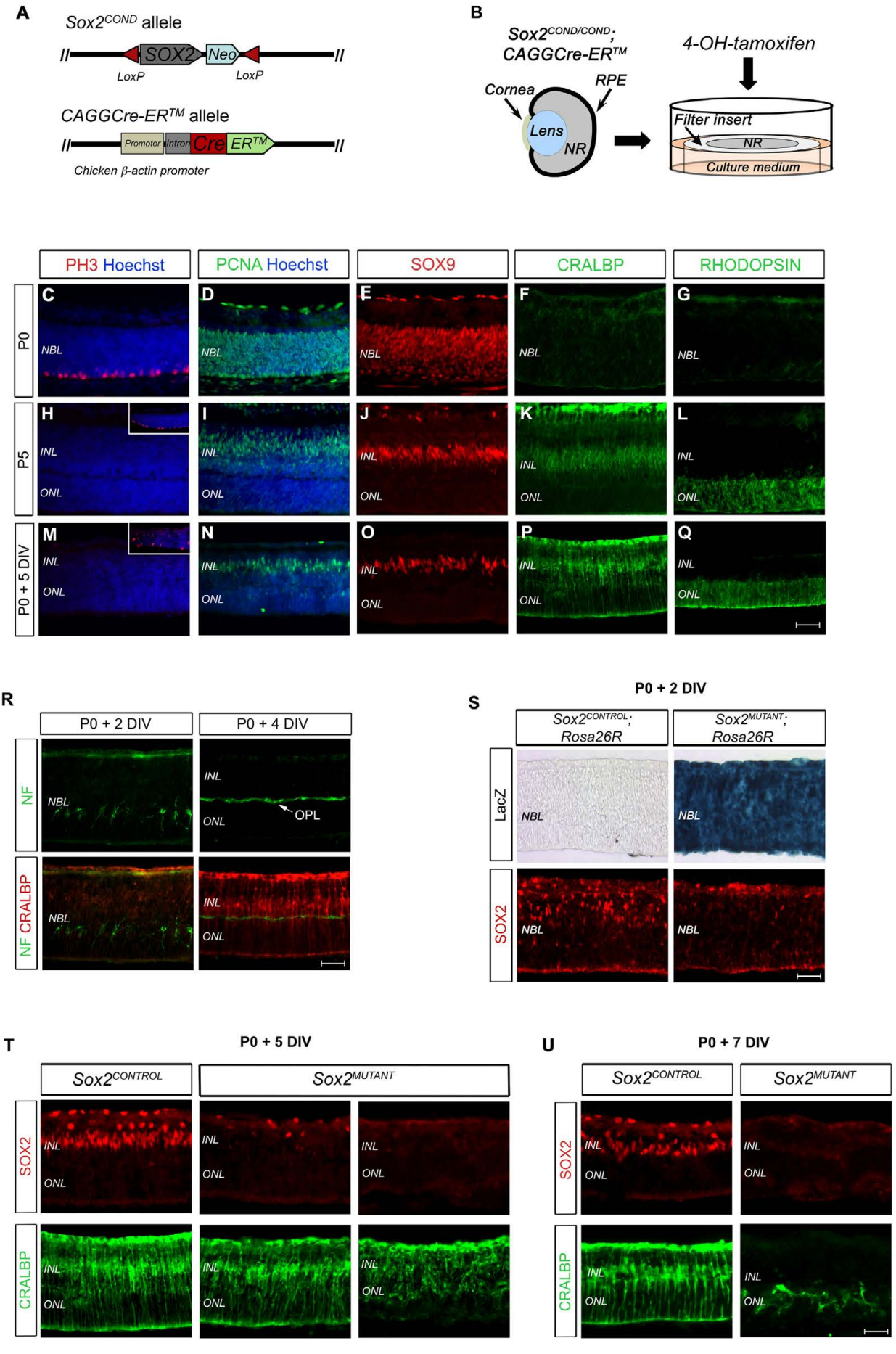

A
Sox2COND allele
SOX2
Neo
LoxP
LoxP
CAGGCre-ERTM allele
Promoter
Intron
Cre
ERTM
Chicken β-actin promoter
B
Sox2COND/COND; CAGGCre-ERTM
Cornea
RPE
Lens
NR
4-OH-tamoxifen
Filter insert
NR
Culture medium
PH3 Hoechst
PCNA Hoechst
SOX9
CRALBP
RHODOPSIN
P0
P5
P0 + 5 DIV
NBL
INL
ONL
R
P0 + 2 DIV
P0 + 4 DIV
NF
NF CRALBP
OPL
S
P0 + 2 DIV
Sox2CONTROL; Rosa26R
Sox2MUTANT; Rosa26R
LacZ
SOX2
T
P0 + 5 DIV
Sox2CONTROL
Sox2MUTANT
SOX2
CRALBP
U
P0 + 7 DIV
Sox2CONTROL
Sox2MUTANT
SOX2
CRALBP

Fig. S1. Temporal and spatial development of neuronal and glial cell types and Cre-mediated ablation of *Sox2* in cultured retinas. (**A**) The *Sox2* conditional allele and *CAGGCre-ER*^{TM} transgene. (**B**) Retinal explant culture technique and ablation of *Sox2* using TM. (**C-Q**) Comparison of the proliferative status and differentiation of postnatal neural progenitor cells into temporally appropriate retinal cell types between P0 (C-G) and P5 (H-L) retinas *in vivo* versus *Sox2*^{CONTROL} retinas isolated at P0, treated with TM and cultured for 5 days *in vitro* (M-Q). (C-G) At P0, neural progenitor cells, marked by PCNA (D) and SOX9 (E), are present throughout the NBL and exhibit PH3 during cell division (C). (F,G) MG cells and rod photoreceptors are not yet formed by P0, as evidenced by low levels of CRALBP (F) and rhodopsin (G) expression, respectively. (H-L) At P5, mitotic progenitor cells are significantly reduced in number in the central retina (H), but still persist in the peripheral retina (inset in H), while PCNA (I) and SOX9 (J) become restricted to the INL. By P5, MG cells and rod photoreceptors have differentiated (K,L). (M-Q) Correct morphology and laminar localization of MG cells and rod photoreceptors in cultured retinas. (**R**) Formation of the OPL, marked by neurofilament, during the culture period and correct localization of MG marked by CRALBP. (**S**) Expression of β-galactosidase (*lacZ*) in *Sox2*^{MUTANT}*;R26R* retinas treated with TM and cultured for 2 days. SOX2 expression is downregulated in *Sox2*^{MUTANT} retinas at day 2 of culture. (**T**,**U**) In control retinas, SOX2 is expressed in the INL and amacrine cells after 5 (T) or 7 (U) days in culture. Expression of SOX2 in *Sox2*^{MUTANT} retinas cultured for 5 days (T) is either absent or detected in a small number of amacrine cells, indicating temporal variation in the efficiency of TM-mediated DNA recombination. Following a 7-day culture period, SOX2 expression is not detected in *Sox2*^{MUTANT} retinas (U). In control retinas, the CRALBP expression pattern reflects typical morphology of MG at both 5 (T) and 7 (U) days in culture. Following a 5-day culture period, MG in *Sox2* mutant retinas are moderately or significantly disorganized (T), and after 7 days in culture a subset of retinas display significant loss of MG cells and retinal degeneration (U). DIV, days *in vitro*; INL, inner nuclear layer; NBL, neuroblast layer; NR, neural retina; ONL, outer nuclear layer; OPL, outer plexiform layer; RPE, retinal pigmented epithelium; TM, 4-hydroxytamoxifen; NF, neurofilament. Scale bars: 65 μm in Q,S; 45 μm in U.

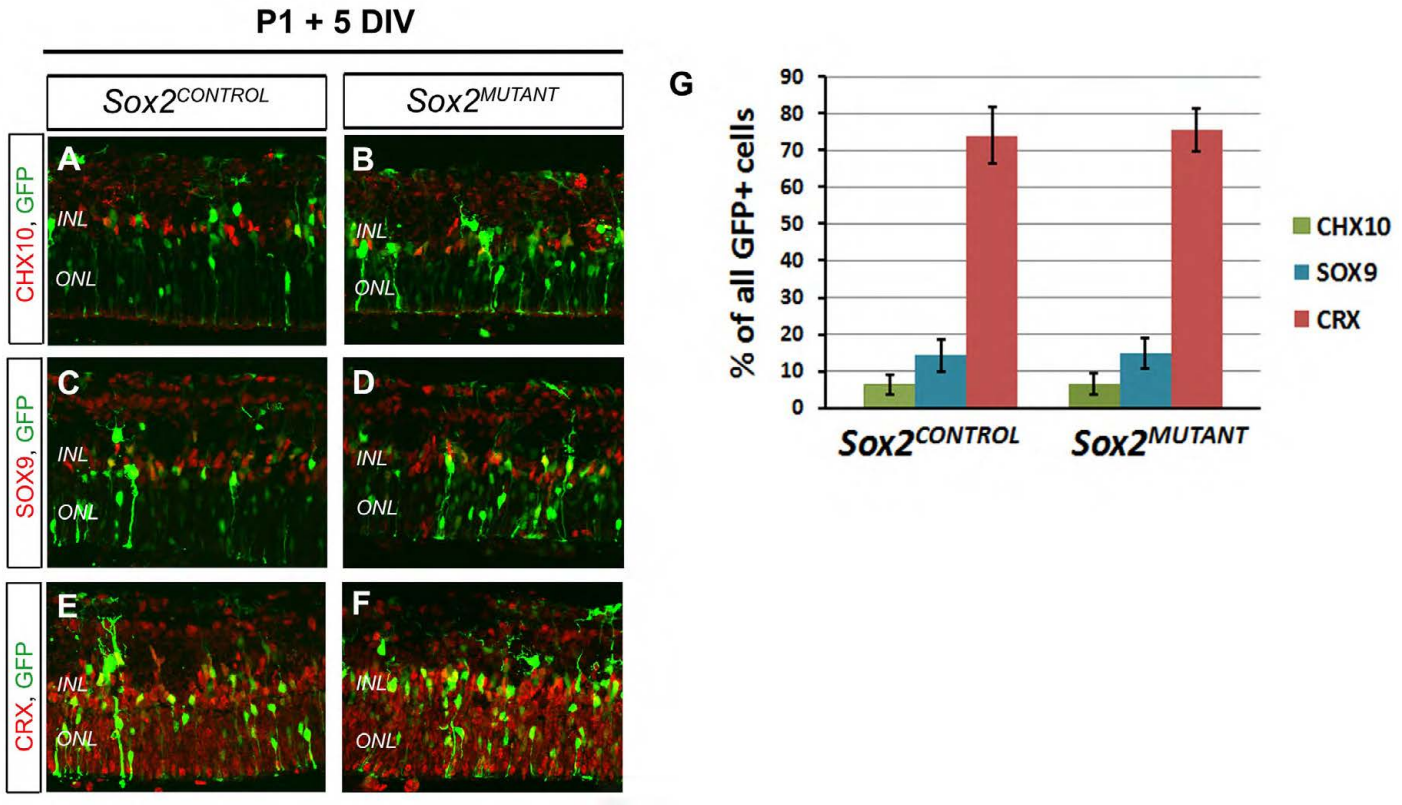


Fig. S2. Fate-mapping analysis of RPCs in *Sox2*^{CONTROL} and *Sox2*^{MUTANT} retinas. (**A-G**) P1 *Sox2*^{CONTROL} and *Sox2*^{MUTANT} retinas electroporated with pCALNL-EGFP, cultured for 5 days and immunostained for markers of late-born cell types. No significant differences (G) are detected in the numbers of labeled bipolar cells (CHX10, A,B), MG (SOX9, C,D), or photoreceptors (CRX, E,F).

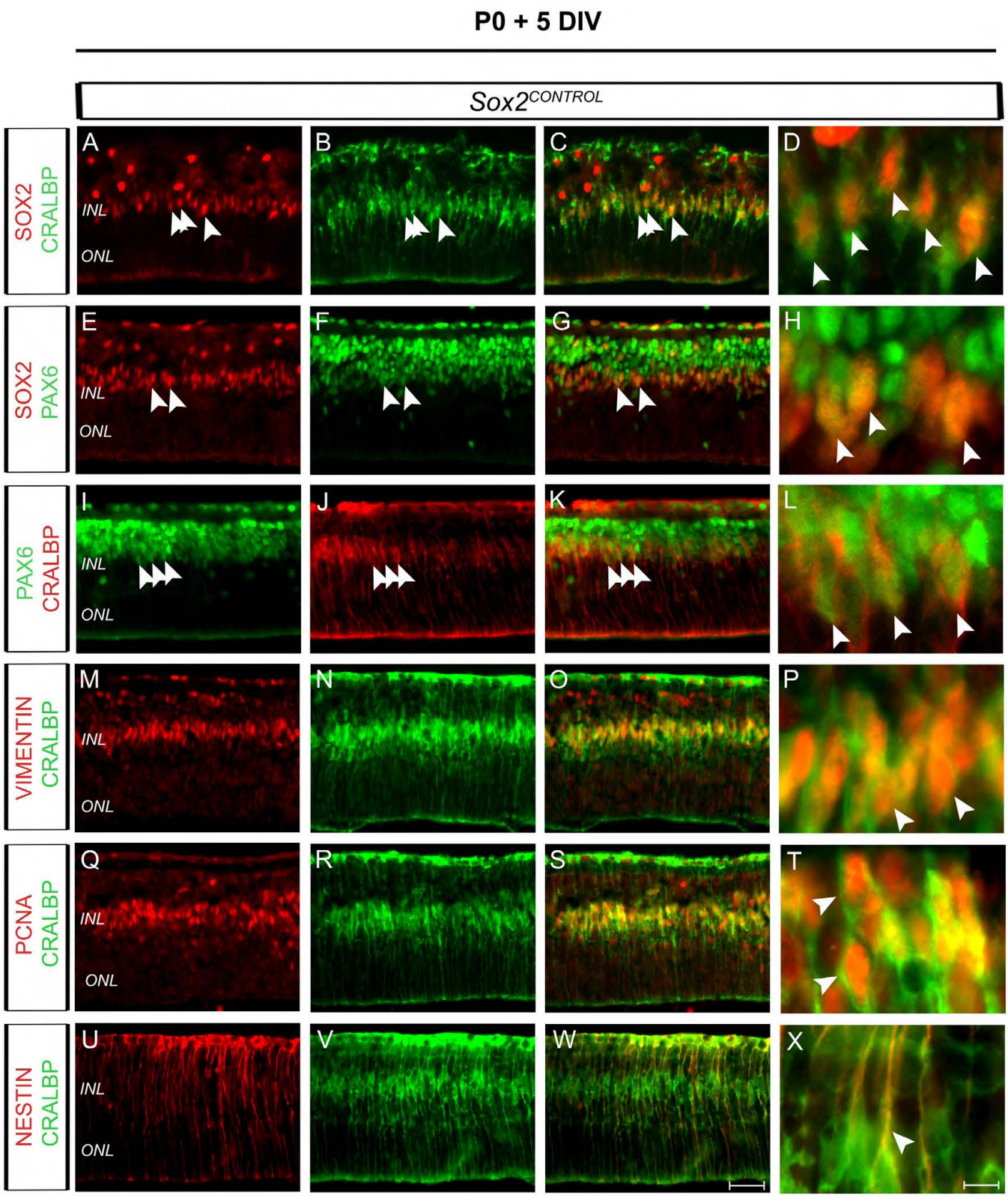


Fig. S3. Expression of markers of differentiating MG in control retinas cultured for 5 days. Expression of MG cell markers was analyzed by immunohistochemistry on sections of TM-treated *Sox2*^{CONTROL} retinas dissected at P0 and cultured for 5 days. (**A-D**) Expression of SOX2 in the INL (A, arrowheads) coincides with CRALBP (arrowheads in B-D). (**E-H**) SOX2-expressing cells within INL (E, arrowheads) express PAX6 at lower levels (F,G, arrowheads). SOX2/PAX6 double-positive cells exhibit the elongated cell body morphology of MG (arrowheads in H). (**I-L**) The subset of INL cells that express PAX6 (I, arrowheads) co-express the MG cell marker CRALBP (arrowheads in J-L). (**M-P**) Vimentin (Histofine) (M) colocalizes with CRALBP (N) in cell bodies of MG (O,P). (**Q-T**) Expression of PCNA (Q) is detected in the majority of CRALBP-positive cells (R) within the INL (S,T, arrowheads). (**U-X**) Nestin (U) is enriched at the basal retinal boundary and localizes to MG cell processes (W,X, arrowheads), marked by CRALBP (V-X). D,H,L,P,T,X are magnifications of C,G,K,O,S,W. Scale bars: 45 µm in W; 15 µm in X.

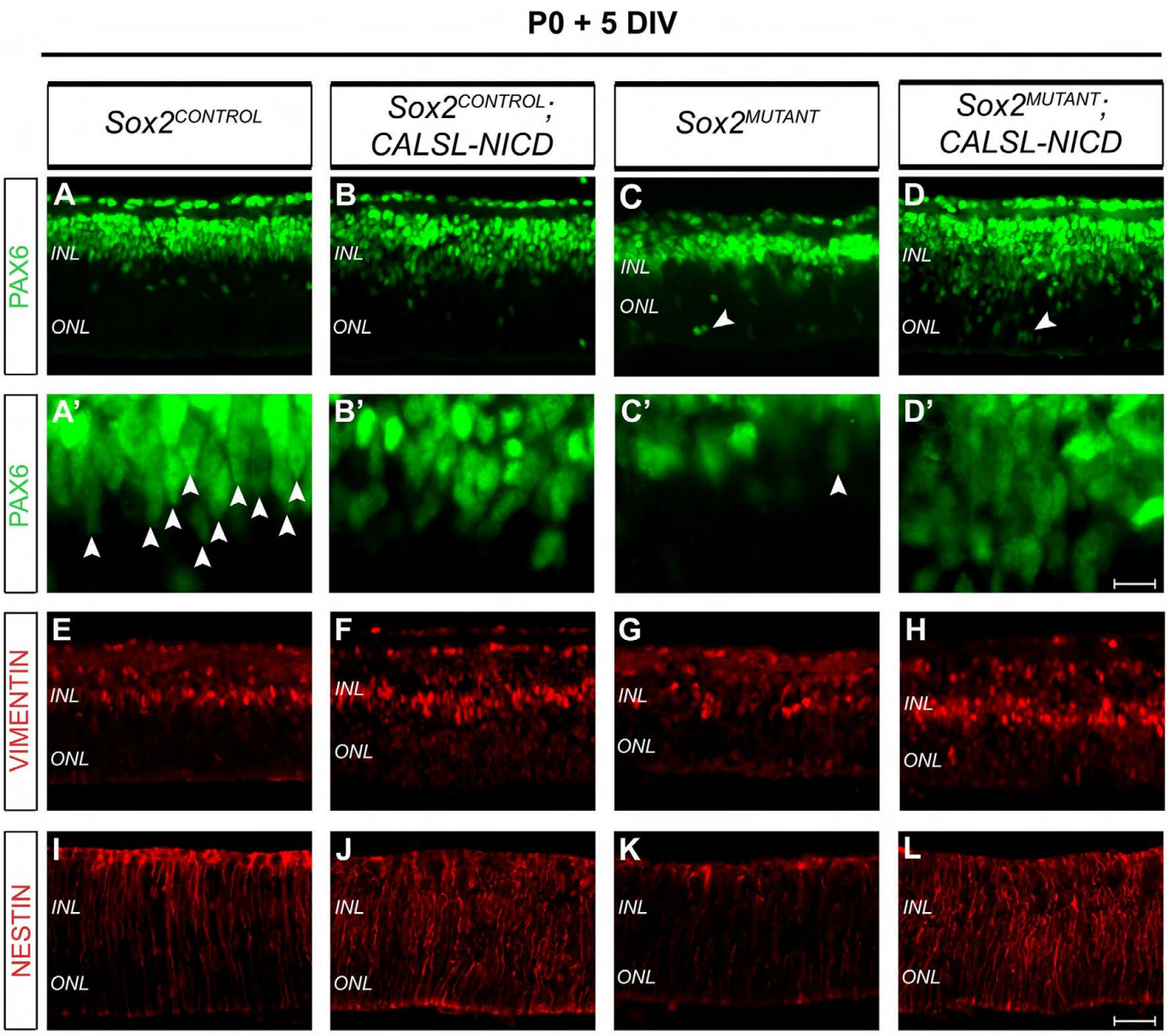


Fig. S4. Expression of MG cell markers is downregulated in *Sox2* mutant retinas but can be restored by activation of NOTCH1 signaling. Expression of PAX6 (A-D′), vimentin (E-H) and nestin (I-L), marking cell bodies (A-H) and radial processes (I-L) of MG cells in the INL, respectively, analyzed in sections of $Sox2^{CONTROL}$ (A,A′,E,I), $Sox2^{CONTROL}$;*CALSL-NICD* (B,B′,F,J), $Sox2^{MUTANT}$ (C,C′,G,K), and $Sox2^{MUTANT}$;*CALSL-NICD* (D,D′,H,L) P0 retinas treated with TM and cultured for 5 days. (**A-D′**) Fewer PAX6-positive cells with elongated cell body morphology are detected in the INL of $Sox2^{MUTANT}$ retinas (C,C′, arrowhead) compared with $Sox2^{CONTROL}$ retinas (A,A′, arrowheads). Genetic activation of NICD causes a moderate expansion of the PAX6 expression domain in $Sox2^{CONTROL}$;*CALSL-NICD* retinas (B,B′). In $Sox2^{MUTANT}$;*CALSL-NICD* retinas, PAX6-positive cells are increased in number (D,D′) and displaced towards the ONL (arrowhead in D). A′-D′ are magnifications of A-D. (**E-H**) Expression of vimentin is reduced in $Sox2^{MUTANT}$ retinas (G), compared with $Sox2^{CONTROL}$ retinas (E), and is increased in $Sox2^{CONTROL}$;*CALSL-NICD* (F) and $Sox2^{MUTANT}$;*CALSL-NICD* (H) retinas. (**I-L**) Expression of nestin, localized to MG cell processes in control $Sox2^{CONTROL}$ retinas (I), is reduced in $Sox2^{MUTANT}$ retinas (K), and is increased in $Sox2^{CONTROL}$;*CALSL-NICD* (J) and $Sox2^{MUTANT}$;*CALSL-NICD* (L) retinas. Scale bars: 15 µm in D′; 45 µm in L.

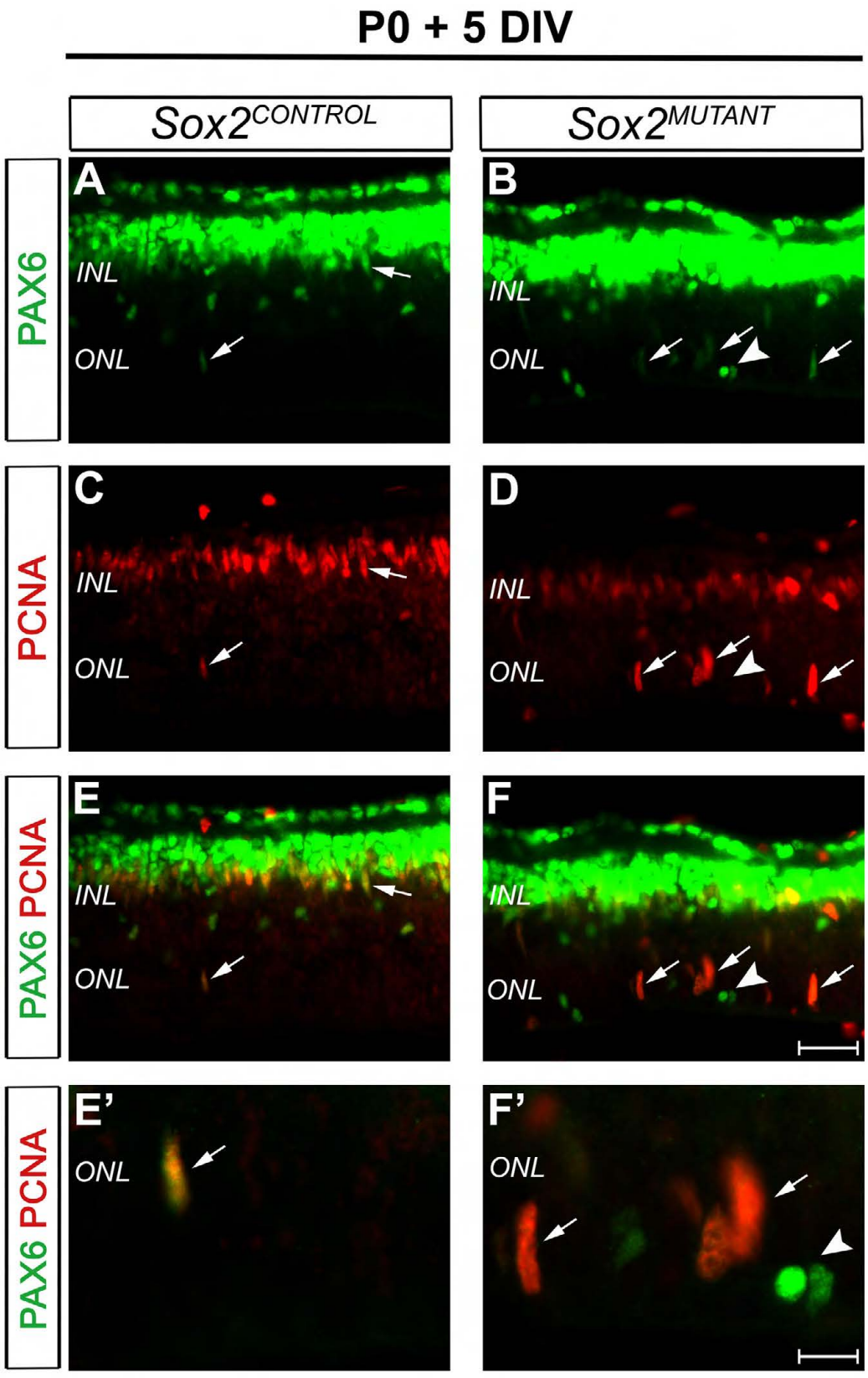


Fig. S5. Co-expression of PAX6 and PCNA identifies dividing MG cells in *Sox2*^{MUTANT} retinas. (**A-F′**) Expression of PAX6 (A,B) and PCNA (C,D) was assessed in sections of TM-treated control (A,C,E,E′) and *Sox2*^{MUTANT} (B,D,F,F′) retinas at day 5 of culture. In *Sox2*^{CONTROL} retinas, co-expression of PAX6 and PCNA marks predominantly cell bodies of MG in the INL (arrows in A,C,E) and occasional cells in the ONL (arrow in E′). Many PAX6/PCNA double-positive elongated cells are found in the ONL of *Sox2*^{MUTANT} retinas (arrows in B,D,F,F′). Cells with round cell body morphology and high PAX6 expression, located in the ONL (arrowheads in B,D,F,F′), do not express PCNA and are found only in *Sox2*^{MUTANT} retinas. E′ and F′ are magnifications of E and F. Scale bars: 45 µm in F; 20 µm in F′.

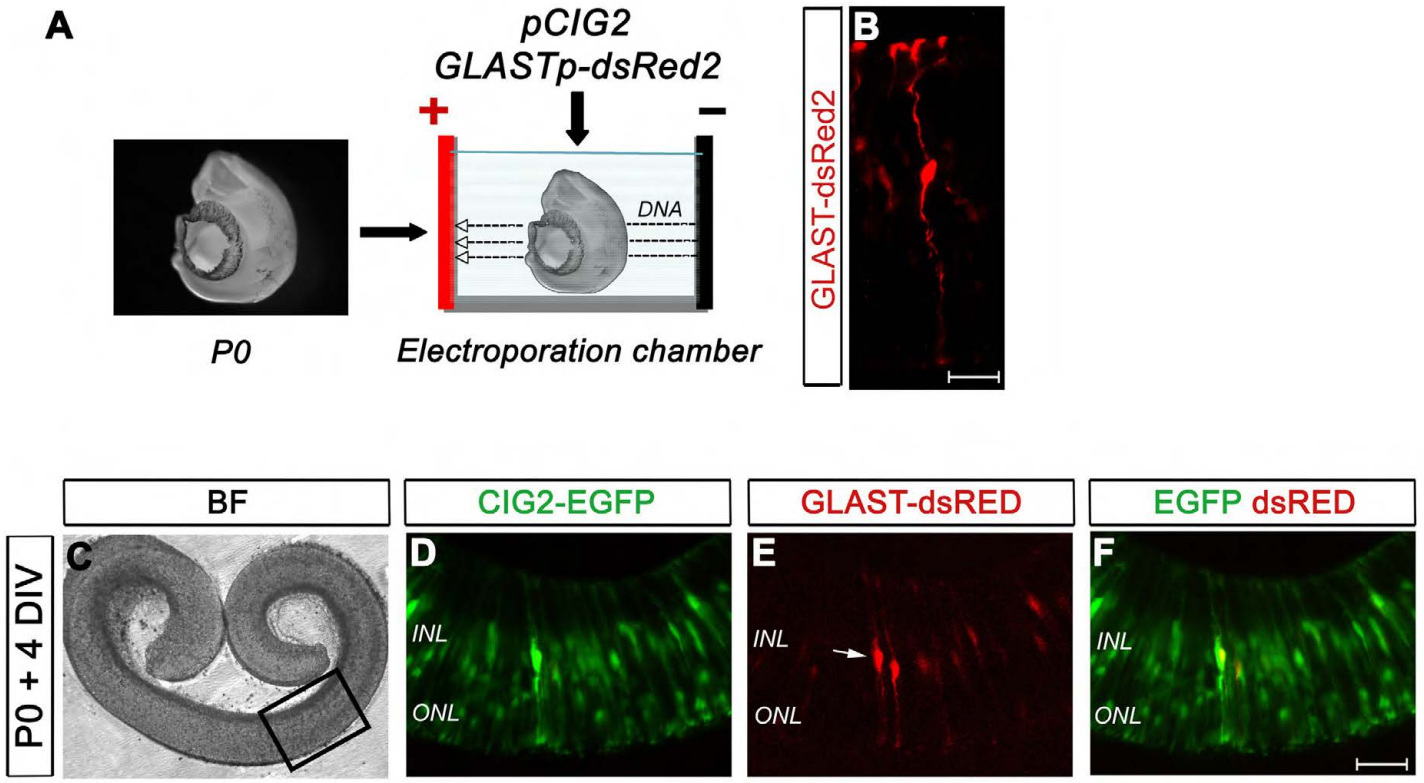


Fig. S6. Visualization of MG cells in cultured retinal slices. (**A**) The *in vitro* electroporation of postnatal retina. (**B**) MG cell expresses GLASTp-dsRED2 3 days following electroporation at P0. (**C**) Slices of electroporated retinas are cultured *in vitro* for 4 days. (D-F, higher magnification images of boxed area in C). Retinas were co-electroporated with pCIG2, encoding EGFP driven by the CAG promoter, and GLASTp-dsRED2, marking MG cells. Confocal image stacks (D-F) were collected from 200 µm retinal slices cultured for 4 days (average intensity *z*-projections are shown). Scale bars: 30 µm in F.

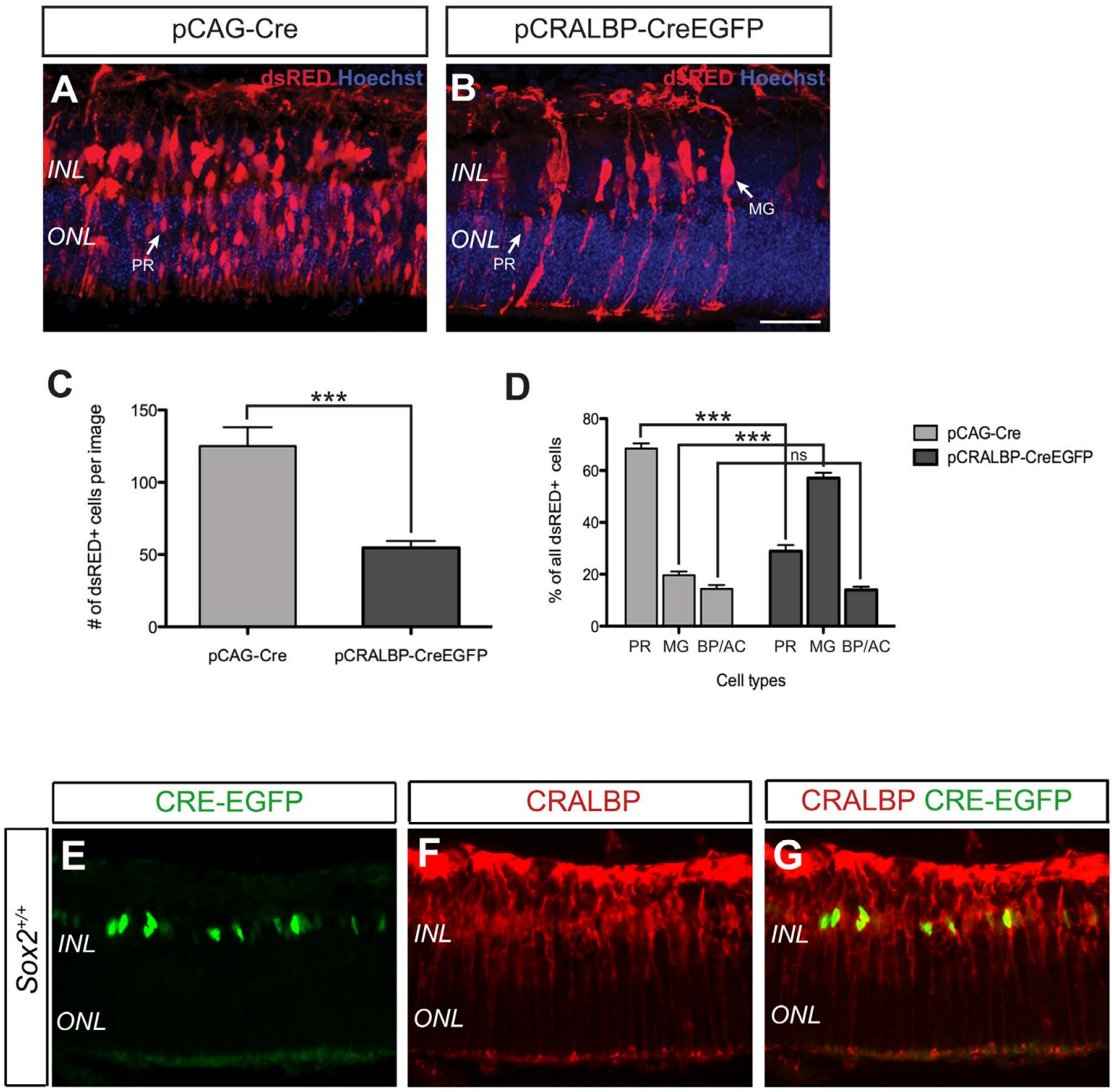
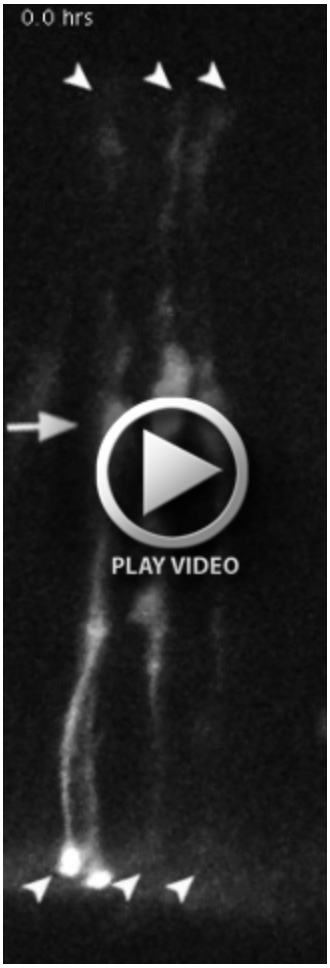
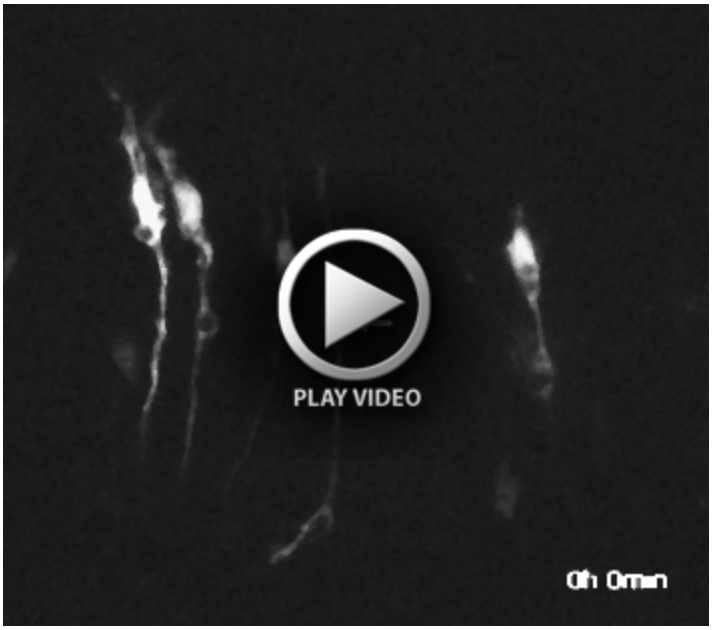


Fig. S7. Fate-mapping analysis of pCRALBP-CreEGFP confirms its expression predominantly in postnatal MG. (**A**,**B**) P1 retinas were co-electroporated with pCAG-Cre (A) or pCRALBP-CreEGFP (B) and Cre reporter construct, pCALNL-dsRED, and cultured for 5 days. In contrast to expression of the dsRED reporter predominantly in rod photoreceptors (PR) in the ONL of retinas electroporated with pCAG-Cre (arrow in A), the majority of dsRED-positive cells in retinas electroporated with pCRALBP-CreEGFP are MG (upper arrow in B). (**C**) The average number of dsRED-positive cells is significantly reduced in retinas electroporated with pCRALBP-CreEGFP (54.70±4.733, mean ± s.e.m.; n=10) compared with retinas electroporated with pCAG-Cre (125.1±13.07; n=10), as determined by unpaired Student's t-test (P<0.0001), suggesting that the *Cralbp* regulatory element is active in a subset of postnatal retinal progenitor cells. Each construct was used at 1 µg/µl. (**D**) The proportion of dsRED-positive MG among all labeled cells is significantly increased in retinas electroporated with pCRALBP-CreEGFP (57.12±2.02; n=9) compared with retinas electroporated with pCAG-Cre (19.65±1.45; n=9) (P<0.0001), whereas the proportion of labeled photoreceptors (PR) is significantly reduced in retinas electroporated with pCRALBP-CreEGFP (28.88±2.40 versus 68.48±1.94; n=9) (P<0.0001). No significant difference was observed in the proportions of labeled bipolar and amacrine cells (BP/AC). (**E-G**) CRALBP-CreEGFP colocalizes with CRALBP in the INL. INL, inner nuclear layer; ONL, outer nuclear layer. Scale bar: 65 µm in B.

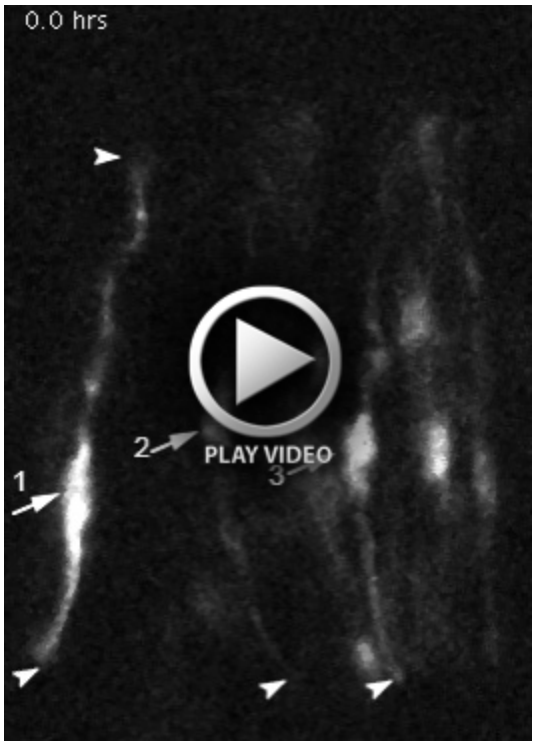


Movie 1. Stability and limited movement of MG in *Sox2*^{CONTROL} retinas. Slices of P0 retinas electroporated with GLASTp-dsRED2 were TM treated and imaged on day 3 of culture over a 17-hour period. MG cells in *Sox2*^{CONTROL} retinas maintain their typical morphology, with cell bodies located in the INL (arrow) and processes extending towards the inner and outer retinal boundaries (arrowheads), and exhibit limited movement.

Movie 2. Visualization of mitotic cell division of MG in *Sox2*^{MUTANT} retinas. GLASTp-dsRED2-labeled MG cell undergoes mitotic cell division over the course of 8.4 hours. The cell body translocates to the apical retinal boundary (arrow), followed by the emergence of two daughter cells (arrows) and accompanied by the splitting of the basal cellular process (top arrowheads).

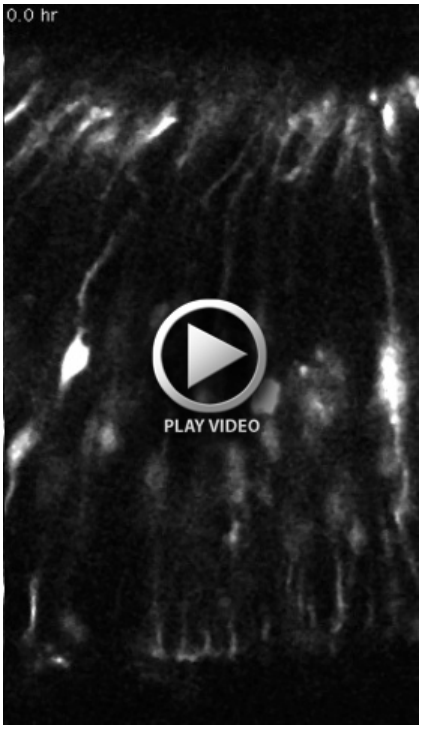


Movie 3. Cell division of MG in *Sox2*^{MUTANT} retinas. P1 *Sox2*^{MUTANT} retinas were electroporated with pCRALBP-dsRED2 and imaged on day 3 of culture over the course of 15 hours. Ds-RED2-expressing MG cell (arrow) travels to the apical surface where it undergoes cell division.



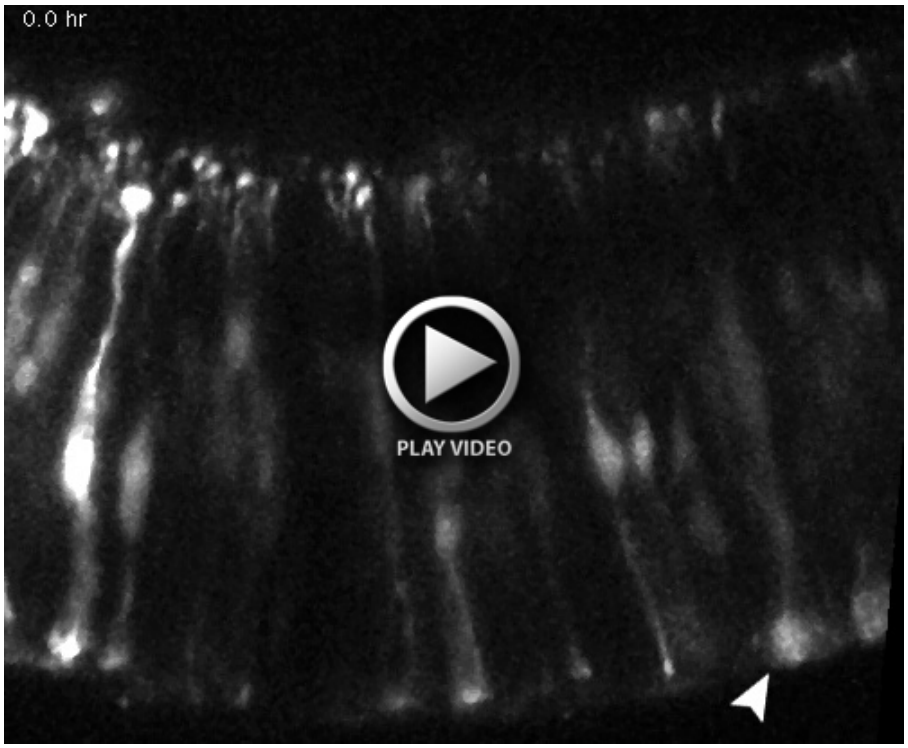
Movie 4. Cell divisions of multiple MG in *Sox2*^{MUTANT} retinas. GLASTp-dsRED2-labeled MG cells were imaged in slices of TM-treated P0 *Sox2*^{MUTANT} retinas on days 3-4 of culture. Cells 1-3 undergo internuclear migration towards the apical retinal surface and divide (arrows). Daughter cells of all three divisions remain closely associated with each other in the ONL.

Movie 5. Mosaic ablation of *Sox2* using pCRALBP-CreEGFP-Nuc leads to mitotic behavior of MG cells. *Sox2*$^{COND/COND}$ retinas were co-electroporated with pCRALBP-CreEGFP-Nuc and pCRALBP-dsRED2 at P1, and dsRED2/CreEGFP double-positive cells were imaged in retinal slices at day 3 of culture over the course of 18 hours. MG cells co-expressing dsRED2 and CreEGFP (arrowheads) undergo interkinetic nuclear migration towards the apical retinal boundary, followed by an aberrant cell division.



Movie 6. Genetic activation of Notch signaling in *Sox2*^{CONTROL}*;CALSL-NICD* retinas does not promote MG cell division. GLASTp-dsRED2-labeled cells in slices of TM-treated *Sox2*^{CONTROL}*CALSL-NICD* retinas isolated at P0 were imaged at days 3-4 of culture. MG display limited movement and do not undergo cell division.

Movie 7. Interkinetic nuclear migration and cell division of MG in *Sox2^{MUTANT};CALSL-NICD* retinas. *Sox2^{MUTANT};CALSL-NICD* retinas were imaged over the course of 14.2 hours. MG cell migrates to the apical retinal boundary, followed by the emergence of two daughter cells, which return back to the INL.



Movie 8. Asynchronous cell divisions of multiple MG cells in *Sox2^{MUTANT};CALSL-NICD* retinas. GLASTp-dsRED2-labeled MG cells were imaged in *Sox2^{MUTANT};CALSL-NICD* TM-treated retinal slices at days 3-4 of culture. Dividing MG cells (arrowheads) are identified by internuclear migration of GLAST-dsRED2-labeled cells towards the apical retinal boundary, followed by the emergence of two daughter cells (arrowheads).

Movie 9. Mitotic behavior of daughter cells resulting from cell divisions of MG in *Sox2^{MUTANT};CALSL-NICD* retinas. Cell nuclei and cytoplasm of MG cells were labeled by co-electroporating P0 *Sox2^{MUTANT};CALSL-NICD* retinas with pCAG-H2BEGFP and GLASTp-dsRED2, and double-labeled cells were imaged over the course of 14.4 hours. MG cell co-expressing H2BEGFP and GLASTp-dsRED2 (arrowhead) undergoes interkinetic nuclear migration towards the outer retinal boundary and divides, giving rise to two daughter cells (arrowheads). One of the resulting daughter cells repeats the cycle of interkinetic nuclear migration and cell division (arrowheads).

Table S1. PCR genotyping primers

Allele	Primers (5′-3′)	Product size
Sox2$^{+/+}$ and *Sox2*^{COND}	CAGAGGACTCGTGTTTGGGAAC TCTTGGATACATAAGGGTGGATGG	*Sox2*^{COND} 345 bp *Sox2*$^{+/+}$ 307 bp
Rosa26 reporter	AAAGTCGCTCTGAGTTGTTAT GCGAAGAGTTTGTCCTCAACC GGAGCGGGAGAAATGGATATG	*R26R* 340 bp *R26R*^{WT} 650 bp
CAGG-CreER^{TM}	GCTAAACATGCTTCATCGTCGG GATCTCCGGTATTGAAACTCCAGC	*Cre* 750 bp
CALSL-NICD	CAACATCCAGGACAACATGG GGACTTGCCCAGGTCATCTA	*NICD* 229 bp

Table S2. Antibodies and dyes

Primary antibodies			
Antigen	**Host**	**Source**	**Dilution**
beta-catenin	Rabbit	Sigma	1:1500
BrdU	Mouse	Becton Dickinson	1:100
cleaved caspase 3	Rabbit	Cell Signaling	1:250
CRALBP	Rabbit	J. Saari, Seattle, WA	1:1500
CRALBP	Mouse	Thermo Scientific	1:500
CRX	Mouse	Abnova	1:500
EGFP	Chicken	Abcam	1:1000
GLAST	Guinea pig	Chemicon	1:1000
Glutamine synthetase	Mouse	Chemicon	1:1000
ISLET1	Mouse	Hybridoma Bank	1:50
Nestin	Mouse	Chemicon	1:150
Neurofilament	Mouse	Sigma	1:1000
NR2E3	Rabbit	J. Nathans, Baltimore, MD	1:100
$P27^{Kip1}$	Mouse	Thermo Scientific	1:1000
PAX6	Mouse	Hybridoma Bank	1:100
PCNA	Mouse	Biosource International	1:500
Rhodopsin	Mouse	Leinco Technologies	1:100
SOX2	Rabbit	Chemicon	1:3000
SOX9	Rabbit	Millipore	1:1000
Vimentin	Mouse	Histofine	1:1
Secondary antibodies			
Antibody	**Host**	**Source**	**Dilution**
Anti-mouse, anti-rabbit CY2 and CY3	Goat	Sigma	1:250
Anti-mouse IgG1, IgG2a Alexa Fluor 488	Goat	Invitrogen	1:2000
Anti-guinea pig Alexa Fluor 546	Goat	Invitrogen	1:2000
Anti-chicken Alexa Fluor 488	Goat	Invitrogen	1:2000
Fluorescent dyes			
Dye		**Source**	**Dilution**
Hoechst 33258		Invitrogen	1:10000
Nissl		Invitrogen	1:2000
Phalloidin		Invitrogen	1:250

Table S3. Representative frequencies of observed severe retinal lamination defects

Days in culture starting at P0	Genotype	Proportion of pups with severe retinal phenotype
4-5	$Sox2^{+/+}$, $Sox2^{+/COND}$, $Sox2^{COND/COND}$	1/9
	$Sox2^{+/+}$;*CAGG-CreER*^{TM}	1/10
	$Sox2^{COND/COND}$;*CAGG-CreER*^{TM}	13/16
7	$Sox2^{+/+}$, $Sox2^{+/COND}$, $Sox2^{COND/COND}$	1/5
	$Sox2^{+/+}$;*CAGG-CreER*^{TM}	1/4
	$Sox2^{COND/COND}$;*CAGG-CreER*^{TM}	8/8