Figure S1

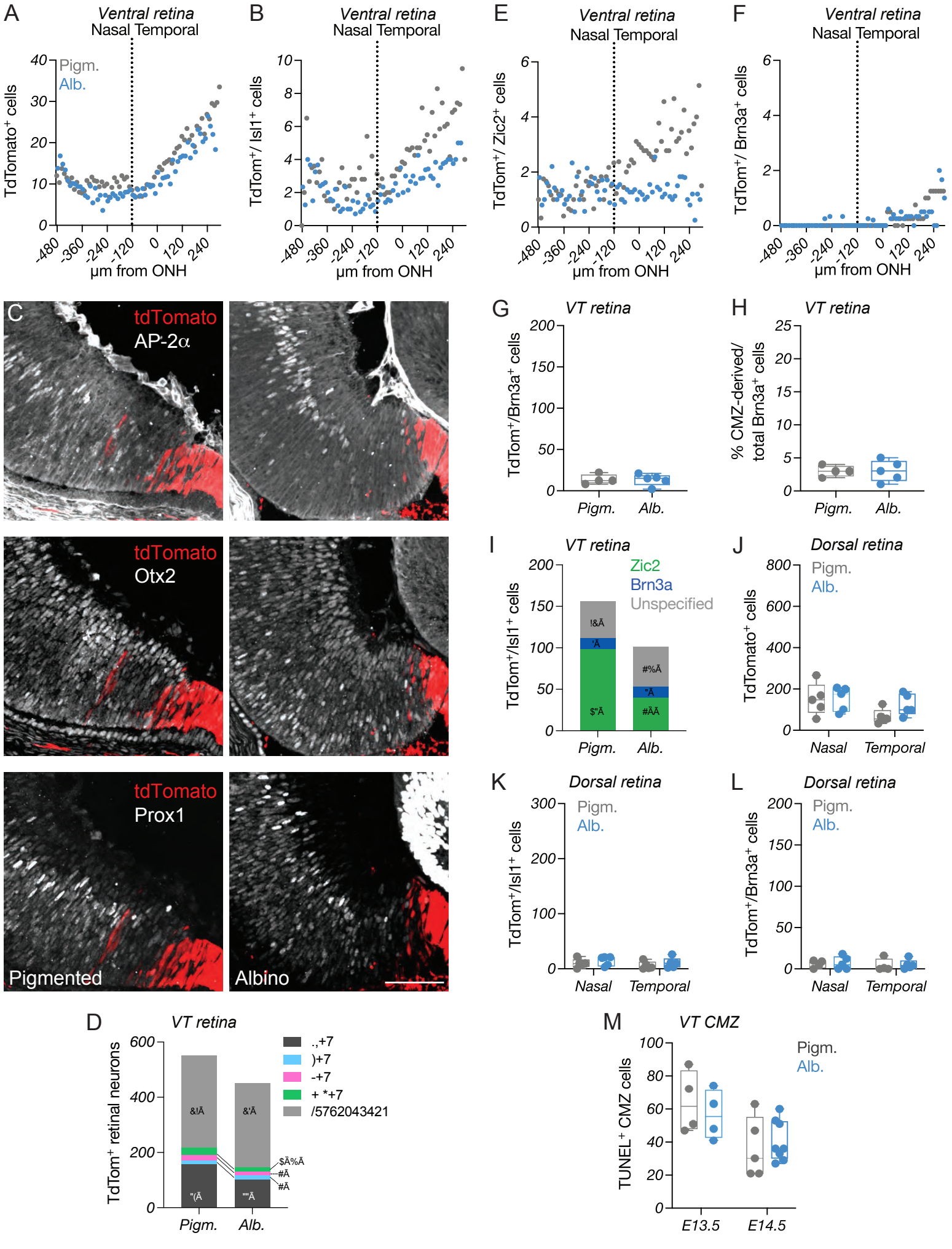

A
Ventral retina
Nasal Temporal
Pigm.
Alb.
TdTomato+ cells
μm from ONH
B
Ventral retina
Nasal Temporal
TdTom+/ Isl1+ cells
μm from ONH
E
Ventral retina
Nasal Temporal
TdTom+/ Zic2+ cells
μm from ONH
F
Ventral retina
Nasal Temporal
TdTom+/ Brn3a+ cells
μm from ONH
C
tdTomato
AP-2α
tdTomato
Otx2
tdTomato
Prox1
Pigmented
Albino
G
VT retina
TdTom+/Brn3a+ cells
Pigm.
Alb.
H
VT retina
% CMZ-derived/ total Brn3a+ cells
Pigm.
Alb.
I
VT retina
Zic2
Brn3a
Unspecified
TdTom+/Isl1+ cells
Pigm.
Alb.
J
Dorsal retina
Pigm.
Alb.
TdTomato+ cells
Nasal
Temporal
K
Dorsal retina
Pigm.
Alb.
TdTom+/Isl1+ cells
Nasal
Temporal
L
Dorsal retina
Pigm.
Alb.
TdTom+/Brn3a+ cells
Nasal
Temporal
D
VT retina
TdTom+ retinal neurons
Pigm.
Alb.
M
VT CMZ
Pigm.
Alb.
TUNEL+ CMZ cells
E13.5
E14.5

Figure S1: Ipsilateral RGC neurogenesis is prominent in the ventral CMZ in pigmented mice but reduced in the albino, Related to Figure 1

A, B, E, F) Number of tdTomato$^+$ (A), tdTomato$^+$/Islet1$^+$ (B), tdTomato$^+$/Zic2$^+$ (E) and tdTomato$^+$/Brn3a$^+$ (F) cells in a full series of 12μm sections collected along the rostro-caudal head axis (nasal, rostral; temporal, caudal). Each point corresponds to an individual section, plotted on the x-axis as a function of distance (in μm) from the first section in the series that contained the optic nerve head opening.

C) Immunostaining of tdTomato, AP-2α (top), Otx2 (middle) and Prox1 (bottom) in the pigmented and albino ventrotemporal retina at E17.5. Co-labeling of tdTomato with either AP-2α, Otx2, or Prox1 is rare in the ventrotemporal retina. Scale bar: 100μm.

D) Quantification of tdTomato$^+$/Islet1$^+$ (RGC), tdTomato$^+$/AP-2α^+ (amacrine), tdTomato$^+$/Otx2$^+$ (cone and bipolar), and tdTomato$^+$/Prox1$^+$ (horizontal) cells in the ventrotemporal retina of pigmented and albino mice at E17.5.

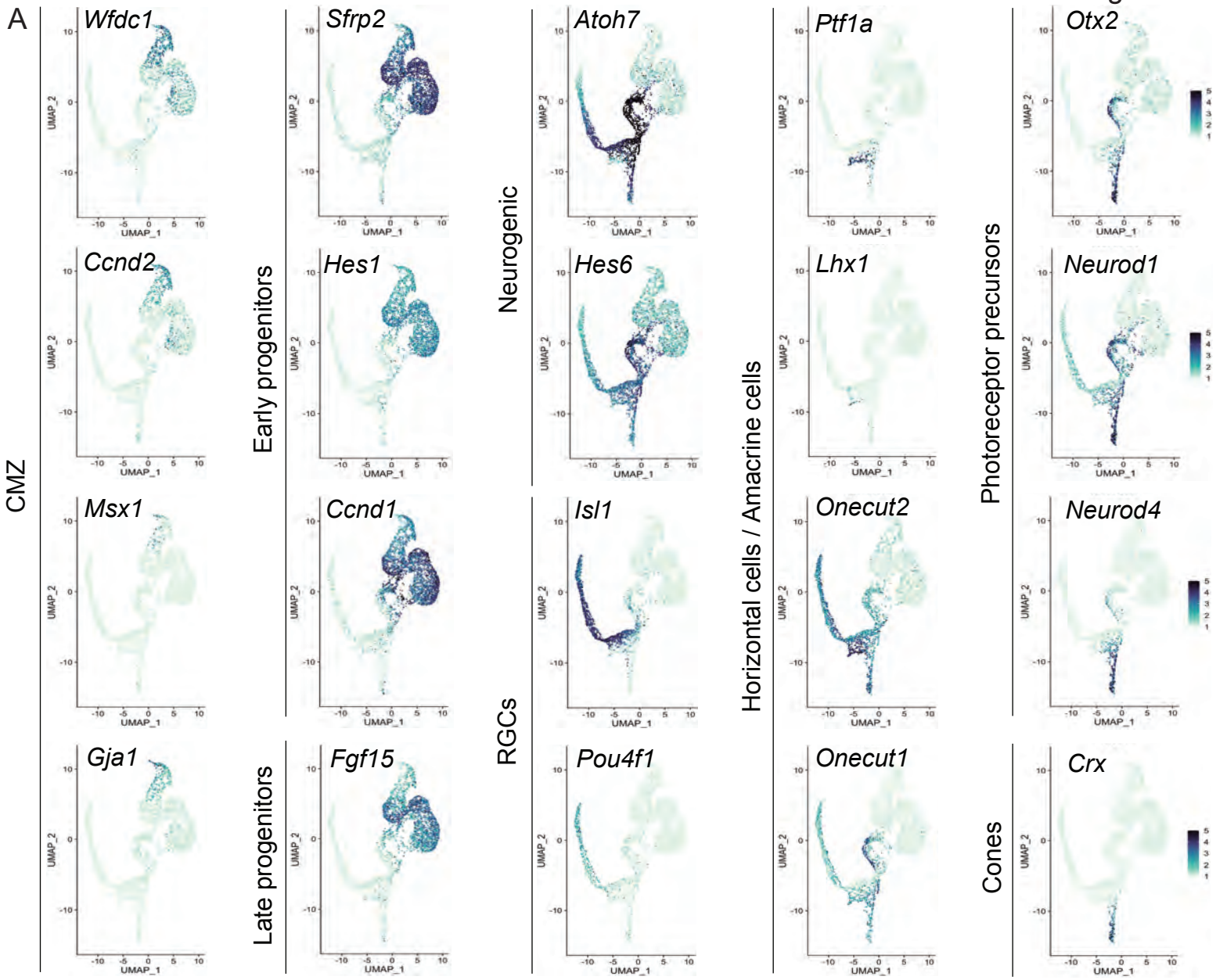
G) Quantification of tdTomato$^+$/Brn3a$^+$ cells in the ventrotemporal retina of pigmented and albino mice at E17.5.

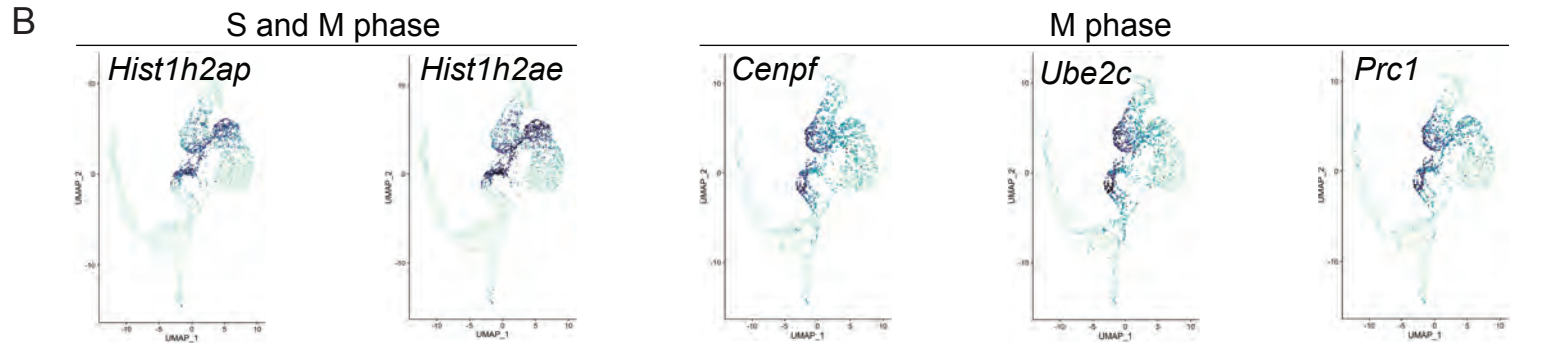
H) Quantification of the proportion (% of total) of Brn3a$^+$ cells deriving from the CMZ.

I) Quantification of tdTomato$^+$/Zic2$^+$ and tdTomato$^+$/Brn3a$^+$ cells as a percentage of the total CMZ-derived RGCs (tdTomato$^+$/Islet1$^+$).

J-L) Quantification of tdTomato$^+$ (J), tdTomato$^+$/Islet1$^+$ (K), tdTomato$^+$/Brn3a$^+$ (L) cells in the nasal and temporal dorsal retina of pigmented and albino mice at E17.5. Very few retinal cells originate from the dorsal CMZ.

M) Quantification of apoptotic (TUNEL$^+$) cells within the pigmented and albino ventrotemporal CMZ at E13.5 and E14.5.


A
CMZ
Early progenitors
Late progenitors
Neurogenic
RGCs
Horizontal cells / Amacrine cells
Photoreceptor precursors
Cones
Wfdc1
Sfrp2
Atoh7
Ptf1a
Otx2
Ccnd2
Hes1
Hes6
Lhx1
Neurod1
Msx1
Ccnd1
Isl1
Onecut2
Neurod4
Gja1
Fgf15
Pou4f1
Onecut1
Crx
UMAP_1
UMAP_2


B
S and M phase
M phase
Hist1h2ap
Hist1h2ae
Cenpf
Ube2c
Prc1

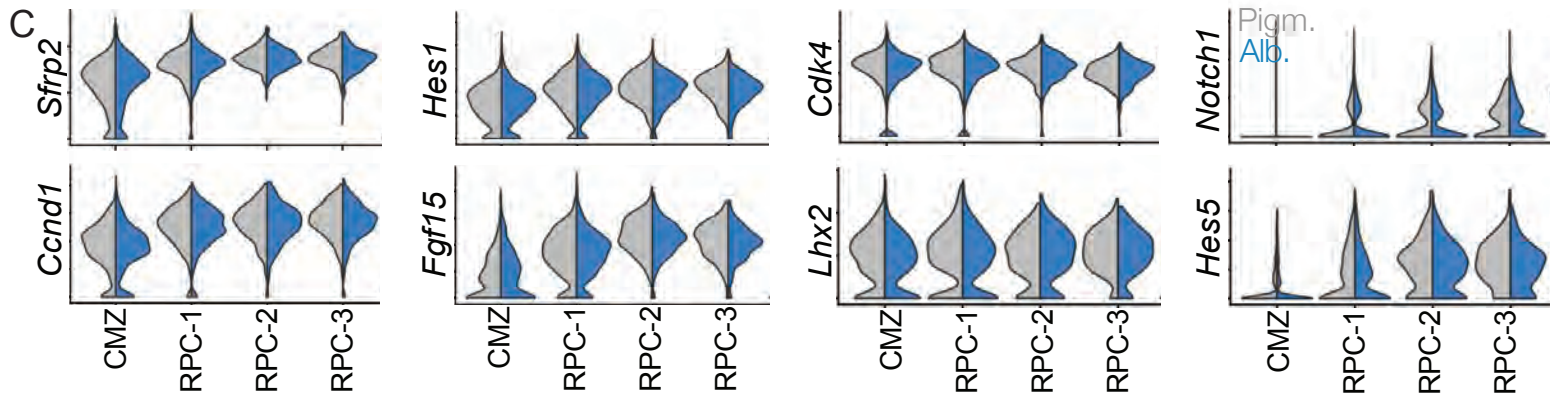

C
Sfrp2
Hes1
Cdk4
Notch1
Pigm.
Alb.
Ccnd1
Fgf15
Lhx2
Hes5
CMZ
RPC-1
RPC-2
RPC-3

Figure S2: scRNA-Seq in the pigmented and albino CMZ, Related to Figure 2

Identification of single-cell clusters by known cell type-specific markers.

A) CMZ cells express *Wfdc1*, *Ccnd2*, *Msx1* and *Gja1*. Early RPCs express *Sfrp2*, *Hes1* and *Ccnd1* while late RPCs express *Fgf15*. Neurogenic cells express *Atoh7* and *Hes6*. RGCs express *Isl1* and *Pou4f1*. The HC/AC cluster expresses *Ptf1a*, *Lhx1*, and *Onecut1/2*. PR precursors express *Otx1*, *Neurod1* and *Neurod4*, and cones express *Crx*.

B) RPC cells were enriched in *Hist1h2ap* and *Hist1h2ae*, marking the S and M cell cycle phases, as well as in *Cenpf*, *Ube2c* and *Prc1* marking the M phase.

C) Violin plots showing comparison of genes that define RPC clusters between pigmented and albino.

Figure S3

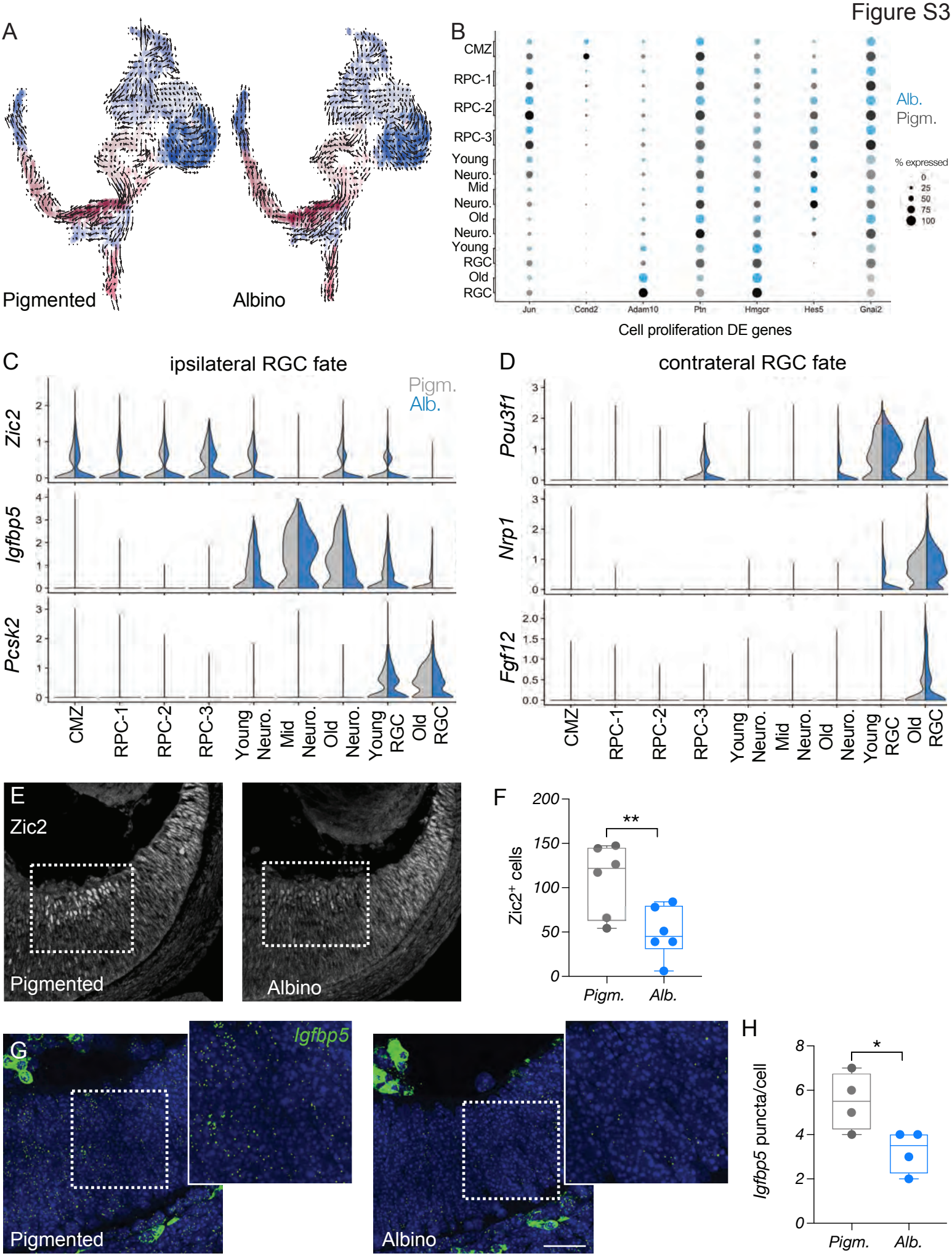

A
Pigmented
Albino
B
CMZ
RPC-1
RPC-2
RPC-3
Young Neuro.
Mid Neuro.
Old Neuro.
Young RGC
Old RGC
Jun
Cond2
Adam10
Ptn
Hmgcr
Hes5
Gnai2
Cell proliferation DE genes
Alb.
Pigm.
% expressed
0
25
50
75
100
C
ipsilateral RGC fate
Pigm.
Alb.
Zic2
Igfbp5
Pcsk2
D
contrateral RGC fate
Pou3f1
Nrp1
Fgf12
E
Zic2
Pigmented
Albino
F
Zic2+ cells
Pigm.
Alb.
**
G
Igfbp5
Pigmented
Albino
H
Igfbp5 puncta/cell
Pigm.
Alb.
*

Figure S3: scRNA-Seq in the pigmented and albino CMZ, Related to Figure 2

A) RNA Velocity vector fields projected on UMAP of single-cell clusters in the pigmented and albino datasets.

B) Differentially expressed genes (DEG) between pigmented and albino, included in the GO term “cell proliferation” across single-cell clusters. Size of the dot represents percentage of cells within a class, while color represents average expression level across all cells within a class.

C-D) Violin plots showing comparison of genes associated with ipsilateral (C) and contralateral (D) fate.

E-F) Validation of Zic2 (E-F) and Igfbp5 (G-H) downregulation with immunohistochemistry and RNAscope, respectively. Scale bar: 25μm.

Figure S4

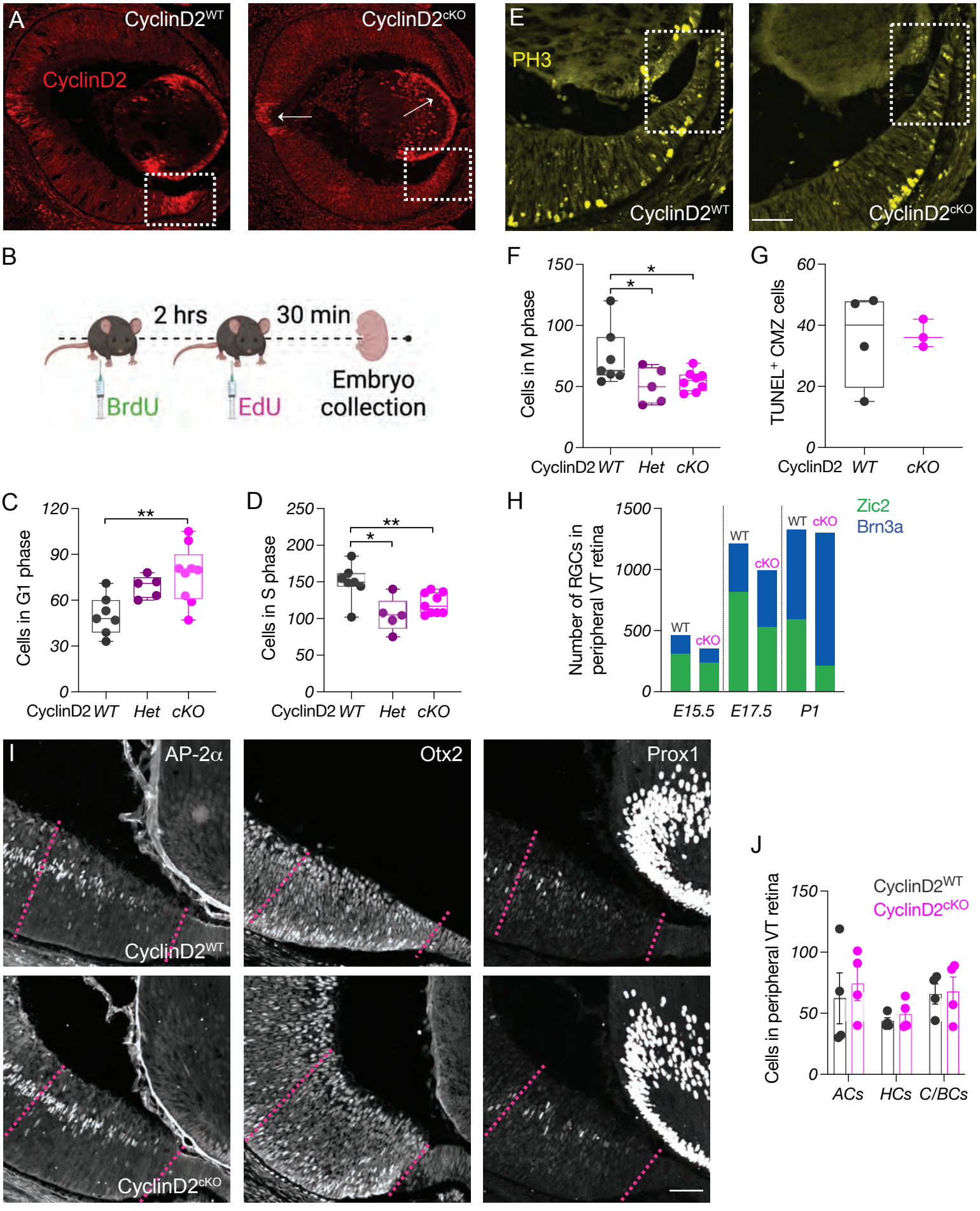

A
CyclinD2WT
CyclinD2
CyclinD2cKO
E
PH3
CyclinD2WT
CyclinD2cKO
B
2 hrs
30 min
BrdU
EdU
Embryo collection
F
Cells in M phase
150
100
50
0
CyclinD2 WT Het cKO
G
TUNEL+ CMZ cells
60
40
20
0
CyclinD2 WT cKO
C
Cells in G1 phase
120
90
60
30
0
CyclinD2 WT Het cKO
D
Cells in S phase
250
200
150
100
50
0
CyclinD2 WT Het cKO
H
Number of RGCs in peripheral VT retina
1500
1000
500
0
WT
cKO
Zic2
Brn3a
E15.5 E17.5 P1
I
AP-2α
Otx2
Prox1
CyclinD2WT
CyclinD2cKO
J
Cells in peripheral VT retina
150
100
50
0
CyclinD2WT
CyclinD2cKO
ACs HCs C/BCs

Figure S4: CyclinD2-dependent G1/S transition regulates ipsilateral RGC neurogenesis, Related to Figure 4

A) Example immunostaining of CyclinD2 in the CyclinD2^{WT} and CyclinD2^{cKO} ventrotemporal CMZ (area in the box) at E14.5. CyclinD2 is conditionally deleted from the CyclinD2^{cKO} CMZ, whereas it is still expressed in the lens and optic nerve head (arrows).

B) Schema of the dual-pulse birthdating experiment.

C-D) Quantification of cells in the G1 and S phases of the cell cycle at E14.5. Het mice: αCre; CyclinD2$^{flox/+}$.

E-F) Immunostaining (E) and quantification (F) of the mitotic marker PH3 in the CyclinD2^{WT} and CyclinD2^{cKO} ventrotemporal CMZ (area in the box) at E14.5. Scale bar: 100μm.

G) Quantification of apoptotic (TUNEL^{+}) cells within the CyclinD2^{WT} and CyclinD2^{cKO} ventrotemporal CMZ at E14.5.

H) Quantification of Zic2^{+} and Brn3a^{+} cells in the CyclinD2^{WT} and CyclinD2^{cKO} ventrotemporal retina at E15.5, E17.5 and P1.

I-J) Immunostaining (I) and quantification (J) of AP-2α (left), Otx2 (middle) and Prox1 (right) in the CyclinD2^{WT} and CyclinD2^{cKO} peripheral ventrotemporal retina at E17.5. Dashed red lines in (I) indicate the 150μm-long peripheral retina segment analyzed. Scale bar: 50μm.

Figure S5

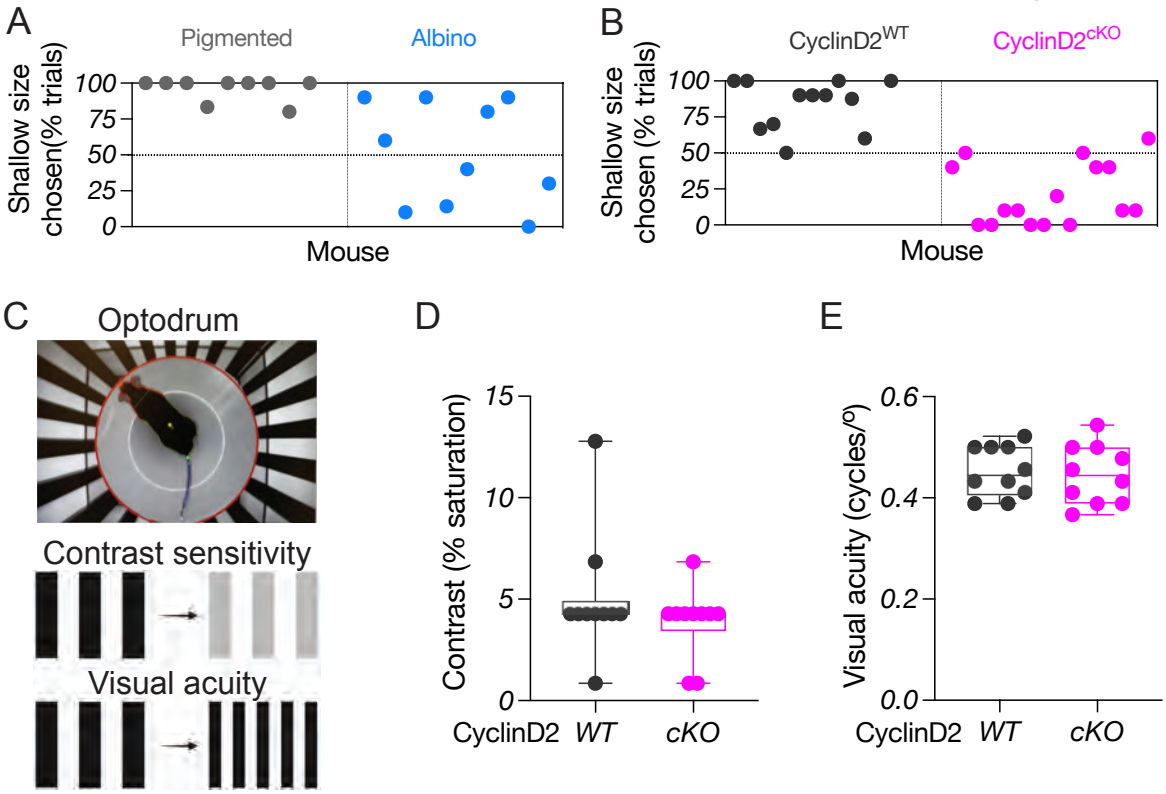

A
Pigmented
Albino
Shallow size chosen(% trials)
100
75
50
25
0
Mouse
B
CyclinD2WT
CyclinD2cKO
Shallow size chosen (% trials)
100
75
50
25
0
Mouse
C
Optodrum
Contrast sensitivity
Visual acuity
D
Contrast (% saturation)
15
10
5
0
CyclinD2 WT cKO
E
Visual acuity (cycles/°)
0.6
0.4
0.2
0.0
CyclinD2 WT cKO

Figure S5: Depth perception requires CyclinD2 expression in the CMZ, Related to Figure 5

A-B) Depth perception, corresponding to the percentage of trials in which the shallow side was chosen by each mouse across all trials in the visual cliff task, comparing pigmented and albino mice (A) and CyclinD2^{WT} and CyclinD2^{cKO} mice (B).

C) Schema of the Optodrum setup, used to measure contrast sensitivity and visual acuity.

D-E) Quantification of contrast sensitivity (D) and visual acuity (E) of CyclinD2^{WT} and CyclinD2^{cKO} mice.

Figure S6

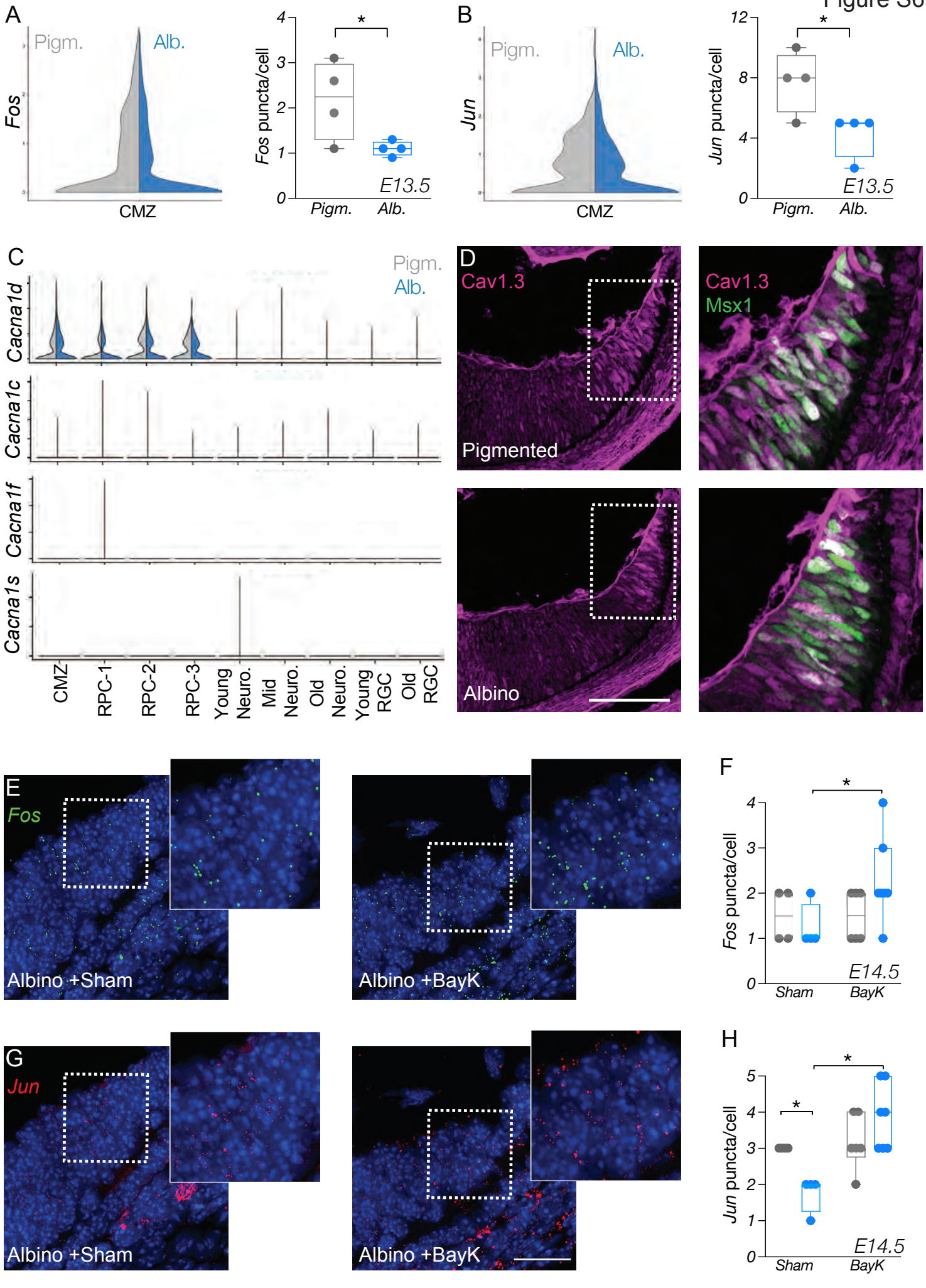

A
Pigm.
Alb.
Fos
CMZ
Fos puncta/cell
E13.5
Pigm.
Alb.
B
Jun
Jun puncta/cell
C
Cacna1d
Cacna1c
Cacna1f
Cacna1s
CMZ
RPC-1
RPC-2
RPC-3
Young Neuro.
Mid Neuro.
Old Neuro.
Young RGC
Old RGC
D
Cav1.3
Msx1
Pigmented
Albino
E
Fos
Albino +Sham
Albino +BayK
F
E14.5
Sham
BayK
G
Jun
H

Figure S6: CyclinD2 upregulation restores binocular circuit formation and function in albino mice, Related to Figure 6

A-B) Violin plots and RNA scope quantification (fluorescent puncta per nucleus) of *Fos* and *Jun* in the CMZ, showing their downregulation in the albino dataset.

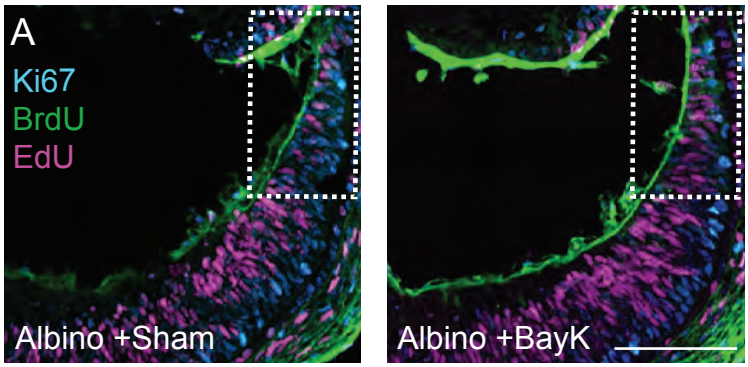
C) Violin plots of *Cacna1* genes in the pigmented and albino scRNA-Seq clusters.

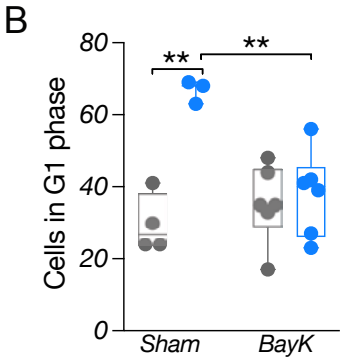
D) Immunostaining of $Ca_V1.3$ and Msx1 in the pigmented and albino ventrotemporal CMZ, showing $Ca_V1.3$ expression within the $Msx1^+$ domain. Panels on the right correspond to the areas in the box (left) in magnification. Scale bar: 100μm.

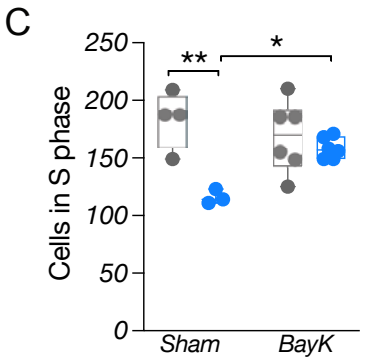
E, G) Fluorescent in situ hybridization (RNA scope) of *Fos* (E) and *Jun* (G) in the CMZ of albino Sham vs BayK treated mice at E14.5.

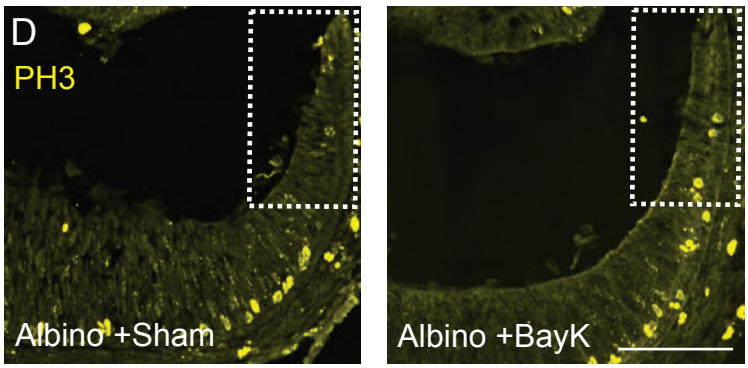
F, H) Quantification of *Fos* (F) and *Jun* (H) in the CMZ of pigmented and albino Sham vs BayK treated mice at E14.5. *Fos* and *Jun* expression are quantified as number of fluorescent puncta per nucleus. Scale bar: 25μm.

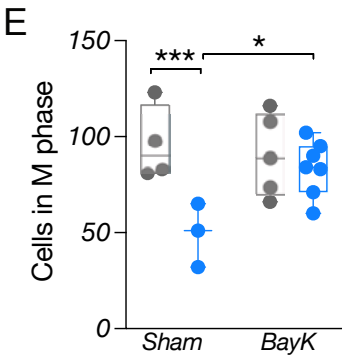
Figure S7

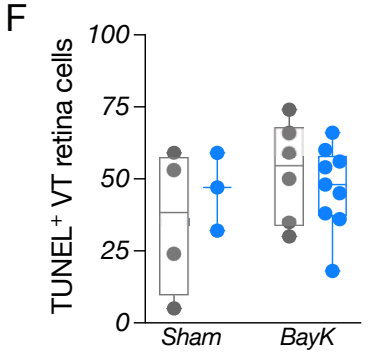

A
Ki67
BrdU
EdU
Albino +Sham
Albino +BayK

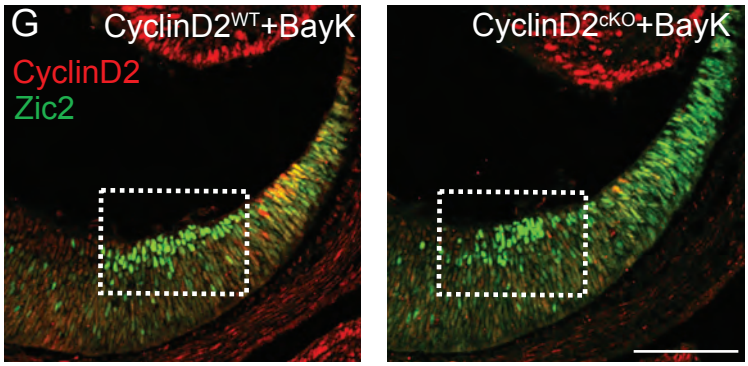

B
Cells in G1 phase
80
60
40
20
0
**
**
Sham
BayK

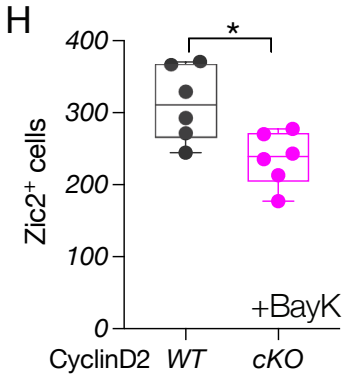

C
Cells in S phase
250
200
150
100
50
0
**
*
Sham
BayK


D
PH3
Albino +Sham
Albino +BayK


E
Cells in M phase
150
100
50
0
***
*
Sham
BayK


F
TUNEL+ VT retina cells
100
75
50
25
0
Sham
BayK


G
CyclinD2WT+BayK
CyclinD2cKO+BayK
CyclinD2
Zic2


H
Zic2+ cells
400
300
200
100
0
*
+BayK
CyclinD2 WT cKO

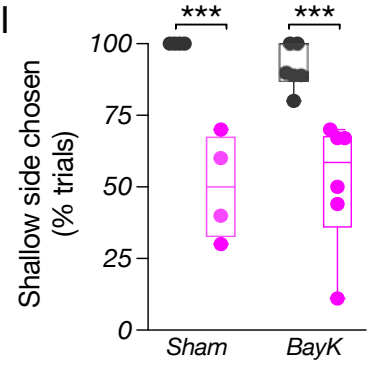

I
Shallow side chosen
(% trials)
100
75
50
25
0
***
***
Sham
BayK

Figure S7: CyclinD2 upregulation restores binocular circuit formation and function in albino mice, Related to Figure 6

A) Immunostaining of Ki67, BrdU, and EdU in the ventrotemporal CMZ (area in the box) of albino Sham vs BayK treated mice at E14.5. Scale bar: 100μm.

B-C) Quantification of cells in the G1 (B) and S (C) phases of the cell cycle at E14.5.

D) Immunostaining of the mitotic marker PH3 in the ventrotemporal CMZ (area in the box) of albino Sham vs BayK treated mice at E14.5. Scale bar: 100μm.

E) Quantification of cells in the M phase of the cell cycle ($PH3^+$) at E14.5.

F) Quantification of apoptotic ($TUNEL^+$) cells within the $Zic2^+$ domain of the ventrotemporal CMZ at E14.5.

G) Immunostaining of CyclinD2 and Zic2 in the $CyclinD2^{WT}$ and $CyclinD2^{cKO}$, BayK-treated ventrotemporal retina and CMZ (area in the box) at E15.5. Scale bar: 100μm.

H) Quantification of $Zic2^+$ cells at E15.5.

I) Quantification of depth perception, corresponding to the percentage of trials in which the shallow side was chosen, comparing $CyclinD2^{WT}$ and $CyclinD2^{cKO}$ Sham vs BayK treated mice.