (G-I) Example of a RFP+ iN (red) derived from CtxAstro reprogrammed with NEUROG2 expressing GLUT (green). (J-L) Example of a RFP+ iN (red) derived from CtxAstro reprogrammed with ASCL1 expressing GABA (green). TUB: TUBULIN, GLUT: GLUTAMATE. Nuclei are stained with DAPI (blue). dpn: days post nucleofection. Scale bars: 20µm. 3 independent experiments/condition.

Figure S6. ***Related to Figure4*****. Most iNs derived from CerebAstro reprogrammed with ASCL1 express the Purkinje-cell markers CALBINDIN and CTIP2.** (A-D) Example of an RFP+ iNs derived from CerebAstro 20 dpn with ASCL1 (A, red) expressing CALB (B, green) and CTIP2 (white). Images are merged in panel D. (E) Quantification of CTIP2+ cells amongst

RFP+/CALB+ iNs. (Student t-test, Mean±SD). CALB: CALBINDIN. Nuclei are stained with DAPI (blue). Scale bars: 20µm. 3 independent experiments/condition.

Figure S7. ***Related to Figure5, 6 and 7.*** **Astroglial cells nucleofected with control plasmid, ASCL1 or NEUROG2 adopts distinct phenotypes in host postnatal cortex and olfactory bulb.** (A-C) Pictures showing GFP+ CtxAstro and CerebAstro nucleofected with control plasmid 20 dpt in postnatal cortex. Note that CtxAstro adopt a protoplasmic-like morphology in the grey matter (A), whereas cells from the same population adopt a fibrous-like morphology in the white matter (B). Note also that CerebAstro do not display morphologies reminiscent of cerebral cortex astrocytes (C). (D-I) Example of CerebAstro nucleofected with ASCL1 20 dpt in the postnatal cortex. Example of iN in layer VI of the cerebral cortex (D, dashed box), showing a GABAergic interneuron-like morphology (E). Note that iN processes are non-spiny (F, magnification of box in E). Example of GFP+ iNs found in layer I of the host cerebral cortex expressing the GABAergic interneuron marker CALB (G, dashed box). High magnification of dashed box in G showing GFP (H) and CALB (I) expression in iNs. (J) Example of GFP+ cells CtxAstro nucleofected with ASCL1 30 dpt in host postnatal cortex. Observe the cell with interneuron-like morphology (arrowhead). (K-O) Coronal sections of brains transplanted with CtxAstro nucleofected with NEUROG2 showing GFP+ axonal processes reaching (K) and within the *corpus callosum* (L-M). Note that some processes are found in the hemisphere contralateral to the transplant (N-O). (P-S) Coronal sections of the OB of animals 30 dpt of CtxAstro nucleofected with control plasmid (P, Q) or NEUROG2 (R, S) in the postnatal SVZ. Note that CtxAstro nucleofected with control plasmids migrated to OB and maintained astroglial morphology and expressed GFAP (P-Q, white arrowhead), but not NEUN (Q, red). In contrast, GFP+ cells adopted typical morphologies of granular (R) and periglomerular (S) OB neurons in

animals transplanted with CtxAstro nucleofected with NEUROG2 (R). Nuclei are stained with DAPI (blue). CALB: CALBINDIN, dpt: days post transplantation. Roman numbers in D represent the cerebral cortex layers. CC: *corpus callosum*, gcc: *genus* of the *corpus callosum*, MC: *motor cortex*, PD: *dorsal peduncular cortex.* Scale bar: 20µm. n=3-5 animals/condition.

Supplemental movies legends

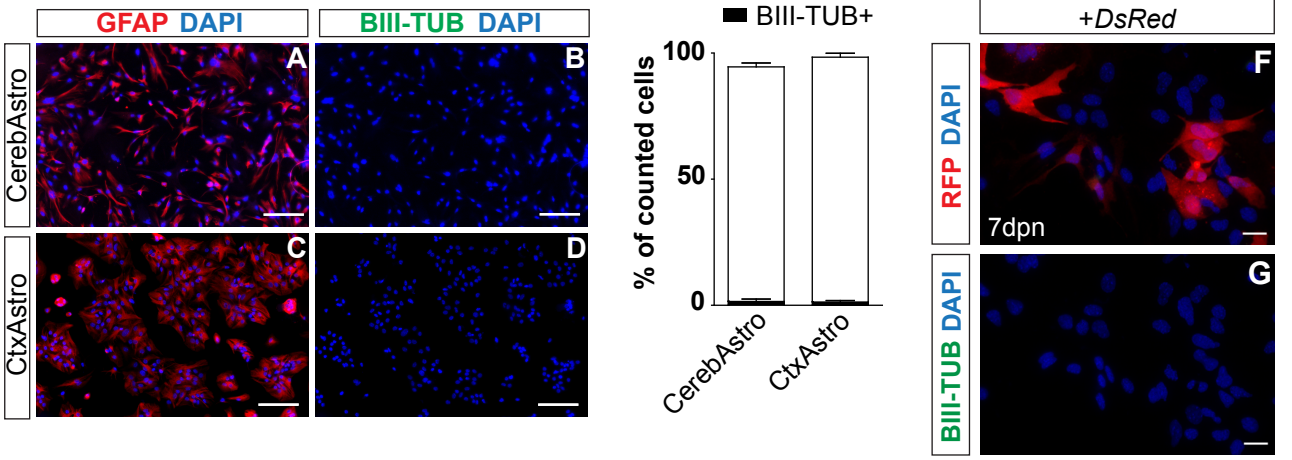
Movie S1. Cerebellum astroglia nucleofected with *control-DsRed*. Frames show fluorescent images taken every 3h. Observe the glial morphology of post mitotic and proliferative cells in the culture. Last frame shows the post-imaging immunocytochemistry for DSRED (red) and BIII-TUBULIN (green). Note that all DSRED positive cells are BIII-TUBULIN negative. Label in the upper left corner indicates the time of observation in days-hours:minutes:seconds.

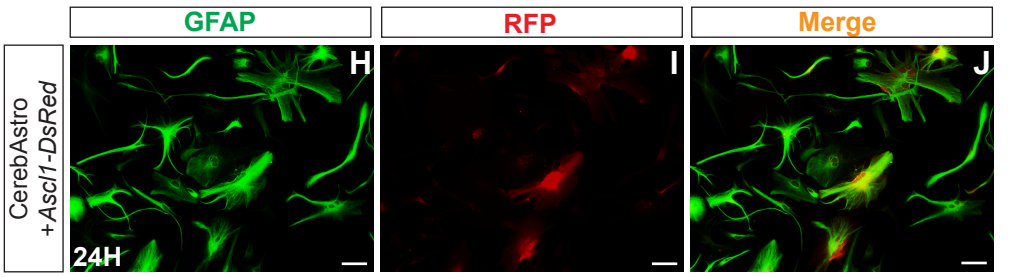
Movie S2. Cerebellum astroglia nucleofected with *Ascl1-DsRed*. Frames show fluorescent images taken every 3h. Observe the progressive transition in the shape of several transfected cells from a glia-like to a neuronal-like morphology. Two cells are indicated in the bottom right corner with red circles to facilitate the observation. Last frame shows the post-imaging immunocytochemistry for DSRED (red) and BIII-TUBULIN (green), where it is possible to observe the high number of DSRED+/BIII-TUBULIN+ cells. Label in the upper left corner indicates the time of observation in days-hours:minutes:seconds.

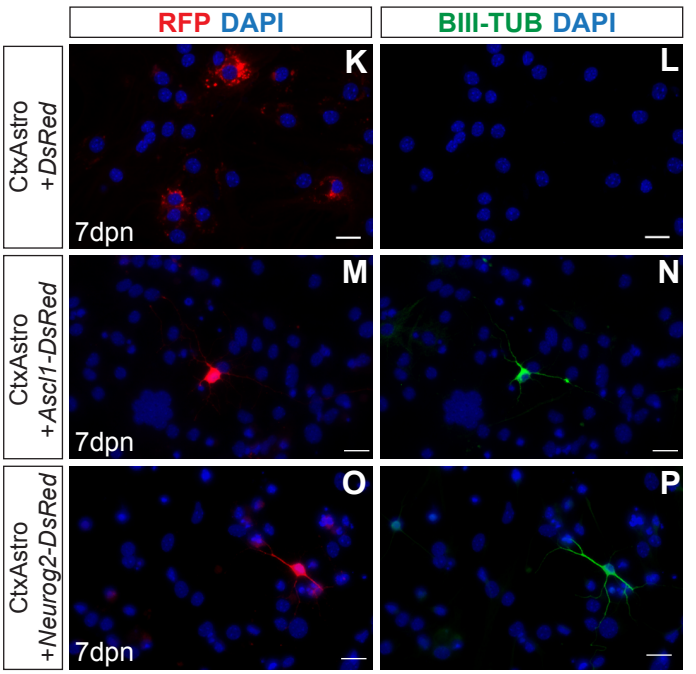
Movie S3. Cerebellum astroglia nucleofected with *Neurog2-DsRed*. Frames show fluorescent images taken every 3h. The cell tracked in the upper left corner (purple circle) undergo one circle of cell division generating two daughter cells. One cell progress to lineage-conversion into neuron and the second dies out. Note also the massive cell death of NEUROG2-transfected cells. Last frame shows the post-imaging immunocytochemistry for DSRED (red) and BIII-TUBULIN (green), where it is possible to confirm the DSRED+/BIII-TUBULIN+ cells nature of the tracked cell. Label in the upper left corner indicates the time of observation in days-hours:minutes:seconds.

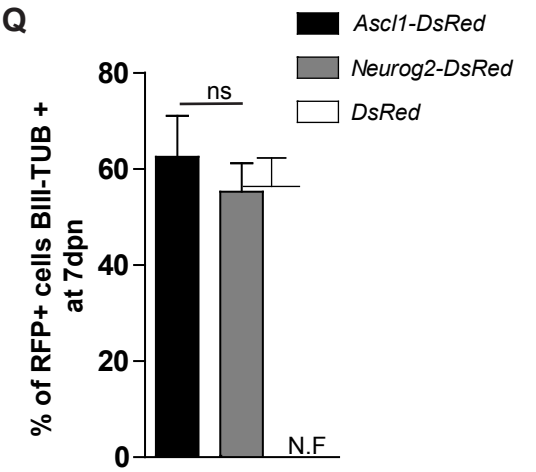
Movie S4. Cerebellum astroglia nucleofected with *Neurog2-DsRed*. Frames show phase contrast images taken every 30min (same field as in MovieS3.). The cell tracked in the upper left corner (purple circle, #1) undergo one circle of cell division generating two daughter cells (white circle, #2 and red circle, #3). One cell progress to lineage-conversion into neuron (#3) and the second dies out (#2). Label in the upper left corner indicates the time of observation in days-hours:minutes:seconds.

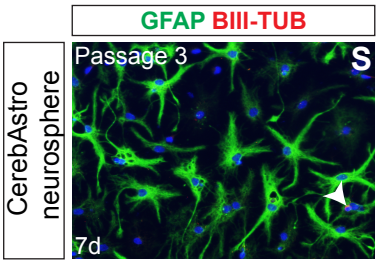
Figure S1











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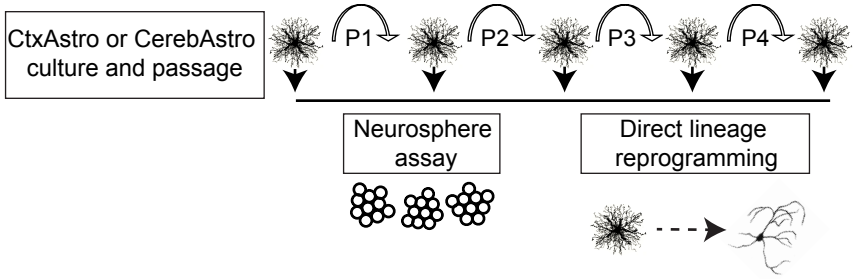

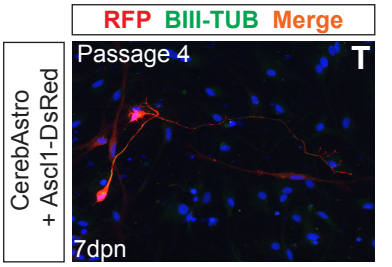

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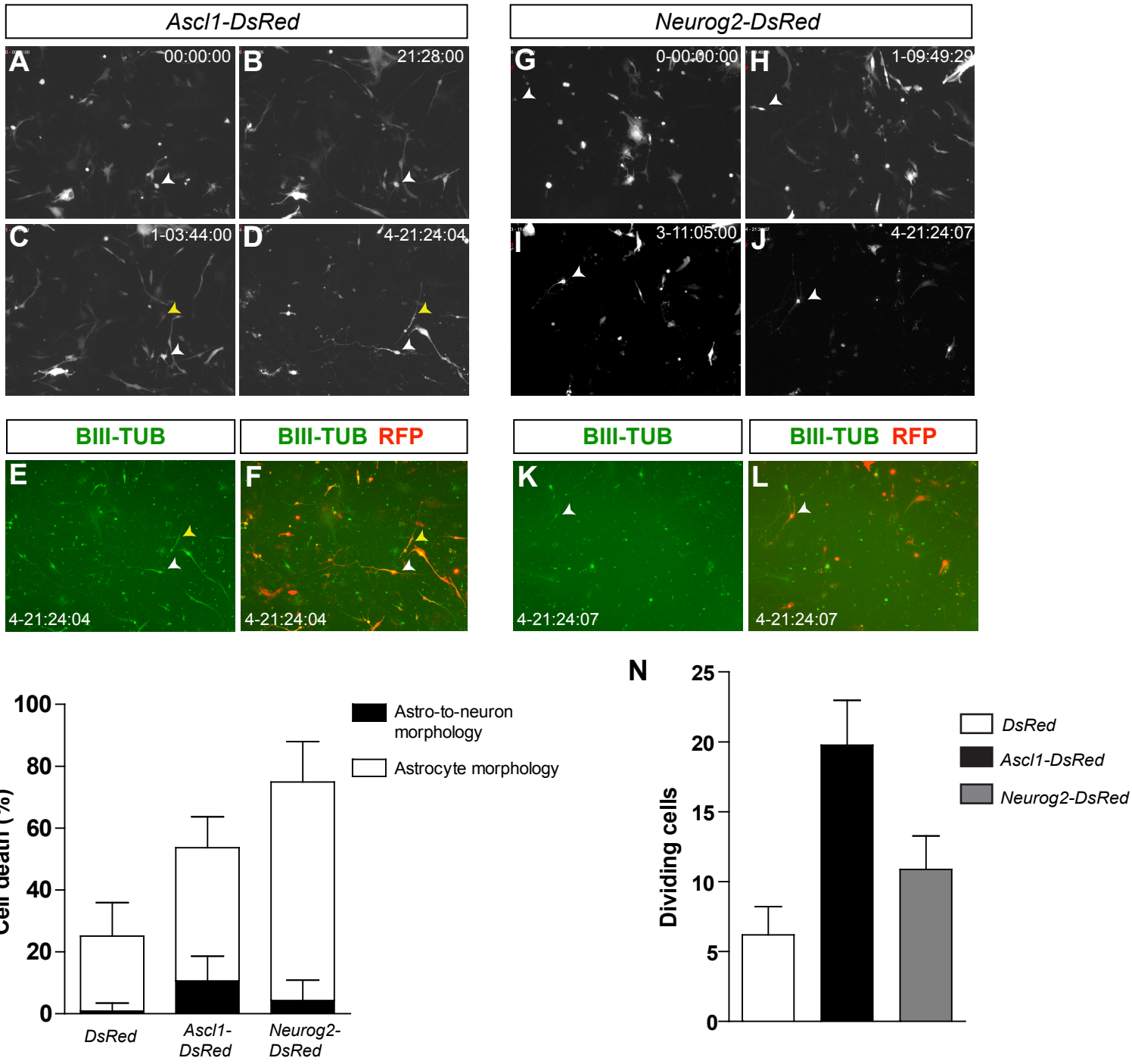
	Cerebral cortex astrocytes				Cerebellum astrocytes			
Passage	P1	P2	P3	P4	P1	P2	P3	P4
Neurosphere formation	++++	++	+	+	++++	+++	++	+
Mean population doubling time (h)	ND	115h	218h*	Plateau	ND	96h	125h	128h
Reprogramming	+++	+	+	ND	+++	+++	+++	++

Figure S2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B
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C
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D
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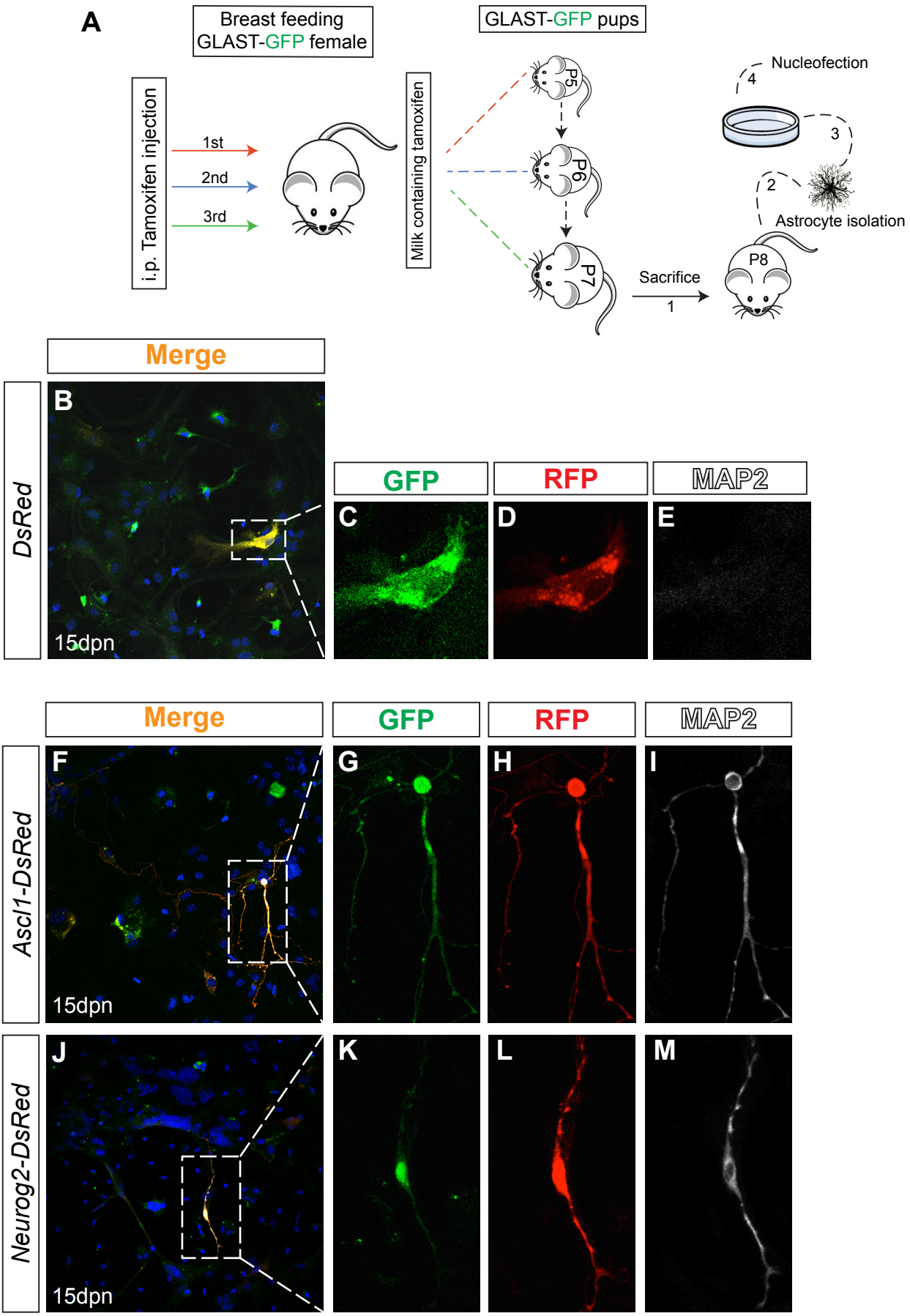
J
4-21:24:07
BIII-TUB
BIII-TUB RFP
E
4-21:24:04
F
4-21:24:04
BIII-TUB
BIII-TUB RFP
K
4-21:24:07
L
4-21:24:07

M
Cell death (%)
100
80
60
40
20
0
Astro-to-neuron morphology
Astrocyte morphology
DsRed
Ascl1-DsRed
Neurog2-DsRed

N
Dividing cells
25
20
15
10
5
0
DsRed
Ascl1-DsRed
Neurog2-DsRed

Figure S3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Breast feeding
GLAST-GFP female
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i.p. Tamoxifen injection
1st
2nd
3rd
Milk containing tamoxifen
P5
P6
P7
P8
Nucleofection
4
3
2
Astrocyte isolation
Sacrifice
1
Merge
DsRed
B
15dpn
GFP
RFP
MAP2
C
D
E
Merge
GFP
RFP
MAP2
Ascl1-DsRed
F
G
H
I
15dpn
Neurog2-DsRed
J
K
L
M
15dpn

Figure S4

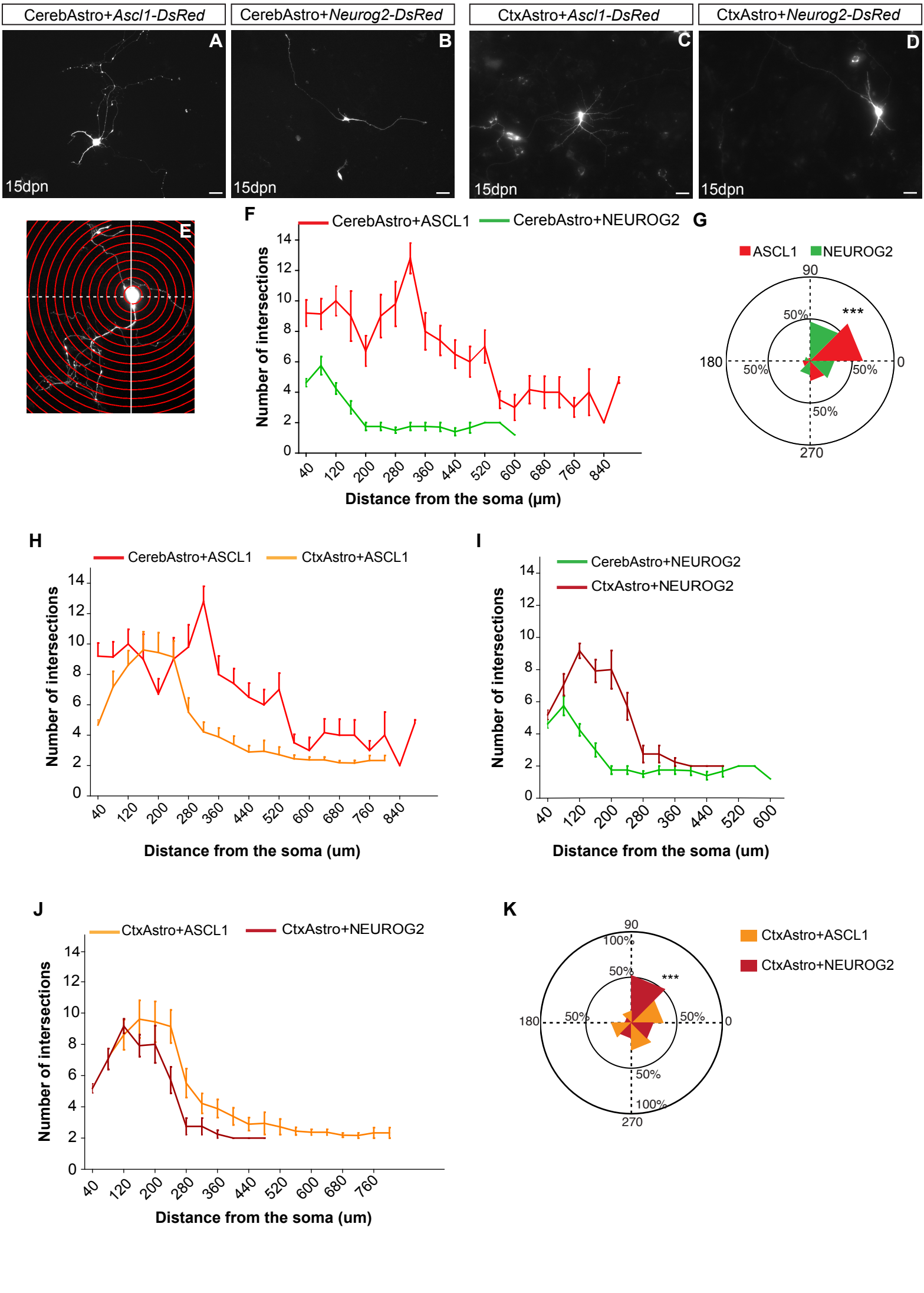

CerebAstro+Ascl1-DsRed
CerebAstro+Neurog2-DsRed
CtxAstro+Ascl1-DsRed
CtxAstro+Neurog2-DsRed
A
B
C
D
15dpn
E
F
CerebAstro+ASCL1
CerebAstro+NEUROG2
Number of intersections
Distance from the soma (μm)
G
ASCL1
NEUROG2
***
H
CerebAstro+ASCL1
CtxAstro+ASCL1
Distance from the soma (um)
I
CerebAstro+NEUROG2
CtxAstro+NEUROG2
J
CtxAstro+ASCL1
CtxAstro+NEUROG2
K

Figure S5

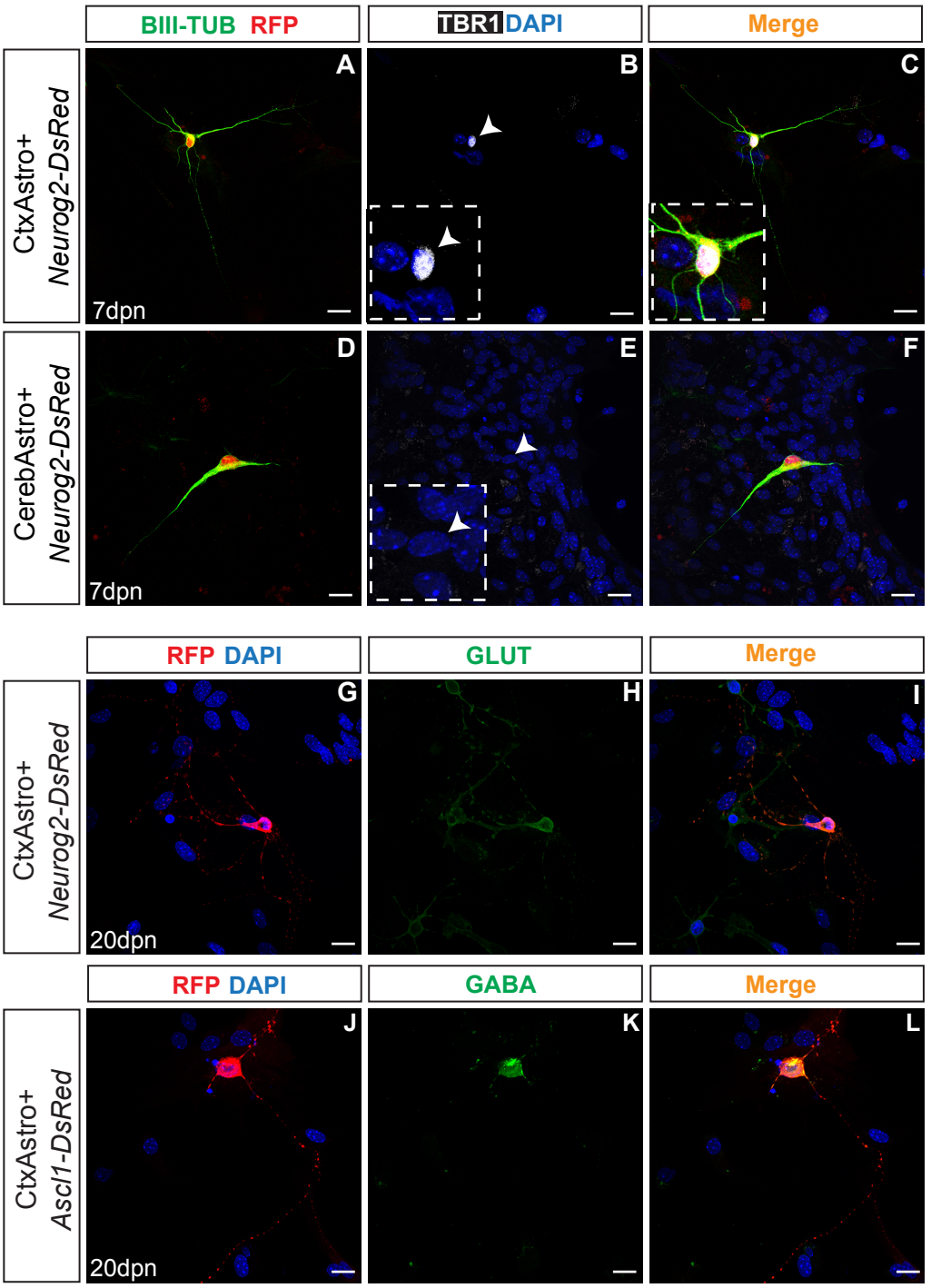

BIII-TUB RFP
TBR1 DAPI
Merge
CtxAstro+ Neurog2-DsRed
CerebAstro+ Neurog2-DsRed
CtxAstro+ Neurog2-DsRed
CtxAstro+ Ascl1-DsRed
RFP DAPI
GLUT
GABA
A
B
C
D
E
F
G
H
I
J
K
L
7dpn
20dpn

Figure S6

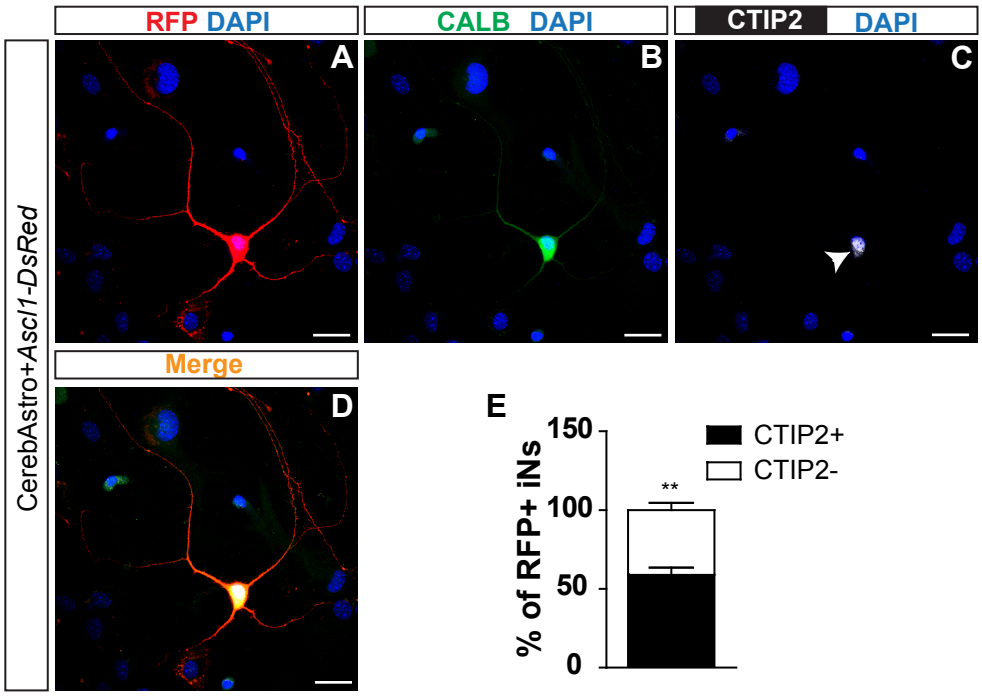
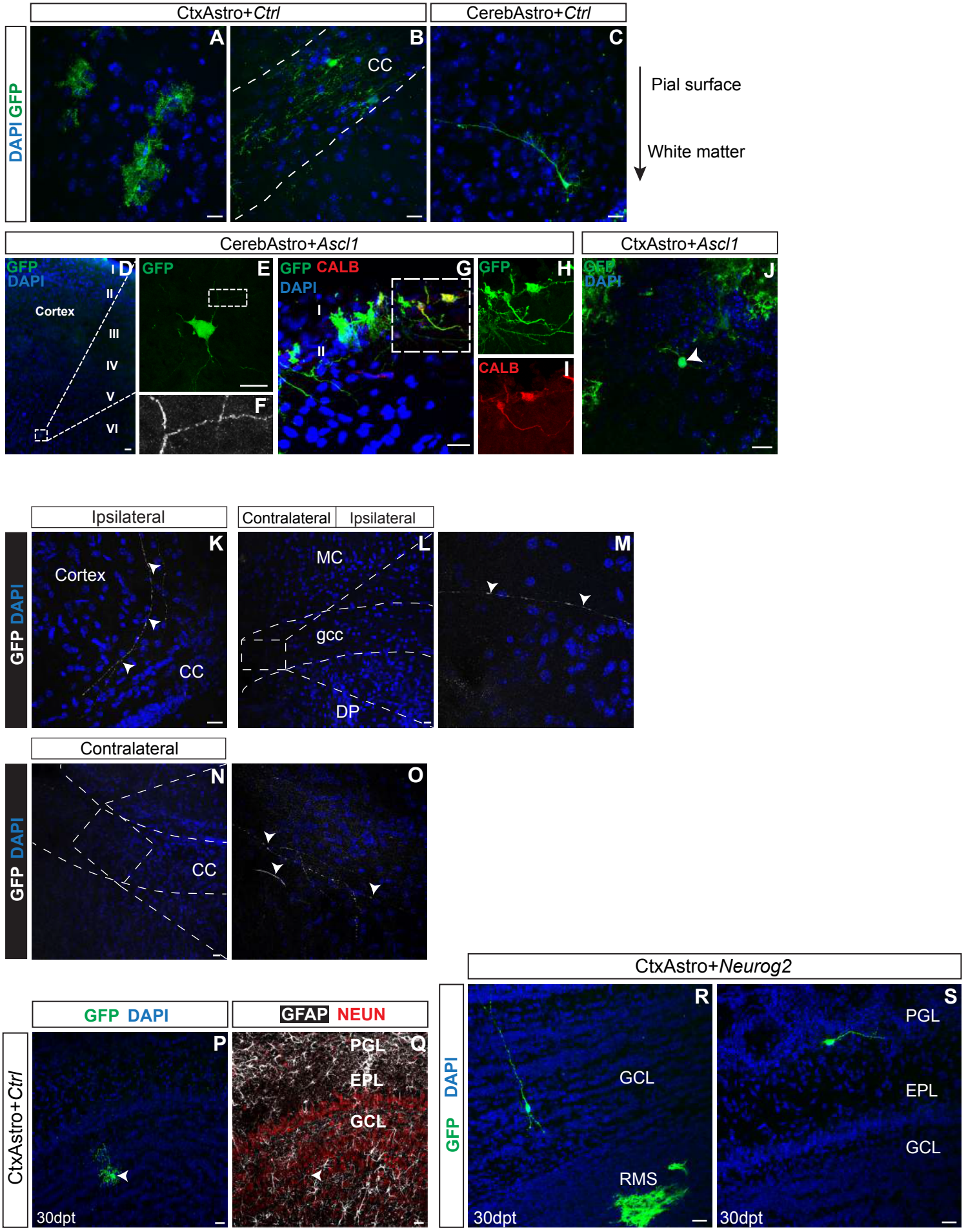

RFP DAPI
CALB DAPI
CTIP2 DAPI
A
B
C
CerebAstro+Ascl1-DsRed
Merge
D
E
150
100
50
0
% of RFP+ iNs
CTIP2+
CTIP2-
**

Figure S7


CtxAstro+Ctrl
CerebAstro+Ctrl
DAPI GFP
A
B
CC
C
Pial surface
White matter
CerebAstro+Ascl1
CtxAstro+Ascl1
GFP DAPI
D
Cortex
II
III
IV
V
VI
GFP
E
F
GFP CALB DAPI
G
I
II
GFP
H
CALB
I
GFP DAPI
J
Ipsilateral
Contralateral
Ipsilateral
GFP DAPI
K
Cortex
CC
L
MC
gcc
DP
M
Contralateral
GFP DAPI
N
CC
O
CtxAstro+Neurog2
GFP DAPI
GFP DAPI
GFAP NEUN
CtxAstro+Ctrl
P
30dpt
PGL
Q
EPL
GCL
R
GCL
RMS
30dpt
S
PGL
EPL
GCL
30dpt