Supplementary Materials:

Materials and Methods

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Supplementary Materials:

Materials and Methods:

Müller Glia Culture

Müller Glia were cultured and purified from post-natal day 12 (P12) C57BL/6 J mouse retinas as described (*1*).

Immunohistochemistry (IHC).

Eyecups or retinal explants were fixed in 4% paraformaldehyde for 25 minutes or 2% paraformaldehyde for 45 minutes, respectively, at room temperature. After a single PBS wash, samples were cryoprotected in 30% sucrose overnight at 4°C. Eyecups or explants were embedded in OCT compound (Sakura Finetek) and sectioned at 12-14 mm. For IHC analysis, sections were blocked with 10% normal horse serum (Vector Labs) containing 0.5% Triton X-100 for 1 hour at room temperature, then primary antibodies were applied and incubated at 4°C overnight. Primary antibodies used are listed in Table 1. Sections were washed three times with PBS, and incubated with secondary antibodies (Life Technologies) for 1.5 hours at room temperature. Sections were then incubated in DAPI (Sigma) for 10 minutes. EdU staining was carried out using the Click-iT EdU Kit (Invitrogen). After two PBS washes and DI water wash, sections were coverslipped, and confocal microscopy was performed using an Olympus FluoView FV1000.

Intravitreal Injections.

Postnatal (P) 12 mice and adult mice were anesthetized with isoflurane and 1.5 or 2 μL respectively of vehicle (PBS) or N-Methyl-D-aspartic acid (NMDA) (100 mM) (Sigma-Aldrich) was injected intravitreally using a Hamilton syringe with a 32 gauge needle (Hamilton). The other eye was uninjected or injected with vehicle.

Light Damage

Adult albino mice were continuously exposed to 10,000 lux light for 8 hours as described previously (*2*). Unanesthetized albino mice were exposed to diffuse, cool, white fluorescent light coming from the top of the cage. Food and water were provided *ad libitum* but were placed in the cage to avoid blocking light exposure. The pupils were not dilated. Average luminance was measured on the cage floor using a light meter, and was approximately 10,000 lux. Mice were exposed to the light for 8 hrs (8am–4pm), and returned under normal lighting (12 hr on/12 hr off cyclic light) for recovery before analysis. Only mice with the genotype Leucine(L)/Methionine(M) or L/L for Retinal Pigment Epithelium-specific 65 kDa (RPE65) were used.

Quantitative RT-PCR (RT-PCR).

Retinas were isolated and RNA isolation was performed using Trizol (Invitrogen). cDNA was made using the iScript cDNA synthesis kit (Bio-Rad), and qPCR was performed using SSO Fast reagent (Bio-Rad). Primers used are listed in Supplementary Table 2.

Nuclei Isolation

Cells were removed by accutase treatment followed by washing with PBS (lacking Ca^{2+} and Mg^{2+}) and centrifugation (600*g*). Cells were resuspended in buffer A (15 mM Tris-HCl, 15 mM NaCl, 60 mM KCl, 1 mM EDTA, 0.5 mM EGTA, 0.5 mM spermidine) with 0.07% IGEPAL and incubated for 10 minutes on ice to extract nuclei, followed by centrifugation (600*g*) and resuspension in buffer A (supplemented with Ca^{2+}) to two million nuclei per mL. DNase I treatment was conducted by incubating nuclei with limiting concentrations of DNase I for 3 minutes at 37°C. The reaction was terminated with stop buffer (50 mM Tris-HCl, 100 mM NaCl, 0.1% SDS, 100 mM EDTA, 1 mM spermidine, 0.5 spermine, pH 8.0) and incubated with proteinase K and RNase A at 55°C for 3 hours.

Sequencing/Mapping/Hotspots

DNA fragments less than 750bp were isolated by sucrose ultracentrifugation followed by end-repair and ligation with Illumina compatible adapters. See ref for details on mapping DNase I hypersensitive sites. Sequence reads of 36bp were mapped to the mouse reference genome (NCBI37) using Bowtie (v 0.12.7) (*3*). Aligned reads were summed within 150bp windows in 20bp step intervals to generate signal tracks using BEDOPS (*4*). The Hotspot algorithm was used to detect local enrichments of sequence tags against a local background model based on a binomial distribution (*5*). See http://www.uwencode.org/proj/hotspot/ for a

detailed description. Enriched regions of DNase I hypersensitivity (hotspots) were scanned internally for maxima (±75bp, defined as peaks). A false discovery rate of 1% was used by generating simulated datasets of random reads at identical sequencing depth.

Generation of *TetO-Ascl1* mice

TetO-Ascl1-IRES-EGFP transgene was constructed by placing the full-length cDNA of mouse *Ascl1* under the control of *tetO* promoter, and the internal ribosome entry site (IRES) and enhanced green fluorescent protein (EGFP) cDNA are further placed downstream of *Ascl1*. Mice carrying the transgene were generated by the pronuclear injection using inbred FVB mice as described previously (*6*). *GLAST-CreER* mice carry the *CreER* transgene knocked into the locus of the glutamate transporter gene *GLAST (7).* Mice carrying *CAG-CAT (CC)-EGFP* Cre-reporter allele was used to track the fate of recombined cells as $EGFP^+$ cells as described previously **(*8*).** P12-P18 mice were injected with 0.8 mg tamoxifen (Sigma) in 100 μL corn oil and adults with 1.5 mg tamoxifen in 100 μL corn oil, intraperitoneally to activate overexpression of Ascl1 and GFP to label recombinant cells.

Figure Legends S1-S7

Figure S1. Expression of progenitor genes in Hes5-GFP FACS sorted progenitors (P0) and Müller glia declines rapidly between P0 and P7 as progenitors become postmitotic and Müller glia are formed. Key proneural transcription factors, like Ascl1, are not expressed in Müller glia and do not get upregulated after retinal damage, either from NMDA or excessive light. Green to red = low to high expression. Note GFAP is increased strongly in Müller glia after either type of injury.

Figure S2. Retinal section of adult Ascl1-GFP mouse after 5 daily tamoxifen injections (1.5mg/100 μL). A substantial fraction of the total Müller glia in the retina express the transgene.

Figure S3. Left panel. Higher magnification of retinal section of adult Ascl1-GFP mouse after 5 daily tamoxifen injections labeled with Ascl1 antibody (red), shows that the Müller glia express detectable levels of Ascl1. Right panel. RT-PCR for Ascl1 and progenitor genes from retinas of adult Ascl1-GFP mouse after 5 daily tamoxifen injections (1.5mg/100 μL). Collected 3 weeks later. Ascl1-GFP cycle difference from control (CC-GFP).

Figure S4. A-D. Intraocular injections of NMDA in either P12 or adult mice leads to the death of the vast majority of ganglion cells (Brn3+, green) seen in representative regions of flatmounted retinas, two days after NMDA treatment. E-F. Sections through the retina of a mouse that received an intraocular injection of NMDA at P12 labeled for HuC/D (red) to reveal amacrine cells. G. Quantitation of amacrine cells in P12 retina showing significant reduction in HuC/D+ cells in the INL after NMDA treatment.

Figure S5. A. Section through retina of an adult Ascl1-GFP mouse that received 6 hours of intense light to induce light damage, labeled for Sox9 (white) and Otx2 (red). Many Ascl1-GFP+ Müller glia express Otx2 after damage and some of these show reduced Sox9 expression. B. Ki67 labeling after NMDA treatment in an adult mouse showing some of the Ascl1-expressing Muller cells (GFP+) remain in the mitotic cell cycle for at least one week after the injury.

Figure S6. A-C. Retinal sections through Ascl1-GFP mouse retina after receiving tamoxifen at P12 and NMDA at P14 after one week survival, to show examples of the range of neuronal cell morphology observed. (A,B). Arrows point to amacrine-like cells, and arrowheads point to cells with bipolar morphology. C. Section through the retina of an Ascl1-GFP mouse (tamoxifen at P12 and NMDA at P14, two week survival) co-labeled for HuC/D, an amacrine cell marker.

Figure S7. A-C. Retinal sections through Ascl1-GFP mouse retina after receiving tamoxifen at P12 and NMDA at P14 after two week survival, to show an example of recoverin labeling (arrow) in Ascl1-GFP+ cells in the ONL (inset shows the boxed region at higher magnification). Otx2, red; Recoverin, white; DAPI, blue.

Figure S8. To further assess whether Muller glial derived neurons were able to make synaptic connections with the host neurons, we used the Zeiss LSA 880 Airyscan (Sheppard et al. 2013), which provides resolution to 140 nm. Labeling the section with Ctpb2, in green and GFP in red, the ribbon synapses appear as small green puncta. If the axon of the bipolar cell is making synaptic contacts with amacrine cells in the plexiform layer, green puncta should overlap with the red axonal process. The arrows point to Ctpb2 puncta that resides within the axon of the regenerated bipolar cell. INL, inner plexiform layer; Scale bar = 1 micron.

Figure S9. A-A’’’. Cryosection through retinal explant 7 DIV, labeled for Ascl1-GFP (green), Otx2 (red), and EdU (white). Arrows show examples of EdU+/Otx2+/Ascl1-mCherry+ cells. Scale bar = 50 um. B. Postnatal

day 12 (P12) retinas from hAscl1-mCherry mice were explanted and cultured in for 7 days (7 DIV) in 1% FBS medium and then processed for IHC. Doxycycline added after one DIV. Flatmount of retina showing large increase in EdU+ cells in Ascl1-mCherry mice. Scale bar = 300 μm. C. Quantification of EdU+ cells from Ascl1-mCherry (n= 5; six fields per retina and control (no Ascl1) retinas (n=3; six fields per retina). Bars = mean and SEM. * p= 0.0055.

Figure S10. A,B. Percent of Ascl1-GFP+ cells that express Sox2/Sox9 and Otx2 after NMDA neurotoxic retinal injury at either P14 or P16/P18. After injury at P14, over 50% of the Ascl1-GFP+ cells express Otx2, and only approximately 30% retain Sox2 or Sox9 expression (we found no difference in the % of cells expressing Sox2 or Sox9 and so the data was combined). A small number of cells are labeled for Sox and Otx2. By contrast, after injury at either P16 or P18, the increase in Otx2+ cells still occurs in the Ascl1-GFP+ cells, but many do not down-regulate Sox9, and as a result there is a large increase in the percentage of cells expressing both markers. C. Percent of Ascl1-GFP+ cells expressing Sox2/9 or Otx2 after NMDA damage as a function of the age of damage. One difference between injury at P14 and P16/18 is that the glial Sox2/9 is not reduced as efficiently at P16/18 or in adult MG as it is at P14. Adult data from Figure 2. D. Examples of Ascl1-GFP+ cells after NMDA injury at P16; the response is more similar to the adult than that observed after P14 injury, in that Ascl1-GFP+ cells do not develop characteristics of retinal neurons, though they show expression of Otx2.

Figure S11. Pie chart showing the degree of overlap between P0-DHS/Ascl1-ChIP-seq peaks that are accessible at P7 and in Müller glia (MG). The majority of the sites for Ascl1 binding that are present at P0 and remain accessible at P7 are still accessible in the P12 Müller glia. B. GREAT analysis of the Gene Ontology enriched categories in the overlapping DHSs between adult retina and Müller glia; neural developmental categories as not enriched in the DHSs common between adult retina and Müller glia.

External Databases:
ENCSR666HFH
GSM1014188
GSM1014198
GSM1014175

Supplemental Table 1. List of antibodies.

Protein	Vendor	Host	Dilution for IHC
Ascl1	BD Biosystems	Mouse	1:100
Brn3	Santa Cruz Biotechnology	Goat	1:100
Cabp5	Gift from Dr. F. Haeseleer	Rabbit	1:200
Ctbp2	BD Biosciences	Mouse IgG1	1:500
GFAP	DAKO	Rabbit	1:500
GFP	Santa Cruz Biotechnology	Chicken	1:500
HuC/D	Invitrogen	Mouse	1:100
Otx2	R&D Systems	Goat/Biotinylated	1:100
Otx2	Abcam	Rabbit	1:250
Pax6	Convance	Rabbit	1:250
PH3	Novus	Rat	1:500
Recoverin	Chemicon	Rabbit	1:1000
Sox2	Santa Cruz Biotechnology	Rabbit	1:1000
Sox9	Millipore	Goat	1:200
VIAAT	Synaptic Systems	Guinea Pig	1:500
PKC	Sigma	Mouse	1:250
AP2a	Developmental Systems Hybridoma Bank	Mouse	1:250

Supplemental Table 2. List of qRT-PCR primers.

Gene	Forward	Reverse
Gapdh	GGCATTGCTCTCAATGACAA	CTTGCTCAGTGTCCTTGCTG
hAscl1	CATCTCCCCCAACTACTCCA	CAGTTGGTGAAGTCGAGAAGC
Glast	ACCAAAAGCAACGGAGAAGAG	GGCATTCCGAAACAGGTAACTC
Glul	GTTCCCACTTGAACAAAGGCA	ACCCAGATATACATGGCTTGGA
Hes5	AGTCCCAAGGAGAAAAACCGA	GCTGTGTTTCAGGTAGCTGAC
Heyl	CAGCCCTTCGCAGATGCAA	CCAATCGTCGCAATTCAGAAAG
Mnfg	ATGCACTGCCGACTTTTTCG	CCTGGGTTCCGTTGGTTCAG
Olig2	ATGCACGACCTCAACATCGCCA	GTGAGCATGAGGATGTAGTTTCGC
Otx2	ACCAAGGATCATGGTCCTGTC	CCTGTGCCTGTACCAGCTC

Supplementary Table 3. Progenitor gene promoters analyzed for DHSs

Chr	start	end	gene
chr1	93309799	93310799	Hes6
chr10	86956405	86957405	Ascl1
chr12	12948642	12949642	Mycn
chr15	78603875	78604875	Mfng
chr16	91224794	91225794	Olig2
chr17	15512787	15513787	Dll1
chr2	119150519	119151519	Dll4
chr3	127335062	127336062	Neurog2
chr4	122909798	122910798	Heyl
chr4	133574731	133575731	Lin28a
chr5	114723770	114724770	Foxn4
chr7	29086804	29087804	Dll3
chr7	152081436	152082436	Fgf15
chr9	91260637	91261637	Zic1
chrX	68808167	68809167	Hmgb3

Supplementary References

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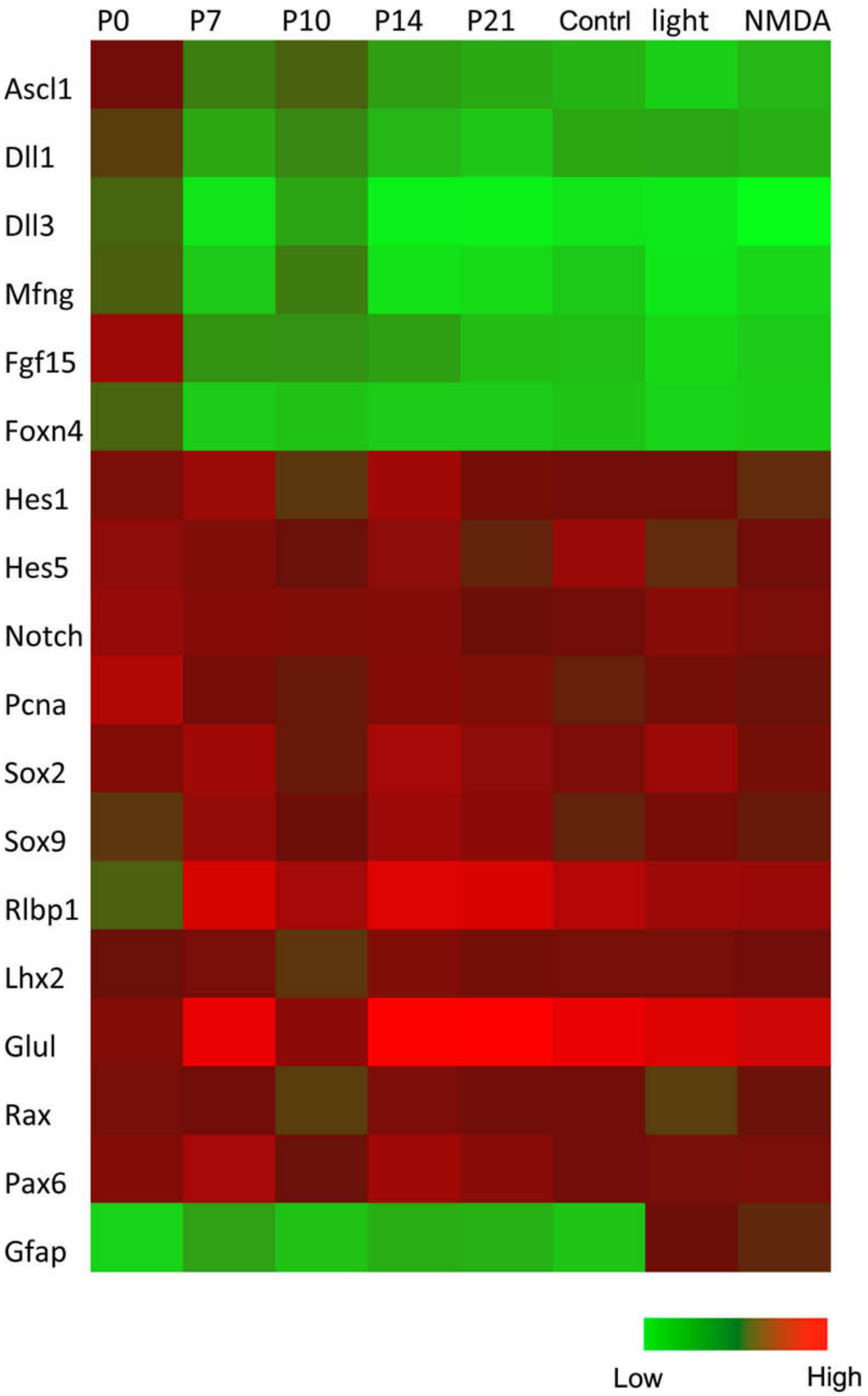
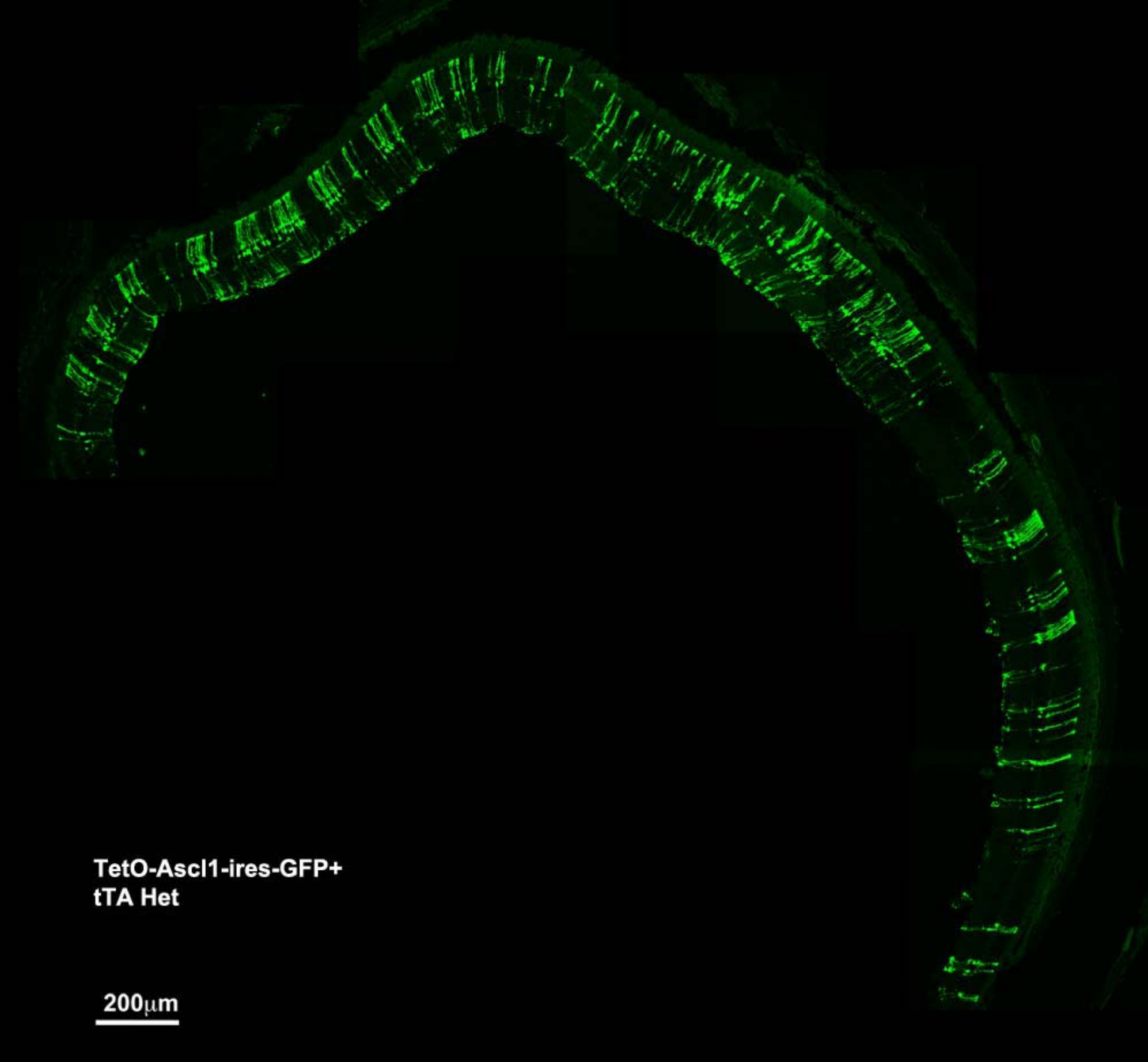
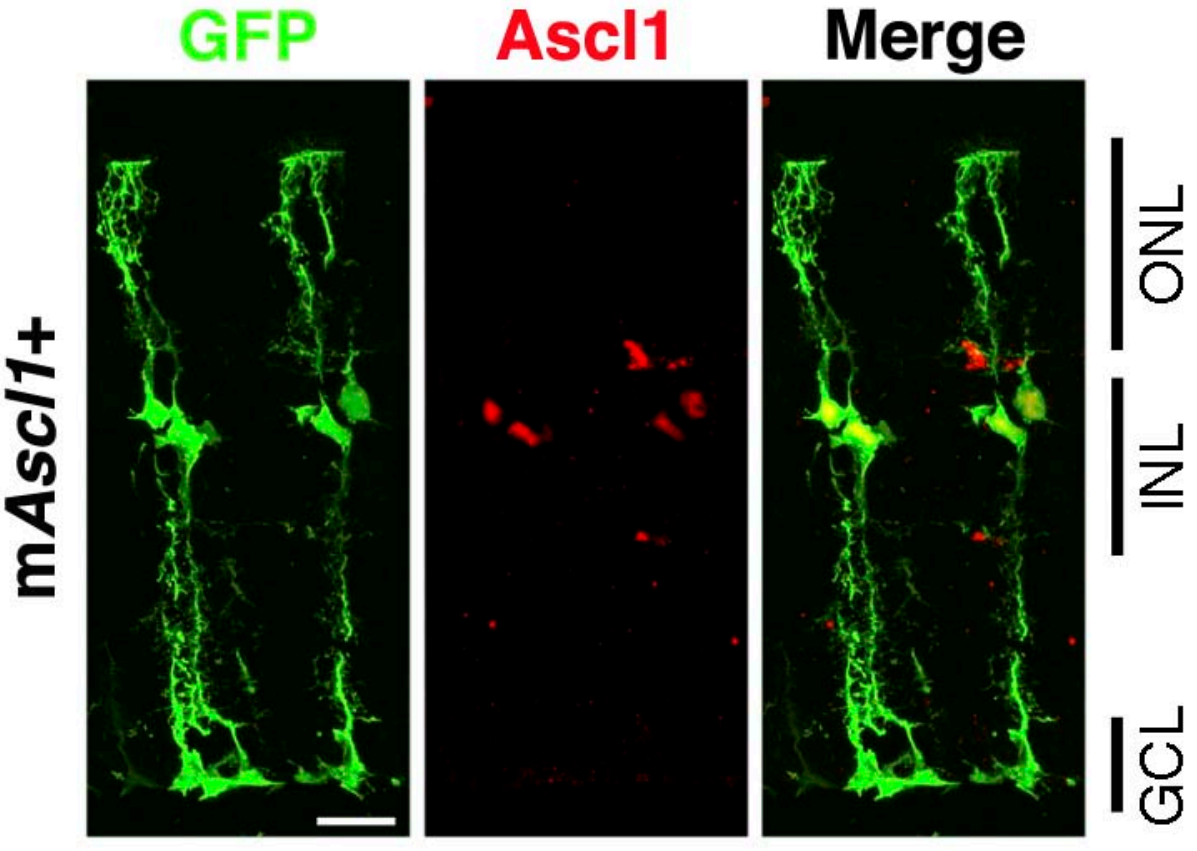

P0
P7
P10
P14
P21
Contrl
light
NMDA
Ascl1
Dll1
Dll3
Mfng
Fgf15
Foxn4
Hes1
Hes5
Notch
Pcna
Sox2
Sox9
Rlbp1
Lhx2
Glul
Rax
Pax6
Gfap
Low
High


TetO-Ascl1-ires-GFP+
tTA Het
200μm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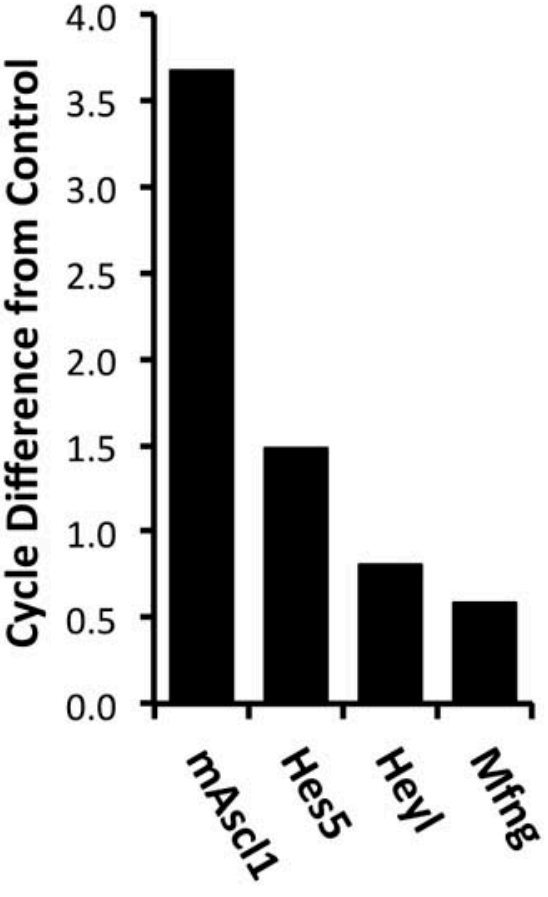
Ascl1
Merge
mAscl1+
ONL
INL
GCL


Cycle Difference from Control
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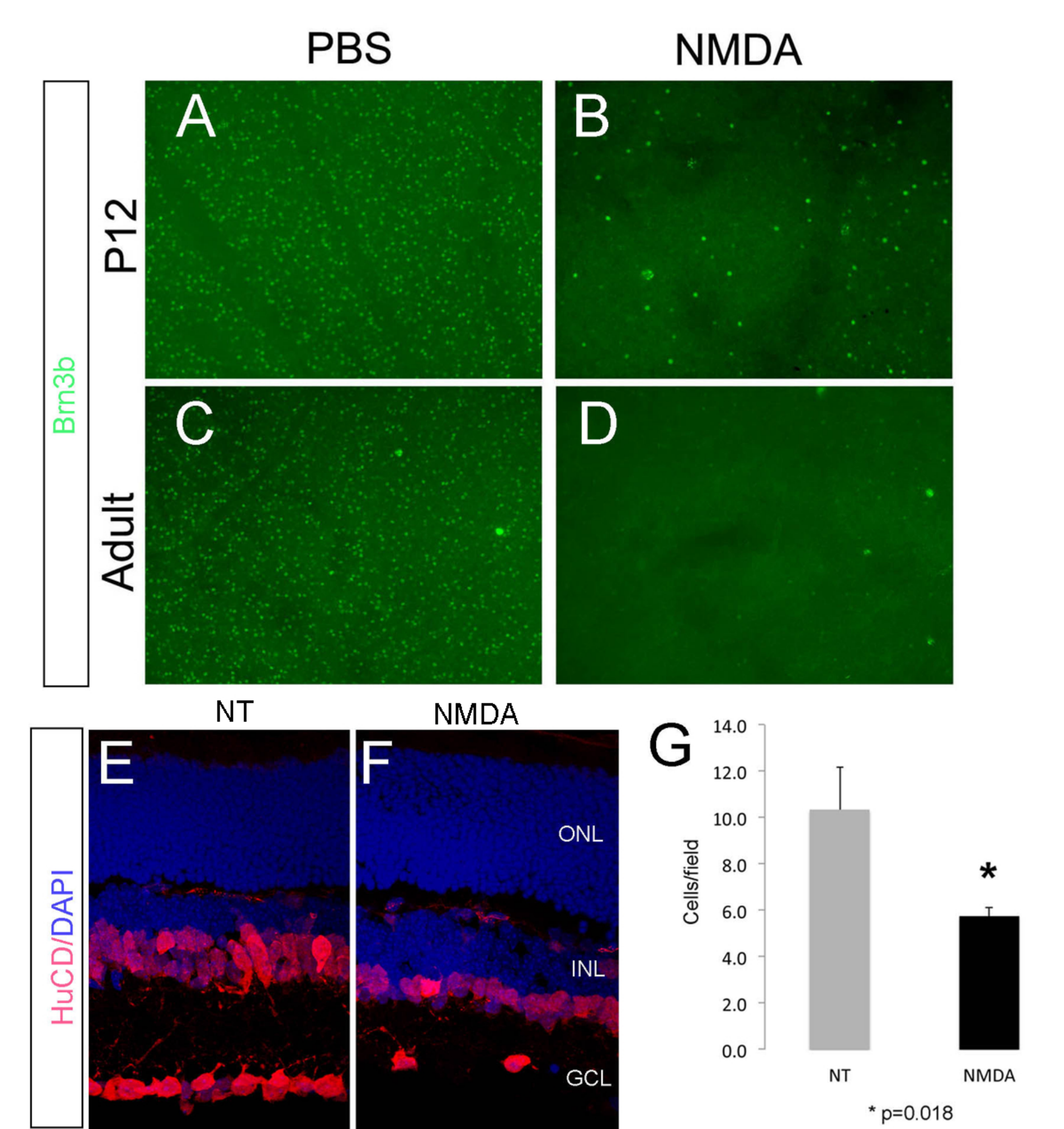
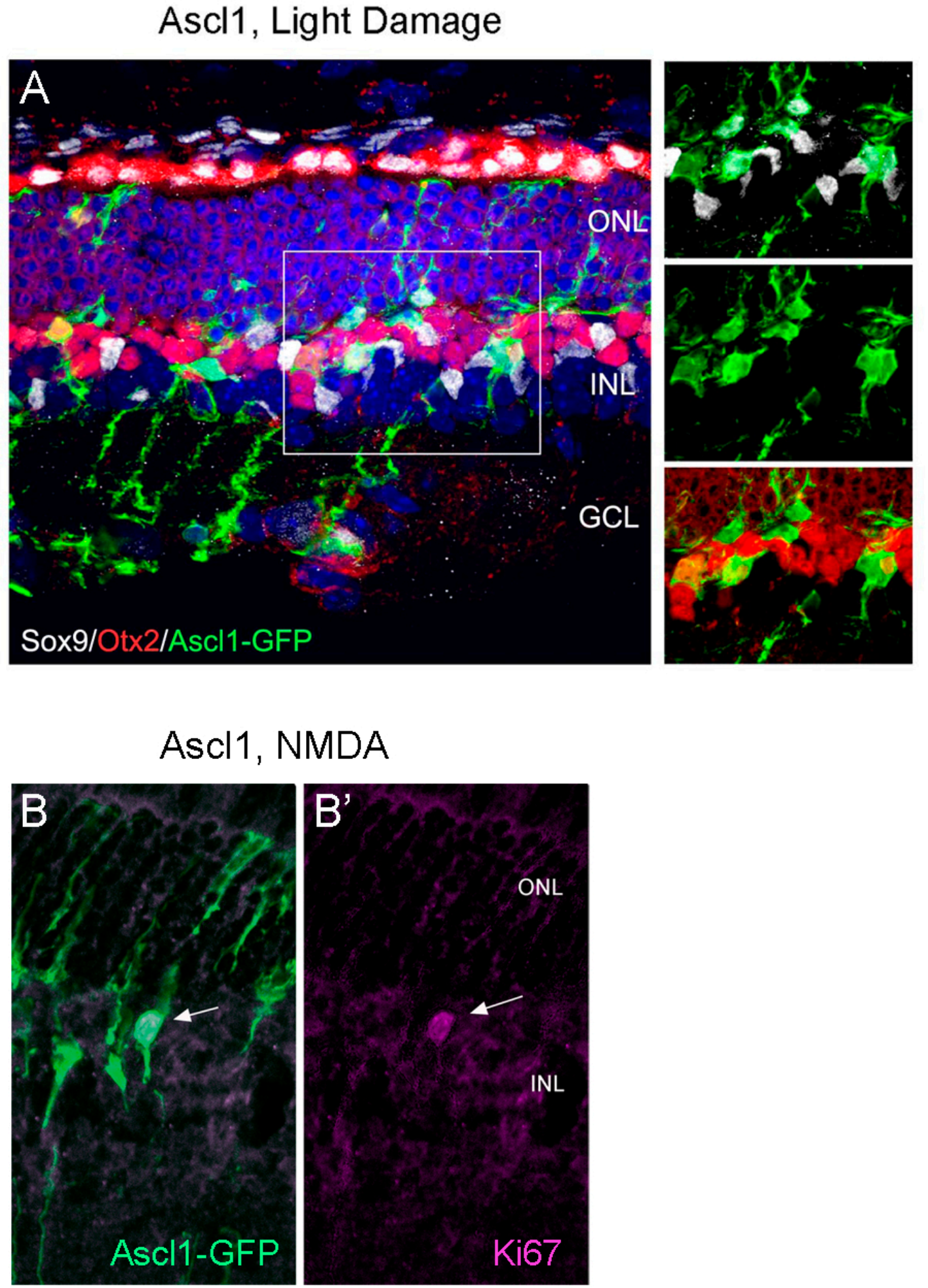
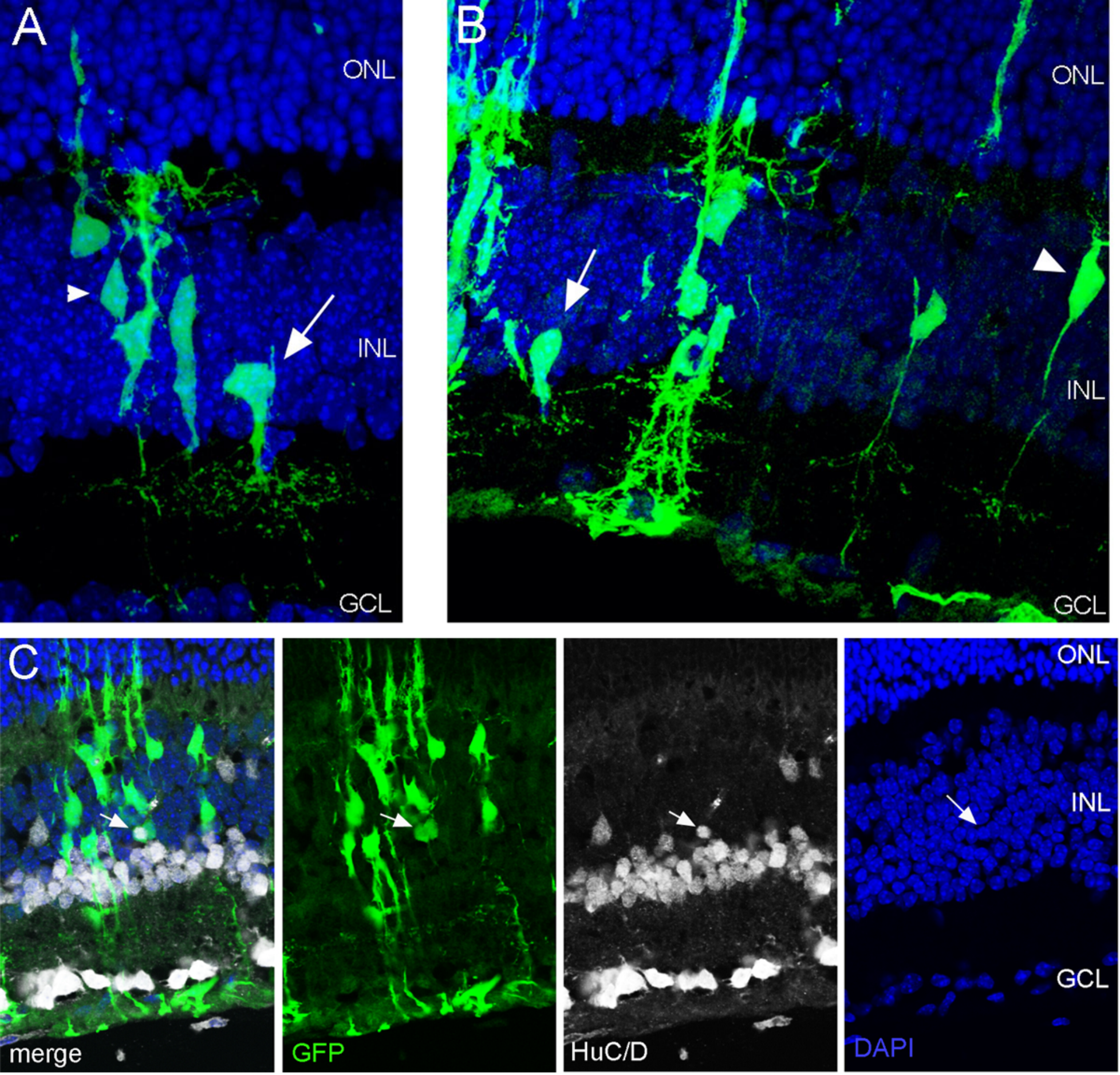
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
mAscl1
Hes5
Heyl
Mfng


PBS
NMDA
Brn3b
P12
Adult
A
B
C
D
NT
NMDA
HuCD/DAPI
E
F
ONL
INL
GCL
G
Cells/field
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
*
NT
NMDA
* p=0.018


Ascl1, Light Damage
A
ONL
INL
GCL
Sox9/Otx2/Ascl1-GFP
Ascl1, NMDA
B
Ascl1-GFP
B'
ONL
INL
Ki67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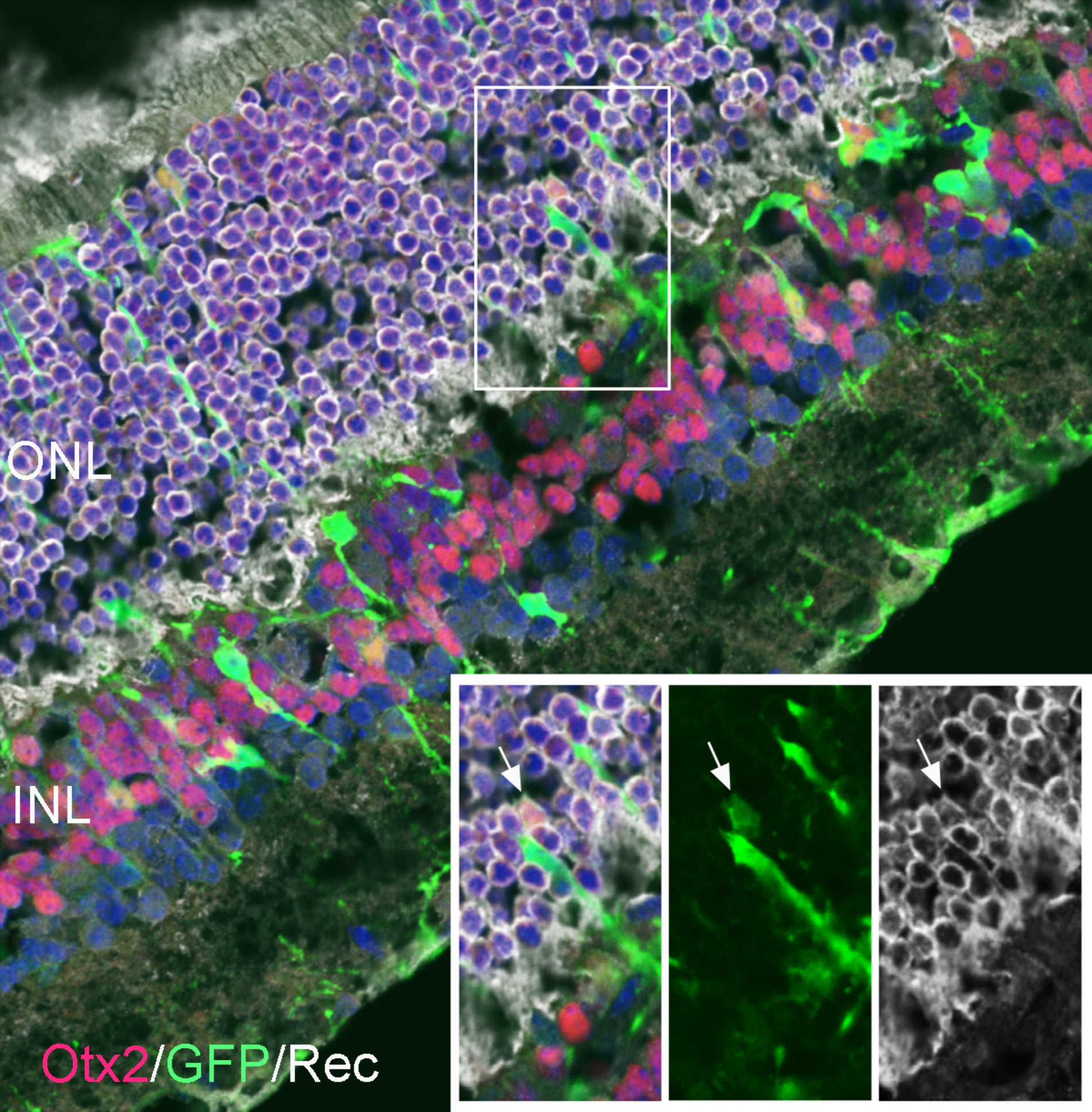
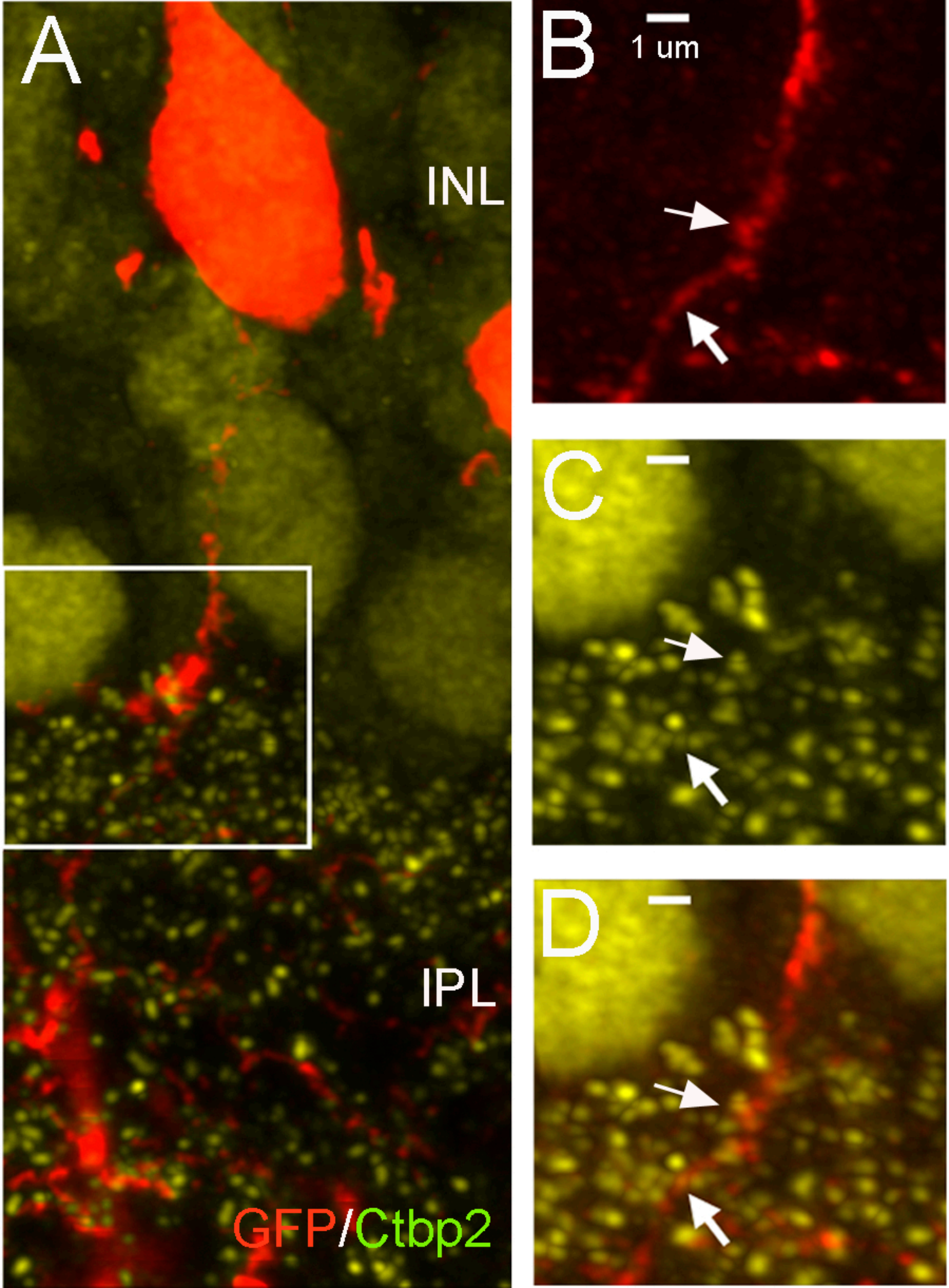
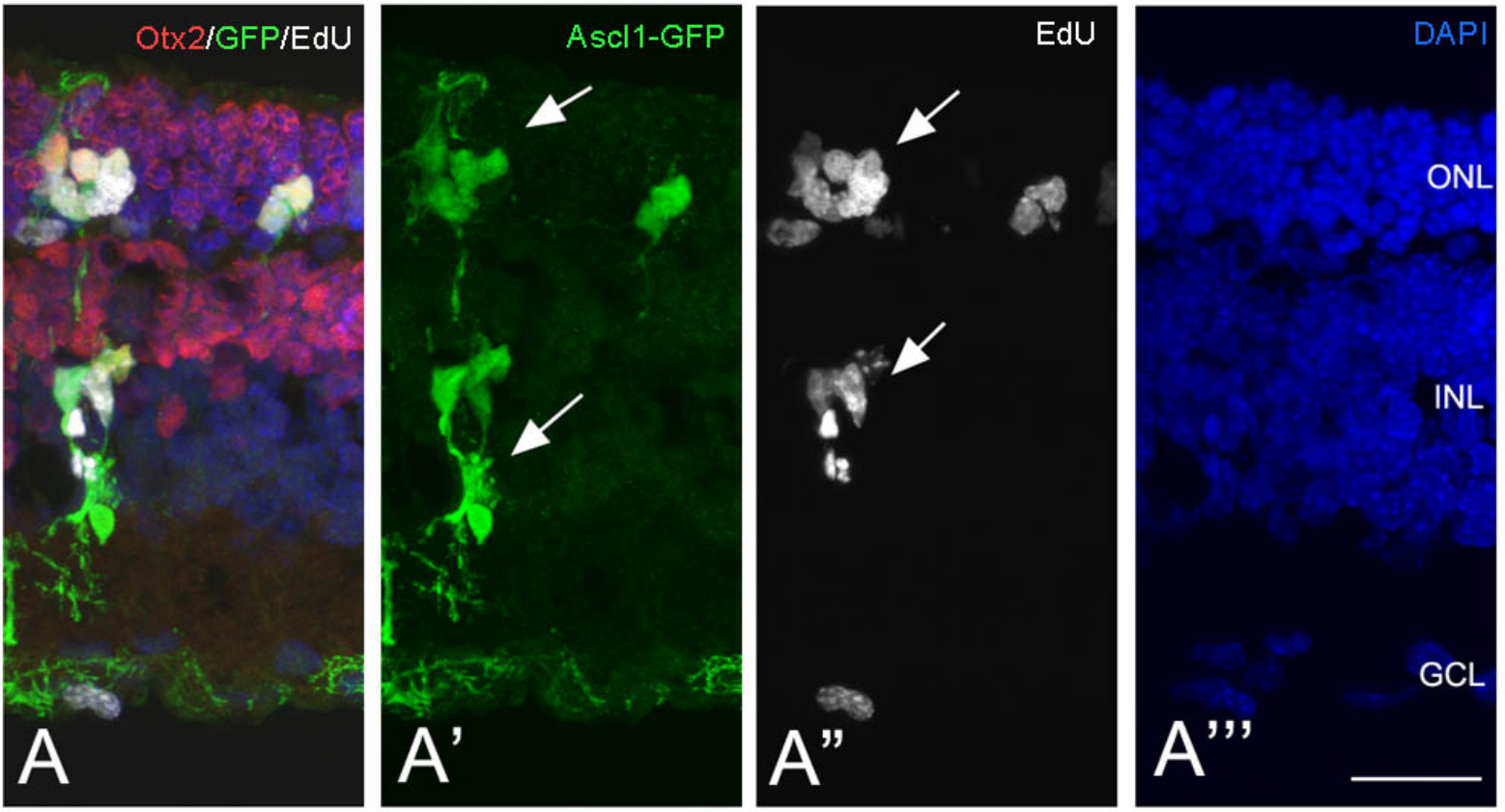
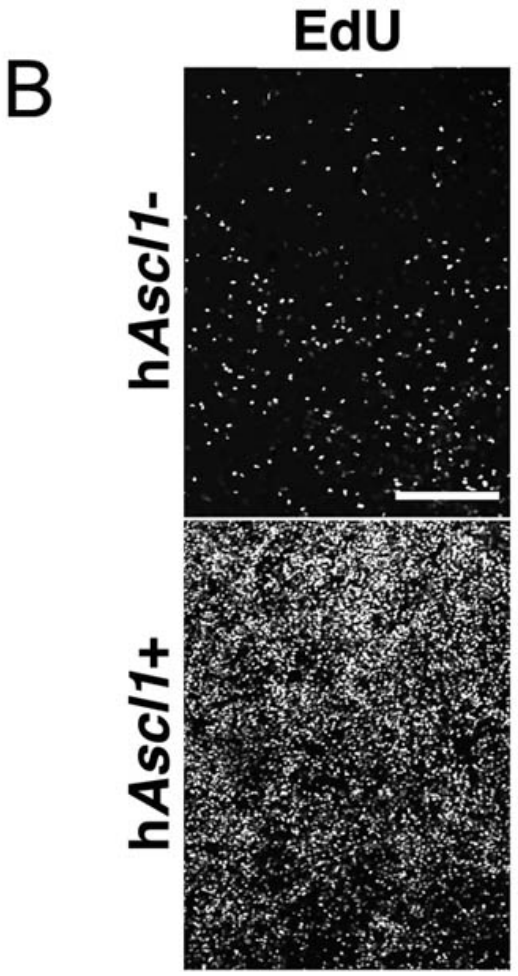
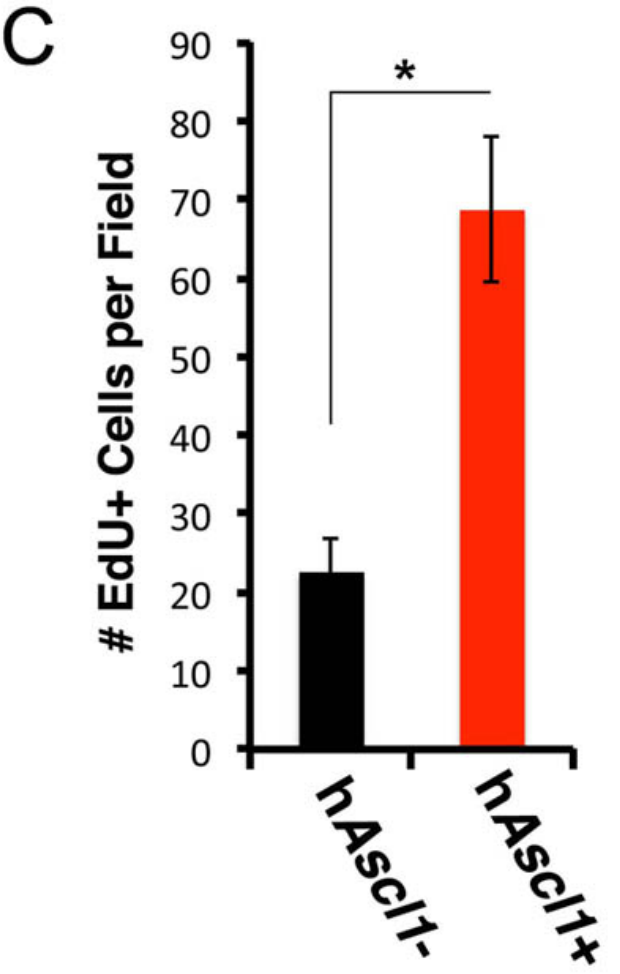
ONL
INL
GCL
B
ONL
INL
GCL
C
merge
GFP
HuC/D
DAPI
ONL
INL
GCL

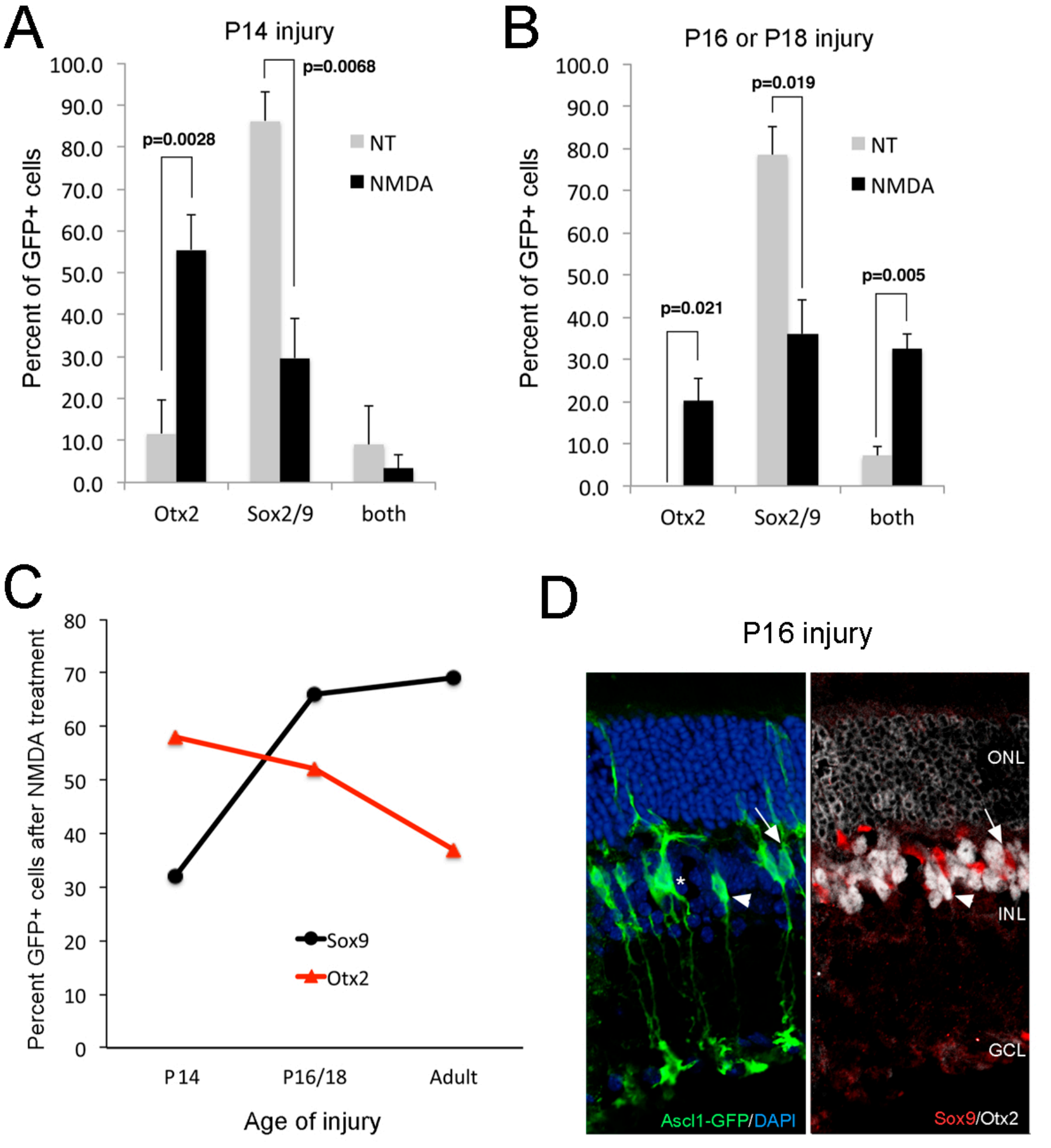

ONL
INL
Otx2/GFP/Rec


A
INL
IPL
GFP/Ctbp2
B
1 um
C
D

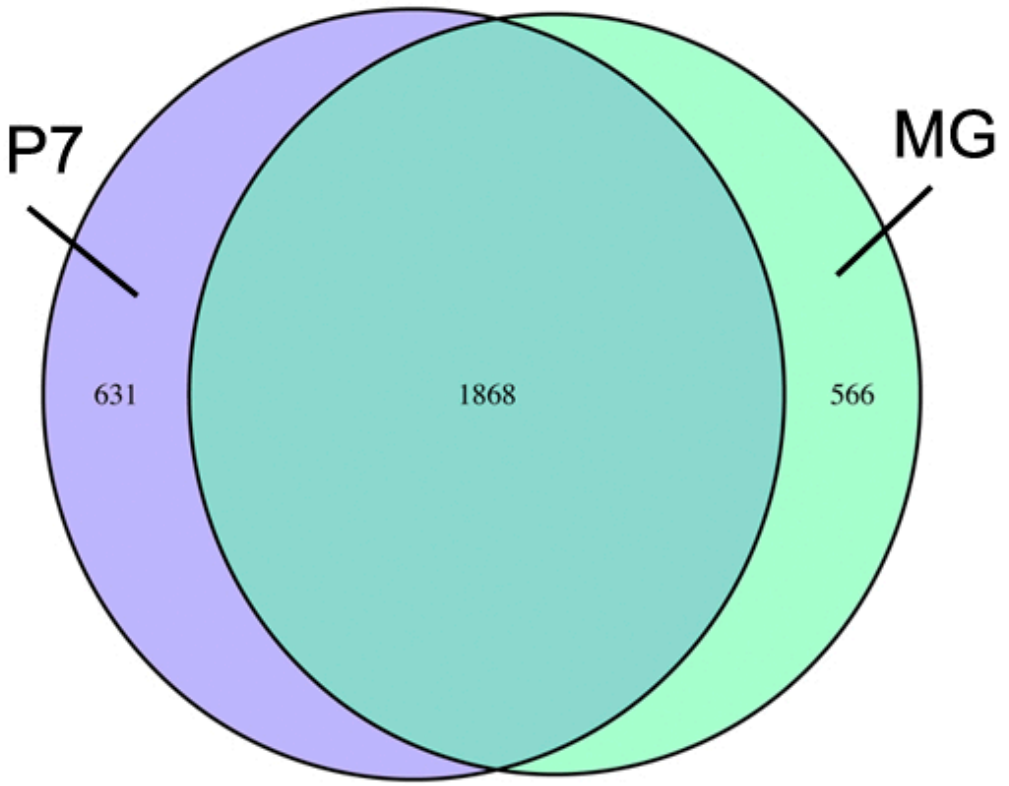

Otx2/GFP/EdU
Ascl1-GFP
EdU
DAPI
ONL
INL
GCL
A
A'
A''
A'''


B
EdU
hAscl1-
hAscl1+

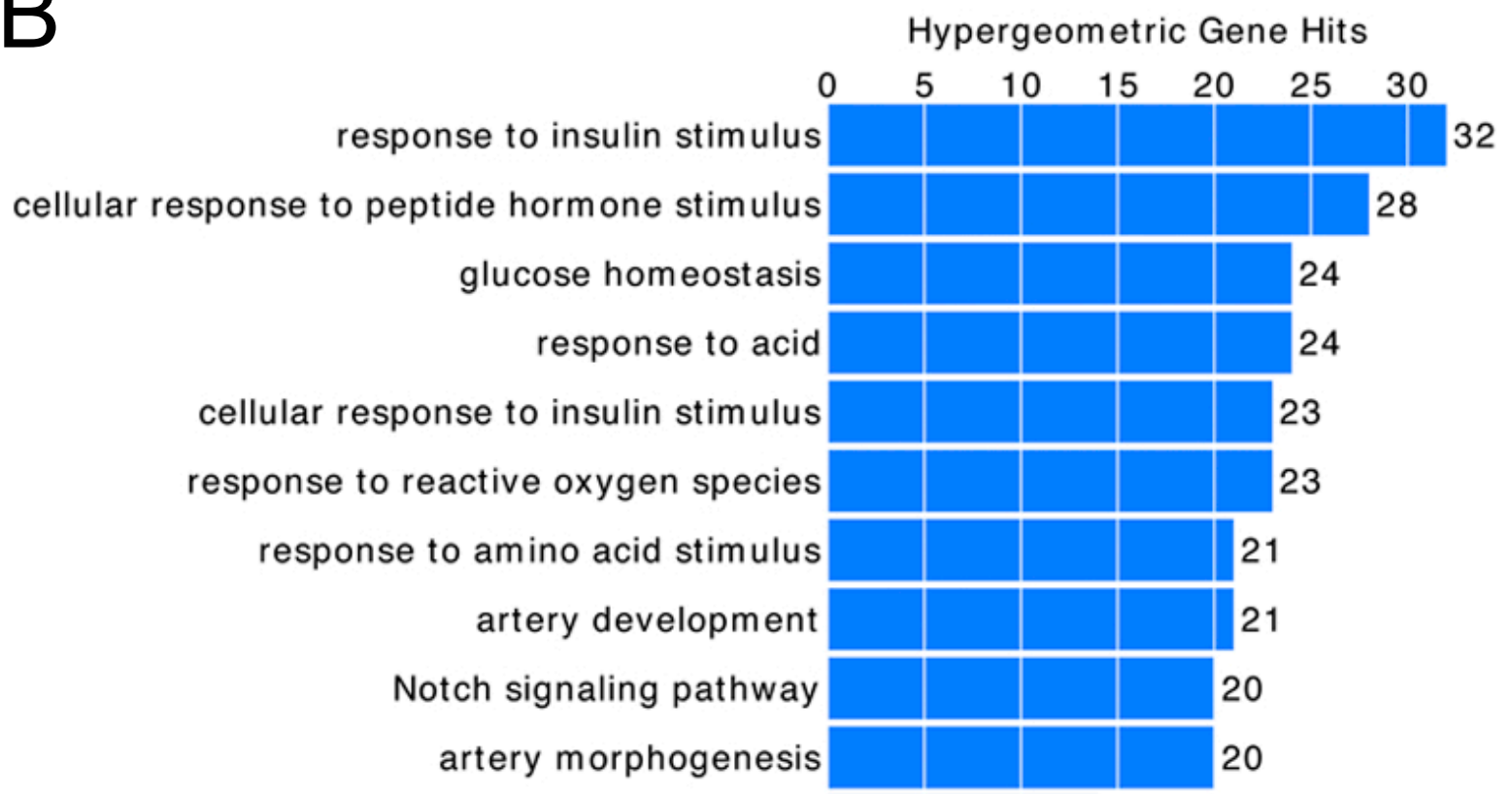

C
EdU+ Cells per Field
90
80
70
60
50
40
30
20
10
0
*
hAscl1-
hAscl1+


A
P14 injury
Percent of GFP+ cells
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
p=0.0028
p=0.0068
NT
NMDA
Otx2
Sox2/9
both
B
P16 or P18 injury
Percent of GFP+ cells
p=0.019
p=0.021
p=0.005
NT
NMDA
Otx2
Sox2/9
both
C
Percent GFP+ cells after NMDA treatment
80
70
60
50
40
30
20
10
0
Sox9
Otx2
P14
P16/18
Adult
Age of injury
D
P16 injury
ONL
INL
GCL
Ascl1-GFP/DAPI
Sox9/Otx2

A


P7
MG
631
1868
566

B


Hypergeometric Gene Hits
0
5
10
15
20
25
30
response to insulin stimulus
32
cellular response to peptide hormone stimulus
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glucose homeostasis
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response to acid
24
cellular response to insulin stimulus
23
response to reactive oxygen species
23
response to amino acid stimulus
21
artery development
21
Notch signaling pathway
20
artery morphogenesis
20