Figure S1

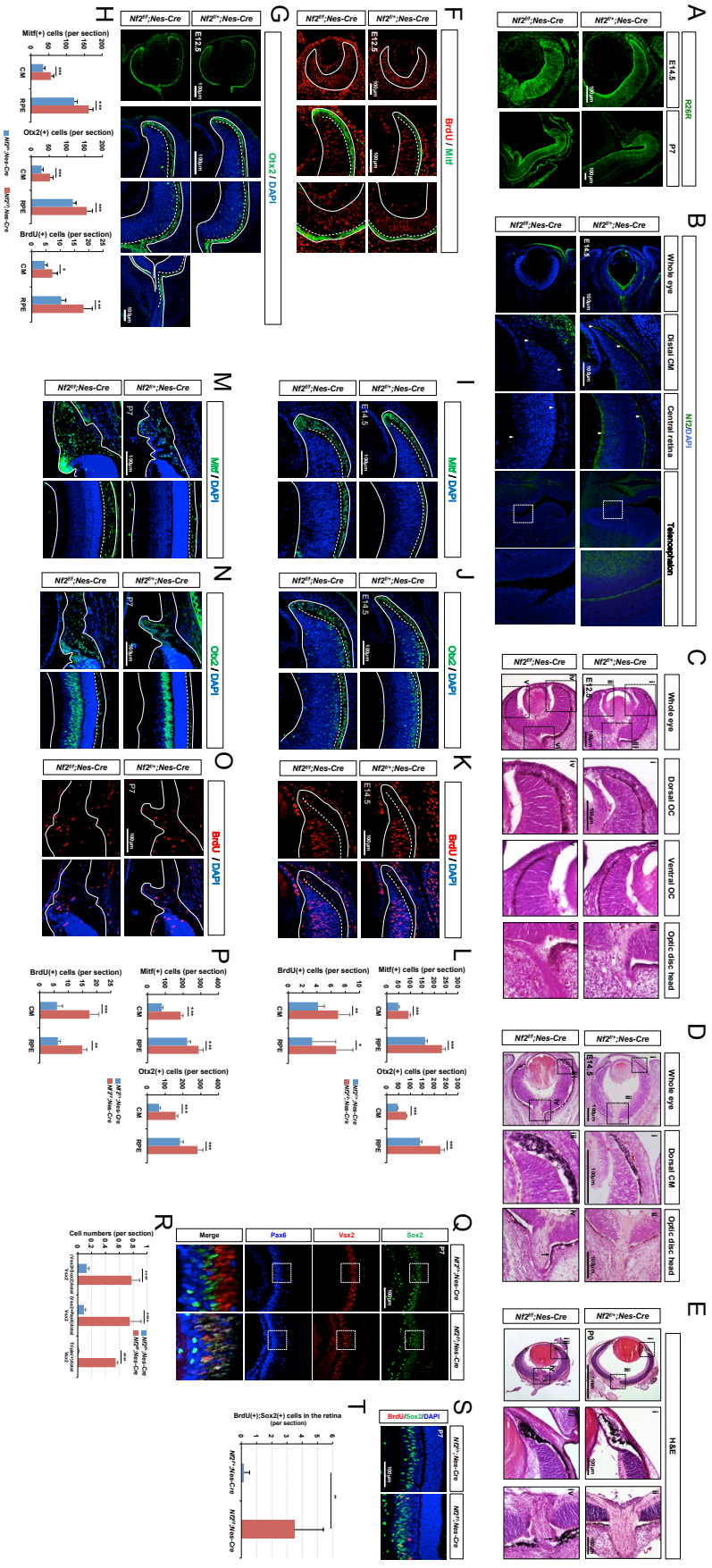

Figure S1 (related to Figure 2). Expansion of pigmented epithelium and RPCs in *Nf2$^{f/f}$;Nes-Cre* mouse eyes. (A**)** Cells underwent Cre recombinase-dependent gene deletion were visualized by immunostaining of ß-galactosidase (R26R), which is expressed at *R26* locus. Note the majority of cells in the retina, CM, and RPE in *Nf2$^{f/f}$;Nes-Cre* mouse eyes are R26R-positive, implicating the deletion of *Nf2* in those cell populations. (**B**) Successful deletion of Nf2 (green) was directly assessed by immunostaining of Nf2 in the eye and brain sections. Nf2 is absent in the optic cup and the brain of E14.5 *Nf2$^{f/f}$;Nes-Cre* mice, but it is present in the cornea of the eye. (**C – E**) H&E staining of eye sections from E12.5 (C), E14.5 (D), P0 (E) *Nf2$^{f/+}$;Nes-Cre* and *Nf2$^{f/f}$;Nes-Cre* littermate mice. Images in right columns are magnified versions of the box areas correspondingly numbered in the images at leftmost columns. Eye sections from E12.5 (F), E14.5 (I), P7 (M) *Nf2$^{f/+}$;Nes-Cre* and *Nf2$^{f/f}$;Nes-Cre* littermate mice were stained with an antibody against a pigmented cell marker Mitf alone (green; **I** and **M**) or together with an antibody against BrdU (red; **F, K,** and **O**). Dot-lines indicate the borders between RPE and retina. The sections were also stained for detecting a RPE marker Otx2 (green; **G, J,** and **N**). Nuclei of the cells were visualized by DAPI staining (blue). (**H, L, P**) Quantification of Otx2-, Mitf-, and Mitf;BrdU-positive pigmented cells in E12.5 (H), E14.5 (L), P7 (P) *Nf2$^{f/+}$;Nes-Cre and Nf2$^{f/f}$;Nes-Cre* littermate mouse eyes (n = 5 from 3 independent litters). (**Q**) Eye sections of P7 *Nf2$^{f/+}$;Nes-Cre* and *Nf2$^{f/f}$;Nes-Cre* littermate mice were also stained for detecting RPCs, which co-express Sox2 (green), Vsx2 (red), and Pax6 (blue). Note that those markers are expressed separately in the Müller glia (positive to Sox2), bipolar cells (positive to Vsx2), and amacrine cells (positive to Pax6 and Sox2 subpopulation) in P7 *Nf2$^{f/+}$;Nes-Cre* mouse retinas. (**R**) Quantification of the RPC population in the retinas (n = 5 from 3 independent litters). (**S**) Proliferation of RPC in P7 *Nf2$^{f/+}$;Nestin-Cre* and *Nf2$^{f/f}$;Nestin-Cre* mouse retinas was determined by detecting cells co-expressing a RPC marker Sox2 (green) and BrdU-labeled DNA (red). (**T**) Quantification of BrdU;Sox2 double-positive proliferating RPCs in P7 *Nf2$^{f/+}$;Nes-Cre* and *Nf2$^{f/f}$;Nes-Cre* littermate mouse retinas (n = 5 from 3 independent litters). Error bars in the graphs of this figure represent mean ± SEM. P-values were obtained by Student's two-tailed unpaired t-test. *p < 0.05 ; **p < 0.01 ; ***p < 0.005; ****p < 0.001.

Figure S2

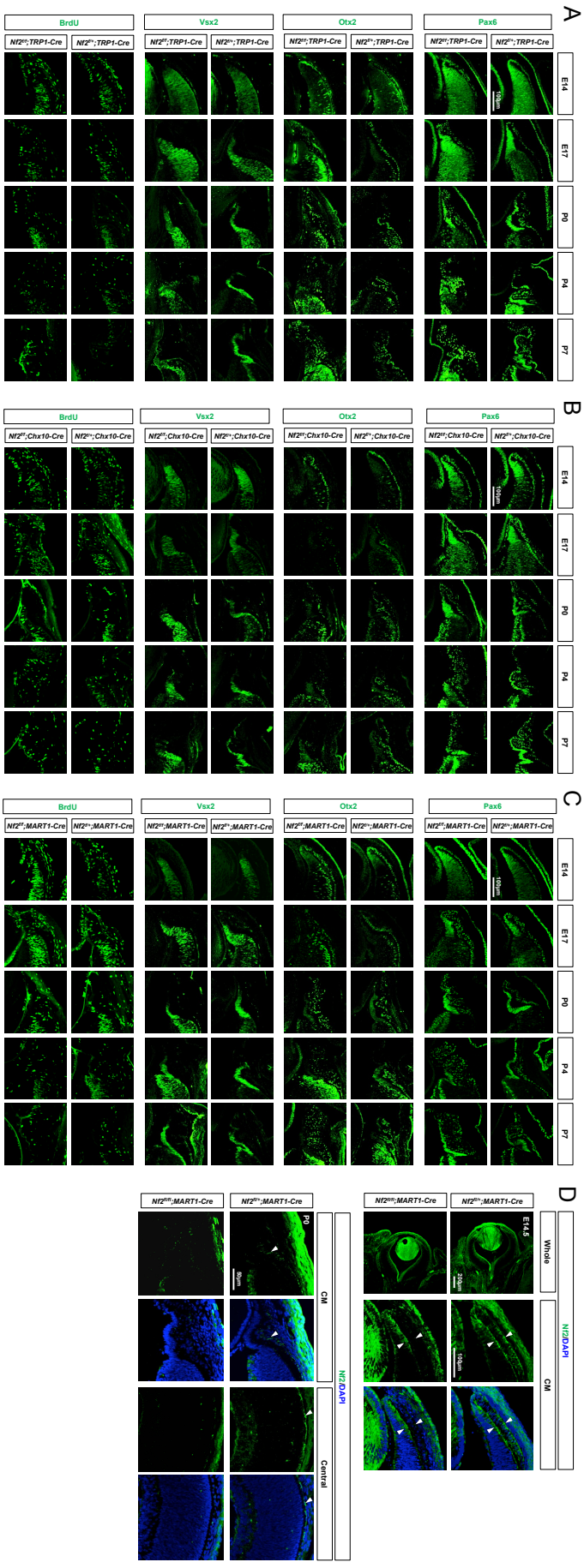
A
BrdU
Vsx2
Otx2
Pax6
Nf2f/f;TRP1-Cre
Nf2+/+;TRP1-Cre
E14
E17
P0
P4
P7
100μm
B
Nf2f/f;Chx10-Cre
Nf2+/+;Chx10-Cre
C
Nf2f/f;MART1-Cre
Nf2+/+;MART1-Cre
D
E14.5
Whole
CM
Central
Nf2/DAPI
50μm
100μm
200μm

Figure S2 (related to Figure 3 and 4). Distribution of CM, ICM, and OCM markers in the mouse eyes deleted *Nf2* in different optic neuroepithelial compartments. (**A, B, C**) Sections of mouse eyes with corresponding genotypes at indicated ages were stained with antibodies detecting pan-CM marker Pax6, OCM/RPE marker Otx2, and ICM/RPC marker Vsx2. Cell proliferation in the eye sections were determined by immunodetection of the cells containing DNA, which were incorporated with BrdU for 2h. (**D**) Successful deletion of Nf2 (green) was confirmed by immunostaining of Nf2 in the *Nf2$^{fl/+}$;MART1-Cre* and *Nf2$^{fl/fl}$;MART1-Cre* eyes at indicated period. Nf2 was absent in the OCM/RPE or PCE/RPE of *Nf2$^{f/f}$;MART1-Cre* mice.

Figure S3

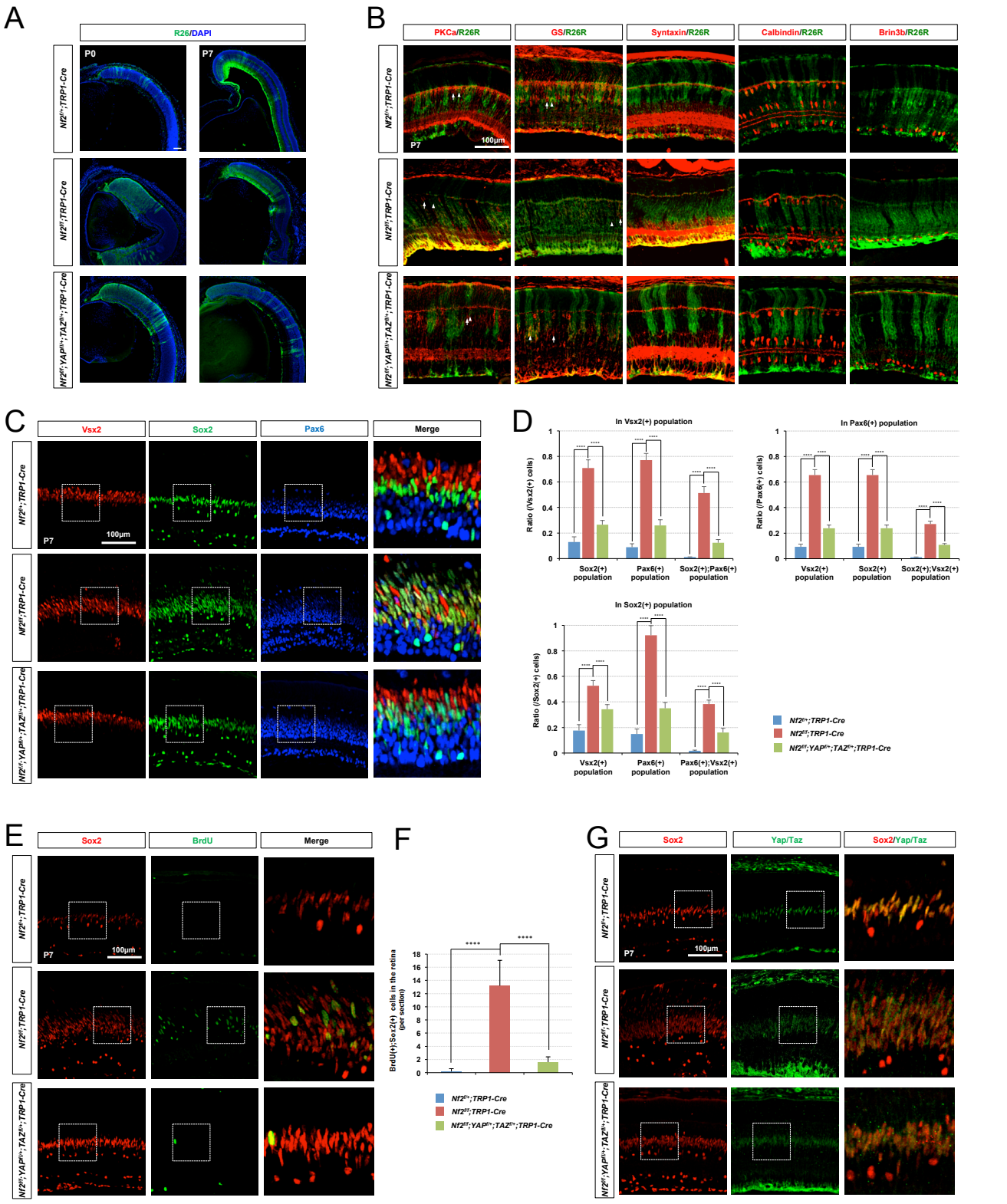
A
R26/DAPI
P0
P7
Nf2f/+;TRP1-Cre
Nf2f/f;TRP1-Cre
Nf2f/f;YAPf/+;TAZf/+;TRP1-Cre
B
PKCa/R26R
GS/R26R
Syntaxin/R26R
Calbindin/R26R
Brin3b/R26R
P7
100μm
C
Vsx2
Sox2
Pax6
Merge
D
In Vsx2(+) population
In Pax6(+) population
In Sox2(+) population
Ratio (/Vsx2(+) cells)
Ratio (/Pax6(+) cells)
Ratio (/Sox2(+) cells)
Sox2(+) population
Pax6(+) population
Sox2(+);Pax6(+) population
Vsx2(+) population
Sox2(+);Vsx2(+) population
Pax6(+);Vsx2(+) population
Nf2f/+;TRP1-Cre
Nf2f/f;TRP1-Cre
Nf2f/f;YAPf/+;TAZf/+;TRP1-Cre
E
Sox2
BrdU
Merge
F
BrdU(+);Sox2(+) cells in the retina (per section)
G
Sox2
Yap/Taz
Sox2/Yap/Taz

Figure S3 (related to Figure 3). Expansion of the CM-derived RPC population in *Nf2$^{f/f}$;TRP1-Cre* mouse retinas. (**A**) Distribution of cells underwent Cre-dependent DNA recombination was examined by immunostaining of ß-galactosidase expressed at *R26R* Cre reporter locus. (**B**) Eye sections of P7 *Nf2$^{f/+}$;TRP1-Cre, Nf2$^{f/f}$;TRP1-Cre,* and *Nf2$^{f/f}$;YAP$^{f/+}$;TAZ$^{f/+}$;TRP1-Cre* mice were stained with the antibodies detecting retinal cell type-specific markers. Note that the *Nf2$^{f/f}$;TRP1-Cre* retinas are devoid of late-born neurons, such as rod bipolar cells (positive to PKCα) and Müller glia (positive to GS) in R26R-positive cell population (arrowheads). However, those two late-born retinal cells were recovered in the *Nf2$^{f/f}$;YAP$^{f/+}$;TAZ$^{f/+}$;TRP1-Cre* mouse retinas. (**C**) The sections were stained for detecting RPCs, which co-express Sox2 (green), Vsx2 (red), and Pax6 (blue). Note that those markers are expressed separately in the Müller glia (positive to Sox2), bipolar cells (positive to Vsx2), and amacrine cells (positive to Pax6 and Sox2 subpopulation) in P7 *Nf2$^{f/+}$;TRP1-Cre* mouse retinas. (**D**) Quantification of the RPCs in the mouse retinas (n = 6 from 3 independent litters). Error bars in those graphs represent mean ± SEM. P-values were obtained by Student's two-tailed unpaired t-test. **** $p < 0.001$. (**E**) Proliferation of RPCs in P7 *Nf2$^{f/+}$;TRP1-Cre, Nf2$^{f/f}$;TRP1-Cre,* and *Nf2$^{f/f}$;YAP$^{f/+}$;TAZ$^{f/+}$;TRP1-Cre* mouse retinas were determined by detecting cells co-expressing a RPC marker Sox2 (red) and BrdU-labeled DNA (green). Note P7 *Nf2$^{f/f}$;TRP1-Cre* retinas maintained Sox2;BrdU double-positive cells in the central part as well as peripheral part, whereas *Nf2$^{f/+}$;TRP1-Cre* littermate mouse retinas showed the BrdU-positive cells only in the peripheral part (data not shown). (**F**) Quantification of the BrdU;Sox2(+) cells in the mouse retinas (n = 6 from 3 independent litters). Error bars in those graphs represent mean ± SEM. P-values were obtained by Student's two-tailed unpaired t-test. **** $p < 0.001$. (**G**) Yap/Taz expression in P7 *Nf2$^{f/+}$;TRP1-Cre, Nf2$^{f/f}$;TRP1-Cre,* and *Nf2$^{f/f}$;YAP$^{f/+}$;TAZ$^{f/+}$;TRP1-Cre* mouse retinas was analyzed by immunostaining. Note Yap/Taz-positive cells (green) are positive to Sox2 (red), which are expressed in Müller glia in the *Nf2$^{f/+}$;TRP1-Cre* mouse retinas but RPCs in the *Nf2$^{f/f}$;TRP1-Cre* mouse retinas.

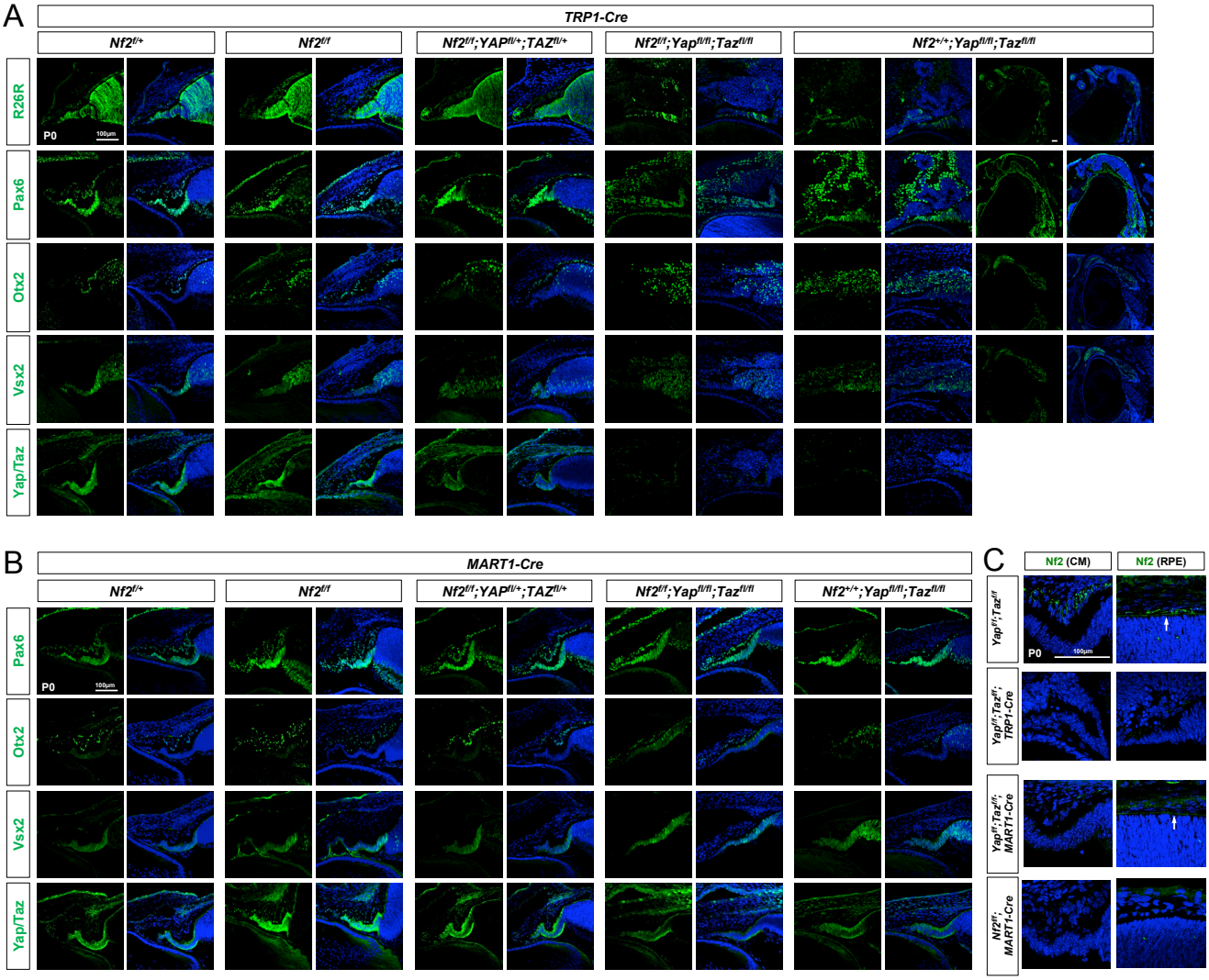

Figure S4 (related Figure 6). Distribution of CM, ICM, and OCM markers in the mouse eyes deleted *Nf2* and *Yap/Taz* in different optic neuroepithelial compartments. (**A** and **B**) Sections of P0 eyes with indicated genotypes were stained with antibodies against Pax6, Otx2, Vsx2, and Yap/Taz. Cre-mediated recombination in the retinas was also visualized by detecting ß-galactsidase expressed at the *R26R* Cre recombinase reporter gene locus (top rows, A). (**C**) The eye sections were stained with an anti-Nf2 antibody (green) to visualize Nf2 in the CM and RPE. Arrowheads indicate Nf2 expressed in the RPE.

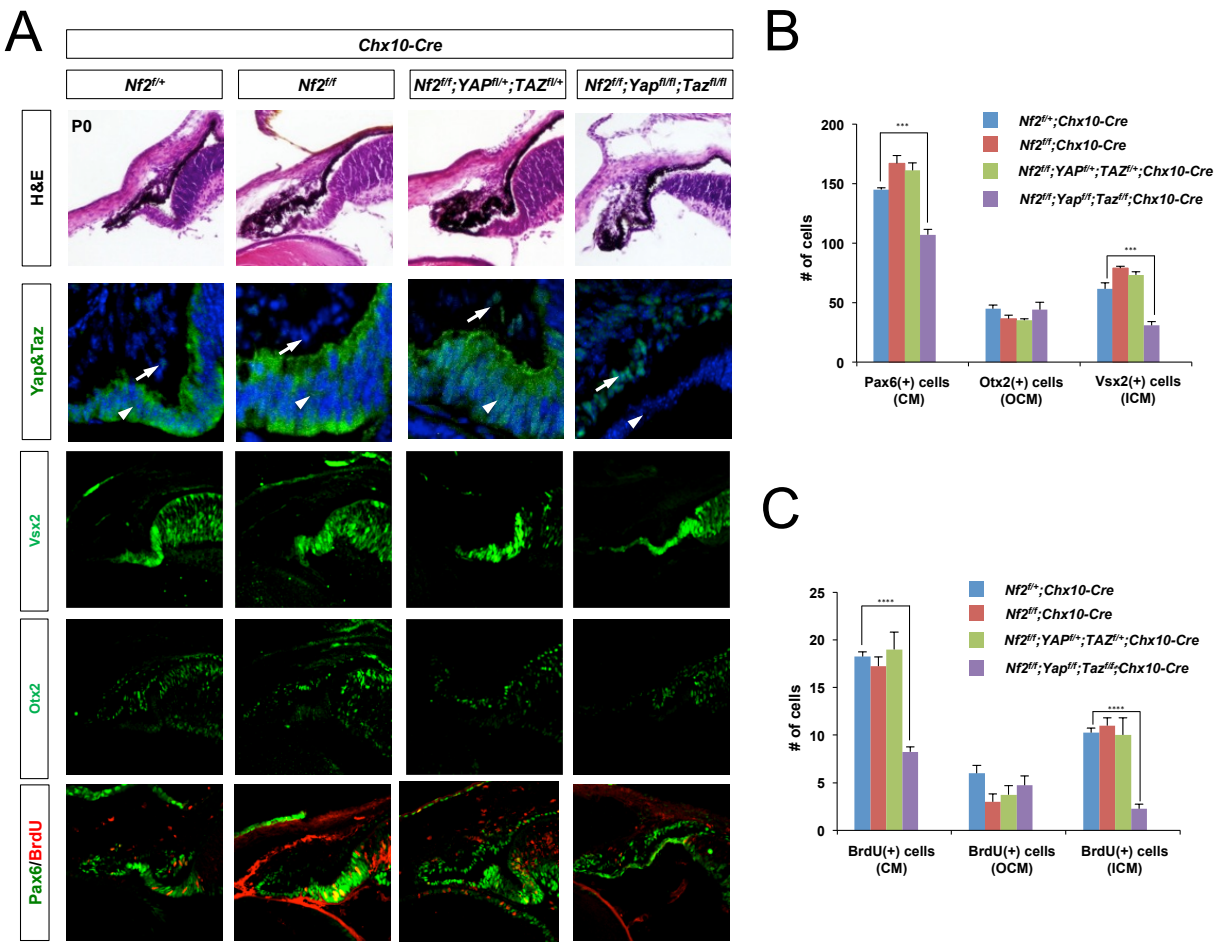

Figure S5 (related to Figure 6). *Yap/Taz* deficiency results in ICM hypoplasia. (**A**) H&E staining images of P0 *Nf2$^{f/+}$;Chx10-Cre, Nf2$^{f/f}$;Chx10-Cre, Nf2$^{f/f}$;YAP$^{f/+}$;TAZ$^{f/+}$;Chx10-Cre,* and *Nf2$^{f/f}$;Yap$^{f/f}$;Taz$^{f/f}$;Chx10-Cre* mouse eye sections. The sections were also stained with antibodies detecting pan-CM marker Pax6, OCM/RPE marker Otx2, and ICM/RPC marker Vsx2. The proliferating cells in the eye sections were determined by detecting cells containing DNA, which were incorporated with BrdU for 2h. (**B**) Pax6-positive total CM cells, Otx2-positive OCM cells, and Vsx2-positive ICM cells in the P0 mouse eyes with indicated genotypes are quantified. (**C**) Quantification of Pax6;BrdU-positive cells among total CM cells, OCM cells, and ICM cells in P0 mouse eyes with indicated genotypes. Error bars in the graphs represent mean ± SEM (n=6, 3 independent litters). P-values were obtained by Student's two-tailed unpaired *t*-test. **p < 0.01; ***p<0.005; ****p < 0.001.

Figure S6

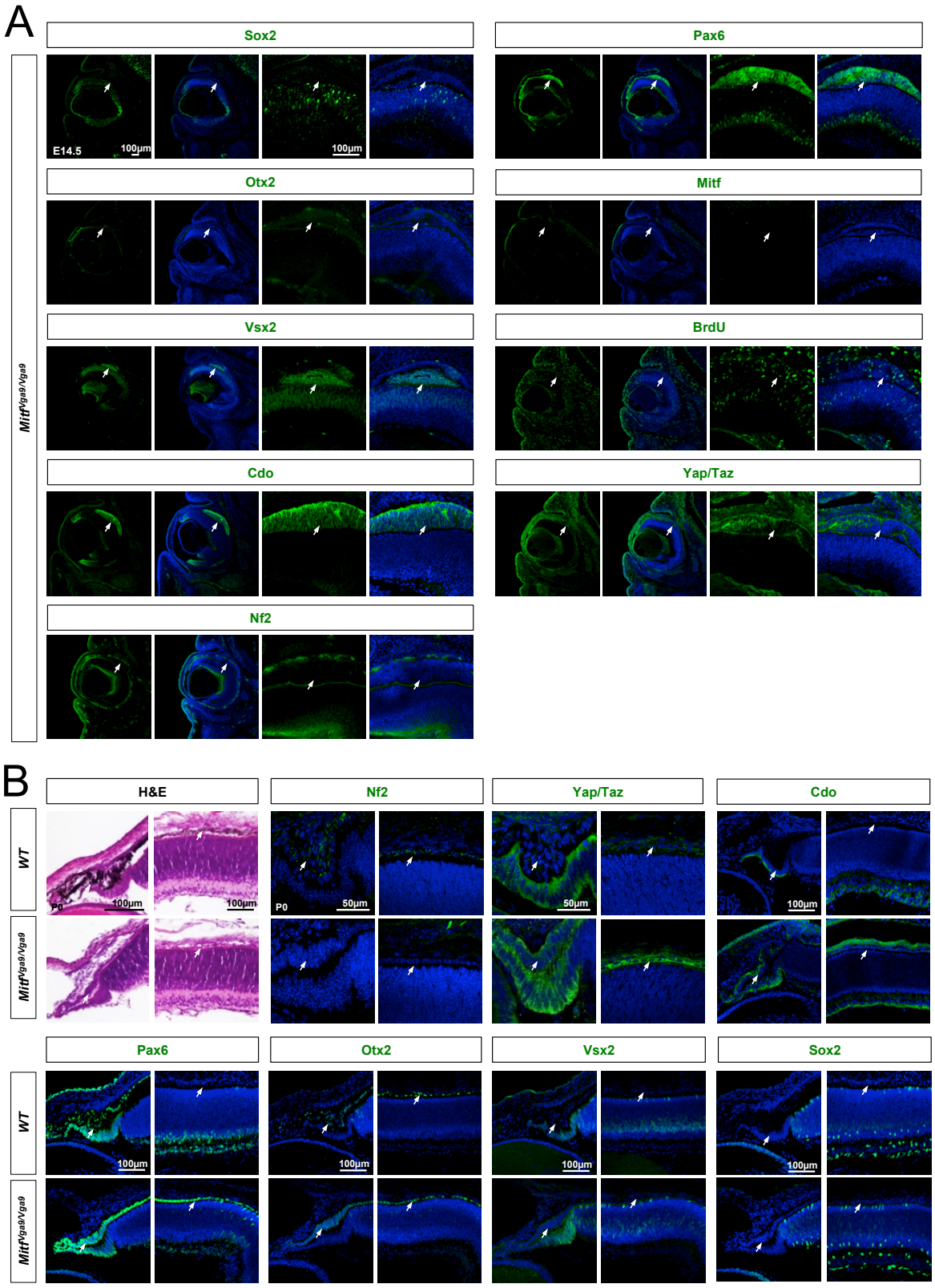
A
Sox2
Pax6
Otx2
Mitf
Vsx2
BrdU
Cdo
Yap/Taz
Nf2
Mitf^Vga9/Vga9
E14.5
100μm
B
H&E
Nf2
Yap/Taz
Cdo
WT
Mitf^Vga9/Vga9
P0
100μm
50μm
Pax6
Otx2
Vsx2
Sox2

Figure S6 (related to Figure 7). Transformation of RPE and OCM into ICM in *Mitf*$^{Vga9/Vga9}$ mouse eyes. (**A**) Sections of E14.5 *Mitf*$^{Vga9/Vga9}$ mouse eyes were stained with antibodies against indicated markers (green). Nuclei of the cells were visualized by DAPI staining (blue). White arrows indicate presence or absence of each marker on the ectopic ICM in the central outer optic cup. (**B**) Sections of P0 *WT* and *Mitf*$^{Vga9/Vga9}$ littermate mouse eyes were stained with antibodies against indicated markers (green). White arrows indicate the ectopic ICM in the central outer optic cup of the *Mitf*$^{Vga9/Vga9}$ mouse eyes.

Figure S7

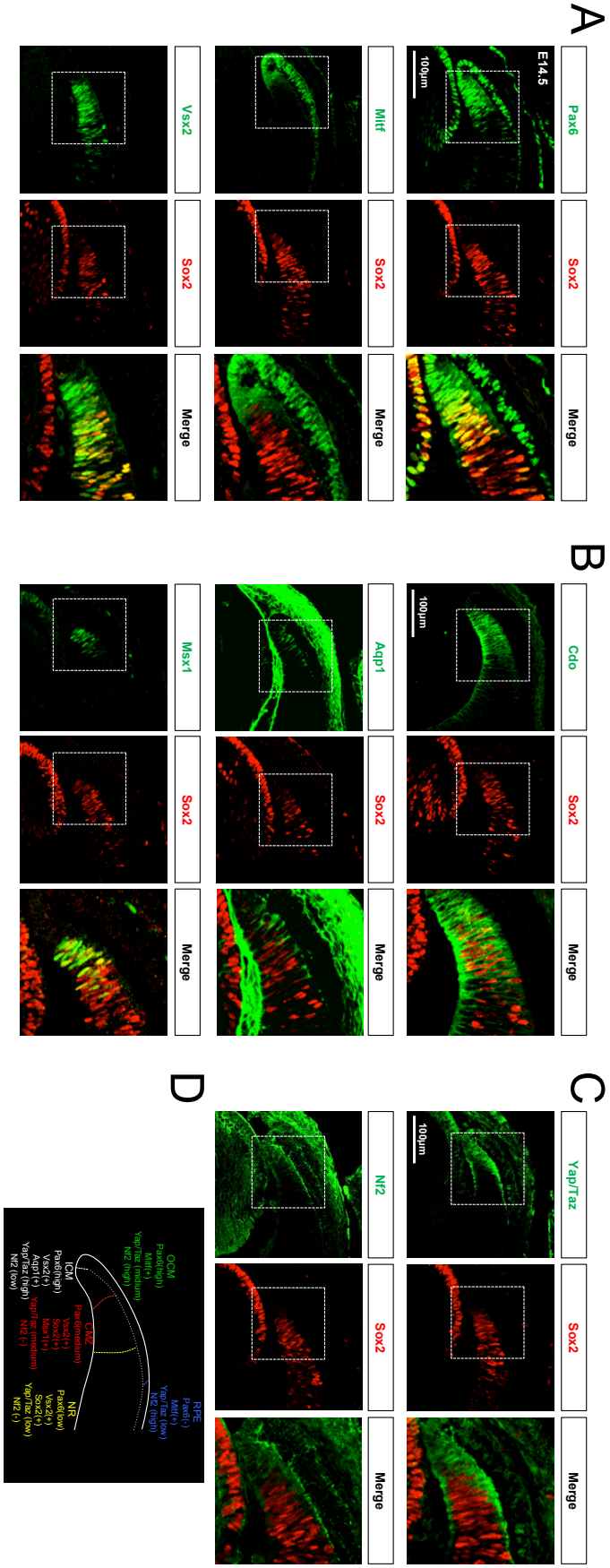
A
Pax6
Sox2
Merge
E14.5
100μm
Mitf
Sox2
Merge
Vsx2
Sox2
Merge
B
Cdo
Sox2
Merge
100μm
Aqp1
Sox2
Merge
Msx1
Sox2
Merge
C
Yap/Taz
Sox2
Merge
100μm
Nf2
Sox2
Merge
D
OCM
Pax6(high)
Mitf(+)
Yap/Taz (medium)
Nf2 (high)
CMZ
Pax6(medium)
Msx1(+)
Yap/Taz (medium)
Nf2 (-)
ICM
Pax6(high)
Vsx2(+)
Aqp1(+)
Yap/Taz (high)
Nf2 (low)
NR
Pax6(low)
Vsx2(+)
Sox2(+)
Yap/Taz (low)
Nf2 (+)
RPE
Pax6(-)
Mitf(+)
Yap/Taz (low)
Nf2 (high)

Figure S7 (related to Figure 7). Sox2 expression in RPC population in the proximal ICM and retina. Sections of E14.5 wild-type mouse eyes (C57BL/6J) were stained with goat anti-Sox2 (red) and antibodies detecting the indicated markers (green). (**A**) Sox2 is expressed in subpopulation of Pax6- or Vsx2-positive cells in the proximal ICM and retina (top and bottom), but is not expressed in Mitf-positive cells in the OCM and RPE (middle). (**B**) Sox2 is detectable at high in subpopulation of Cdo-positive ICM, but at low in Aqp1-positive distal ICM subpopulation. Majority of Msx1-positive cells in the proximal ICM co-express Sox2. (**C**) Sox2 is largely negative in ICM cells expressing Yap/Taz and Nf2. (**D**) Schematic diagram displays the markers expressed in each optic neuroepithelial compartment of mouse embryonic eye.