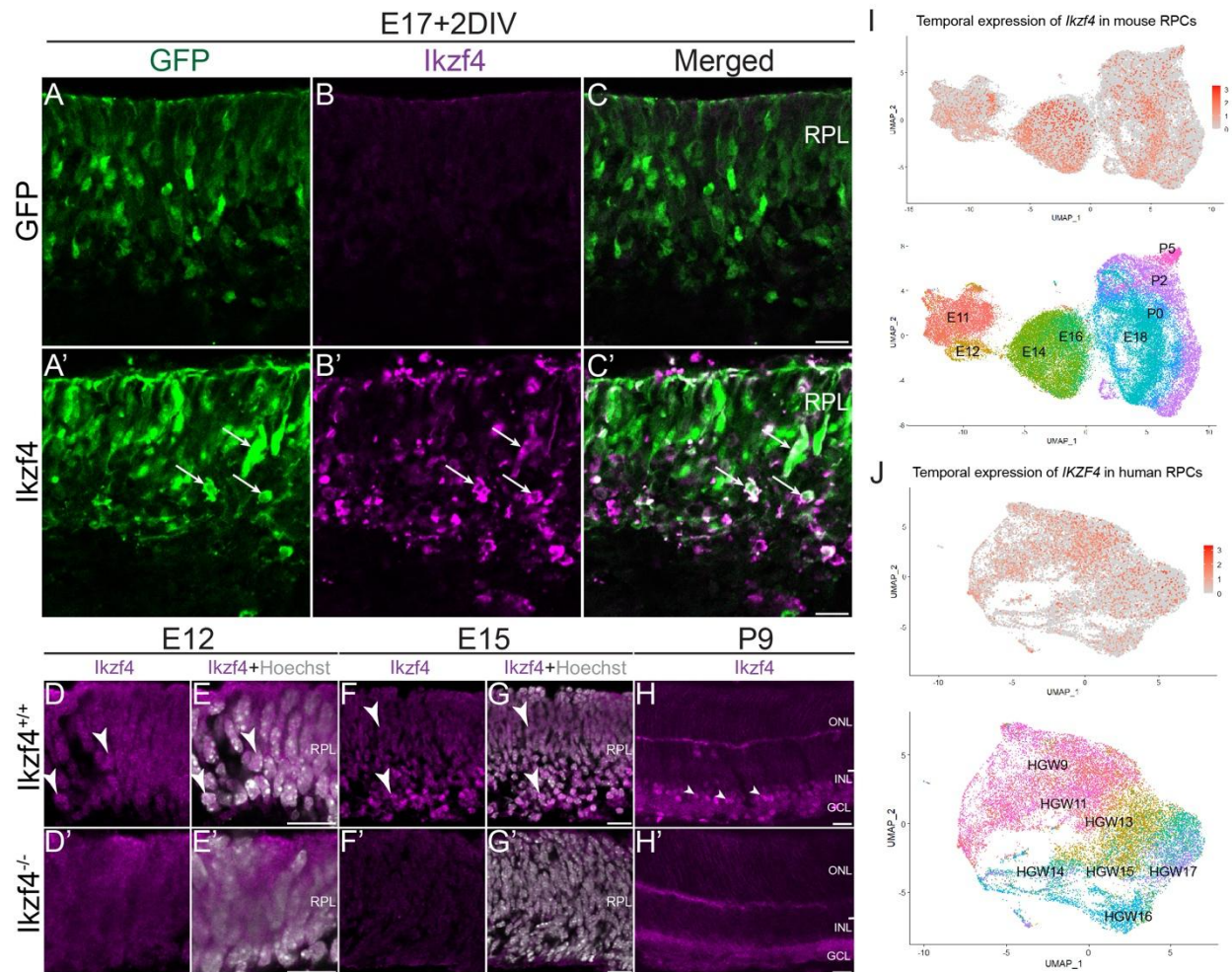Figure S1, Javed et al.

Fig. S1. Specificity of Ikzf4 antibody and scRNA-seq re-analysis of human and mouse RPCs.

(A-C') Examples of electroporation of either GFP (A-C) or Ikzf4-IRES-GFP (A'-C') in E17 retinas and immunostained with Ikzf4 antibody 2 days later. Note that these samples were fixed for 1 hour, which does not allow detection of endogenous Ikzf4 as reported in Figure 1 (fixed 2 mins). White arrows point to overexpressed Ikzf4, not endogenous. White arrows indicate GFP^{+ve}Ikzf4^{+ve} cells. (D-H') Validation of the Ikzf4 antibody in Ikzf4$^{+/+}$ retinas (D-H) compared to Ikzf4$^{-/-}$ retinas (D'-H') at E12 (D-E'), E15 (F-G') and P9 (H-H'). White arrows show Ikzf4^{+ve} cells with nuclear immunostaining. (I-J) Re-analysis of previously published single cell RNA-seq datasets from mouse (Clark et al. 2019) and human fetal (Lu et al. 2020) retinas. RPL: Retinal progenitor layer. ONL: Outer nuclear layer. INL: Inner nuclear layer. GCL: Ganglion cell layer. RPC: Retinal Progenitor Cell. HGW: Human Gestational Week. Scale bars: 10μm (A-H').
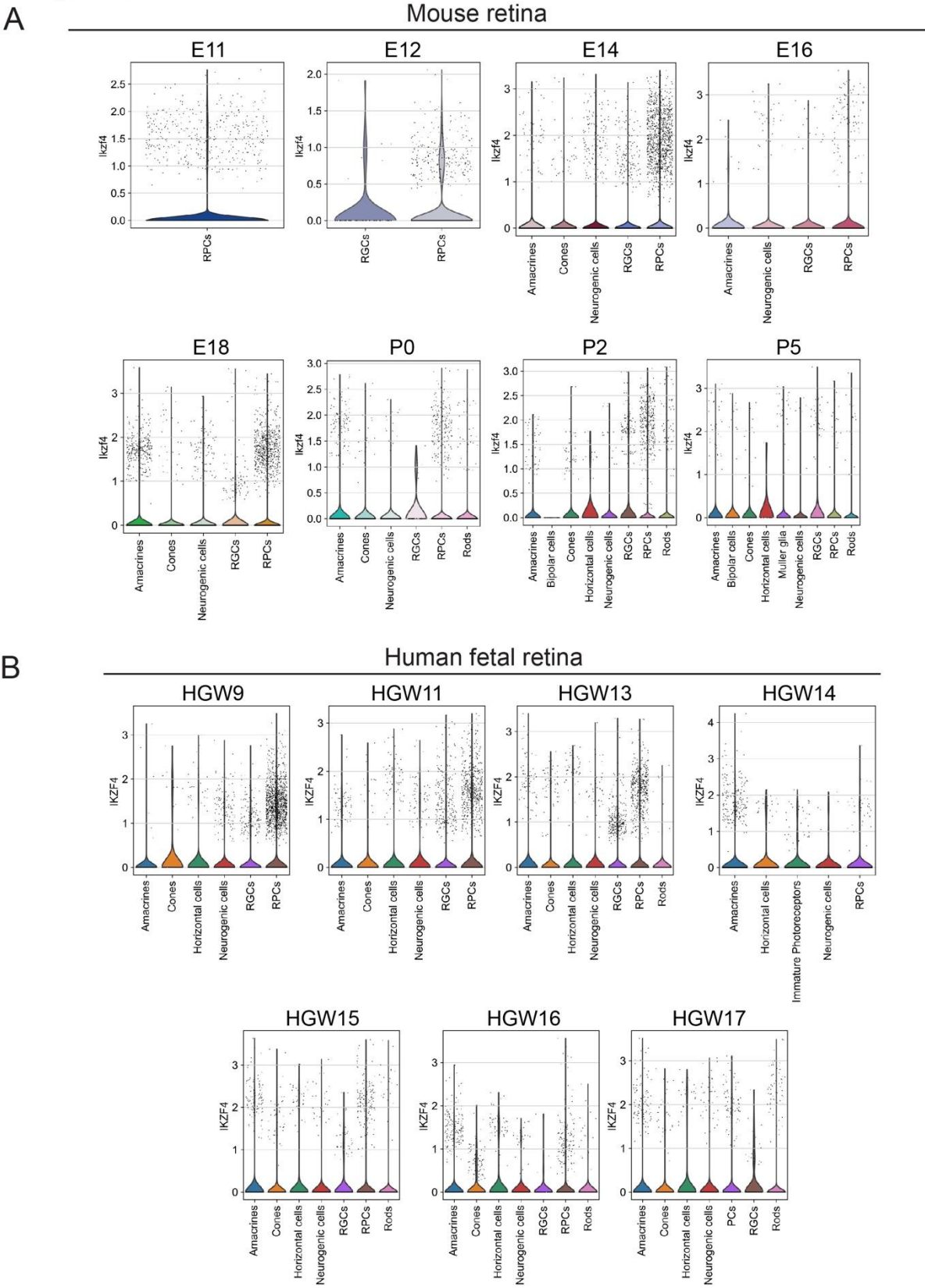
Figure S2, Javed et al.

A

Mouse retina

B

Human fetal retina

Fig. S2. *Ikzf4* mRNA is expressed in some differentiated cell types in mouse and human retina.

(A) Violin plot showing *Ikzf4* mRNA expression during mouse retinogenesis from previously published scRNA-seq dataset (Clark et al. 2019). (B) Violin plot showing *IKZF4* mRNA expression during early to late human fetal retinogenesis from previously published scRNA-seq dataset (Lu et al. 2020).
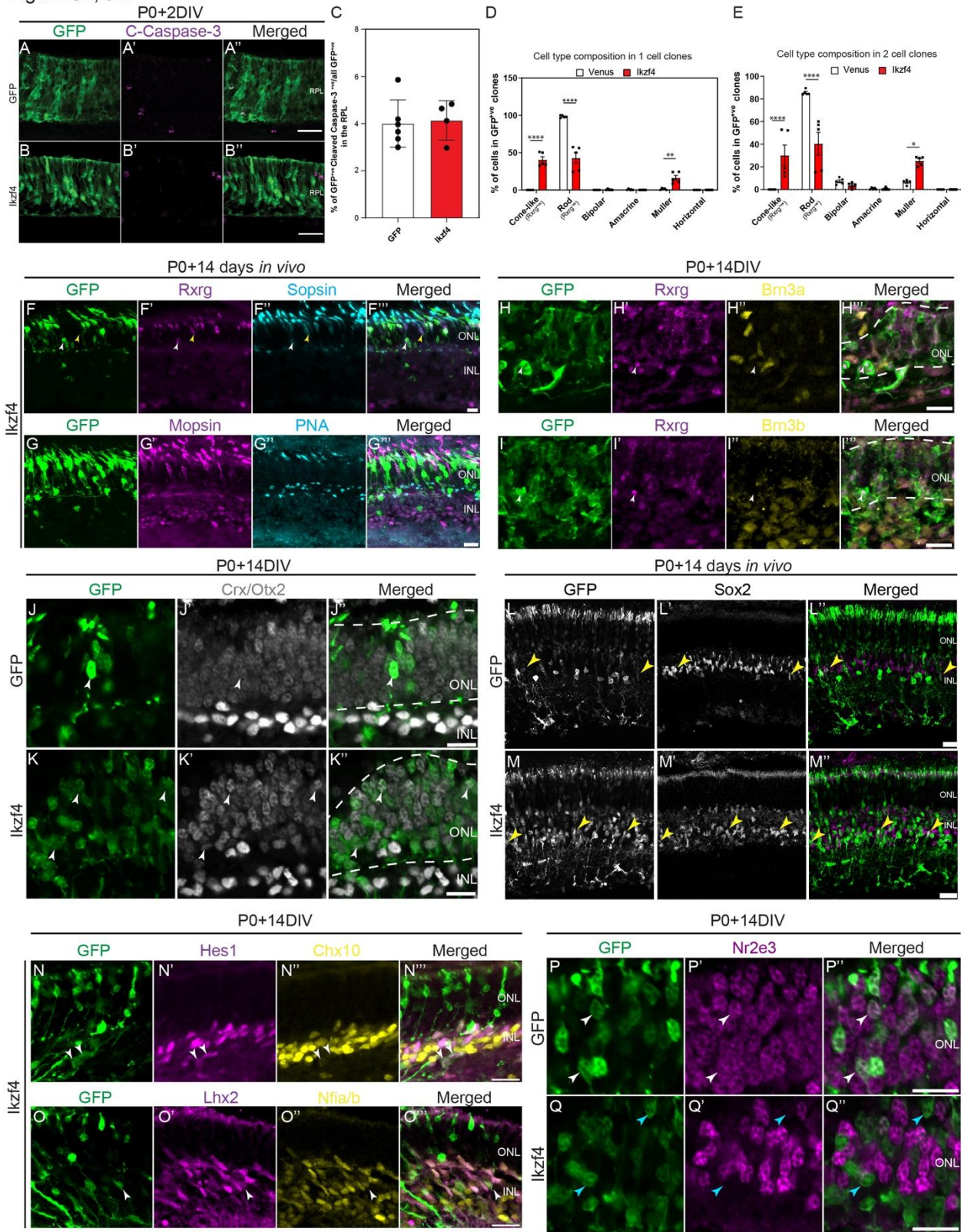
Figure S3, Javed et al.

Fig. S3. Ikzf4 promotes cones and Müller glia from late RPCs by inducing early cell cycle exit rather than apoptosis.

(A-B') Examples of retinal explants electroporated at P0 with either GFP (A-B) or Ikzf4-IRES-GFP (A'-B') and immunostained with Cleaved Caspase-3 (B-B') 2 days later. (C) Quantification of GFP^{+ve} cells expressing Cleaved-Caspase-3 shown in (A-B'). (D-E) Retroviral clonal analysis of Venus or Ikzf4 as shown in (Fig. 2A-G) focusing on cell type composition of 1 cell (D) or 2 cell (E) clones. (F-G''') Examples of *in-vivo* electroporations at P0 with Ikzf4-IRES-GFP (F-G''') and immunostained 14 days later with Rxrg (F'), S-opsin (F''), M-opsin (G') or PNA (G''). White arrows denote GFP^{+ve}Rxrg^{+ve}S-opsin^{-ve} cells whereas yellow arrow represent endogenous non-electroporated Rxrg^{+ve}S-opsin^{+ve} cones. (H-I''') Examples of Ikzf4-IRES-GFP (H-I''') electroporations of retinal explants at P0 and co-immunostained 14 days later with Rxrg (H'-I') with either Brn3a (H'') or Brn3b (I''). (J-K'') Examples of retinal explants electroporated at P0 with either GFP (J-J'') or Ikzf4-IRES-GFP (K-K'') and immunostained with Crx/Otx2 (J-K') after 14 days of culture. Dashed lines highlight the outer nuclear layer. (L-M'') Examples of *in-vivo* electroporation of P0 retinas with either GFP (L-L'') or Ikzf4-IRES-GFP (M-M'') and immunostained with Sox2 (L'-M') 14 days later. (N-O''') Examples of retinal explants electroporated at P0 with Ikzf4-IRES-GFP (N-O''') and immunostained with Hes1 (N'), Chx10 (N''), Lhx2 (O') or Nfia/b (O''). White arrows represent either Hes1^{+ve}Chx10^{-ve} cells (N-N''') or Lhx2^{+ve}Nfia/b^{+ve} cells (O-O'''). (P-Q') Examples of retinal explants electroporated at P0 with either GFP (P-P'') or Ikzf4-IRES-GFP (Q-Q'') and immunostained with Nr2e3 (P'-Q') after 14 days of culture. White arrows represent GFP^{+ve}Nr2e3^{+ve} cells whereas cyan arrows show GFP^{+ve}Nr2e3^{-ve} cells. *p<0.05, **p<0.01, ****p<0.0001. Statistics: Two tailed unpaired t-test (D-E). ONL: Outer nuclear layer. INL: Inner nuclear layer. RPL: Retinal progenitor layer. Scale bars: 10µm (A-B', F-K'', N-Q''), 20µm (L-M'').

Figure S4, Javed et al.
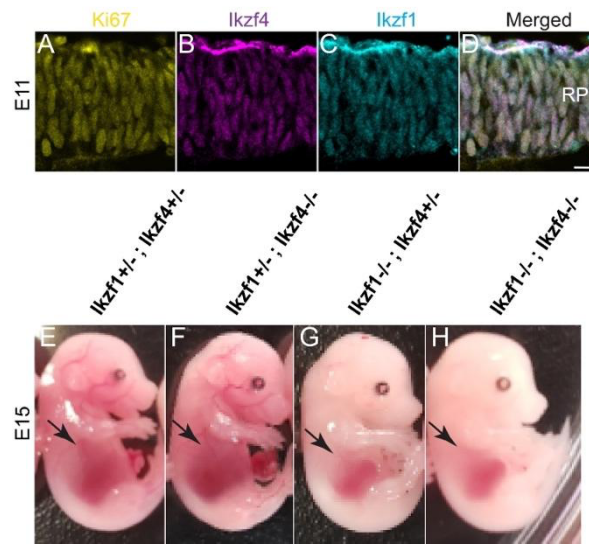
Fig. S4. Ikzf1 and Ikzf4 are expressed in the same cells during early retinogenesis and redundantly required for fetal liver size.

(A-D) Co-immunostaining of Ki67 (A), Ikzf4 (B), Ikzf1 (C) in E11 retinas. (E-H) Examples of E15 embryos with genotypes, Ikzf1$^{+/-}$;Ikzf4$^{+/-}$ (E), Ikzf1$^{+/-}$;Ikzf4$^{-/-}$ (F), Ikzf1$^{-/-}$;Ikzf4$^{+/-}$ (G) or Ikzf1$^{-/-}$;Ikzf4$^{-/-}$ (H). Black arrows denote the fetal liver. RPL: Retinal progenitor layer. Scale bars: 10µm.

Figure S5, Javed et al.

A

B

GO Biological process of upregulated genes in Ikzf1 RNA-seq

	GO_term_ID	GO_term_def	P	P_FDR_adj
1	GO:0007275	multicellular organism development	0.00E+00	1.00E-10
2	GO:0009966	regulation of signal transduction	0.00E+00	3.80E-09
3	GO:0048856	anatomical structure development	0.00E+00	1.00E-10
4	GO:0007399	nervous system development	0.00E+00	3.00E-10
5	GO:0048731	system development	0.00E+00	2.00E-10

Bhlhe22
Tfap2b
Nr4a2
Pax6

GO Biological process of downregulated genes in Ikzf1 RNA-seq

	GO_term_ID	GO_term_def	P	P_FDR_adj
1	GO:0007155	cell adhesion	0.00E+00	3.00E-10
2	GO:0007420	brain development	0.00E+00	2.20E-09
3	GO:0007154	cell communication	0.00E+00	2.00E-10
4	GO:0045664	regulation of neuron differentiation	0.00E+00	1.00E-10
5	GO:0032270	positive regulation of cellular protein metabolic process	0.00E+00	3.00E-10

Casz1
Hes1
Irx3
Rbpj
Sox2/9

C

CUT&RUN Feature Distribution

D

Ikzf1 CUT&RUN

Log p-value= -4.945e+00

E

F

Ikzf1 bound genes in Ikzf1 OE RNA-seq

G

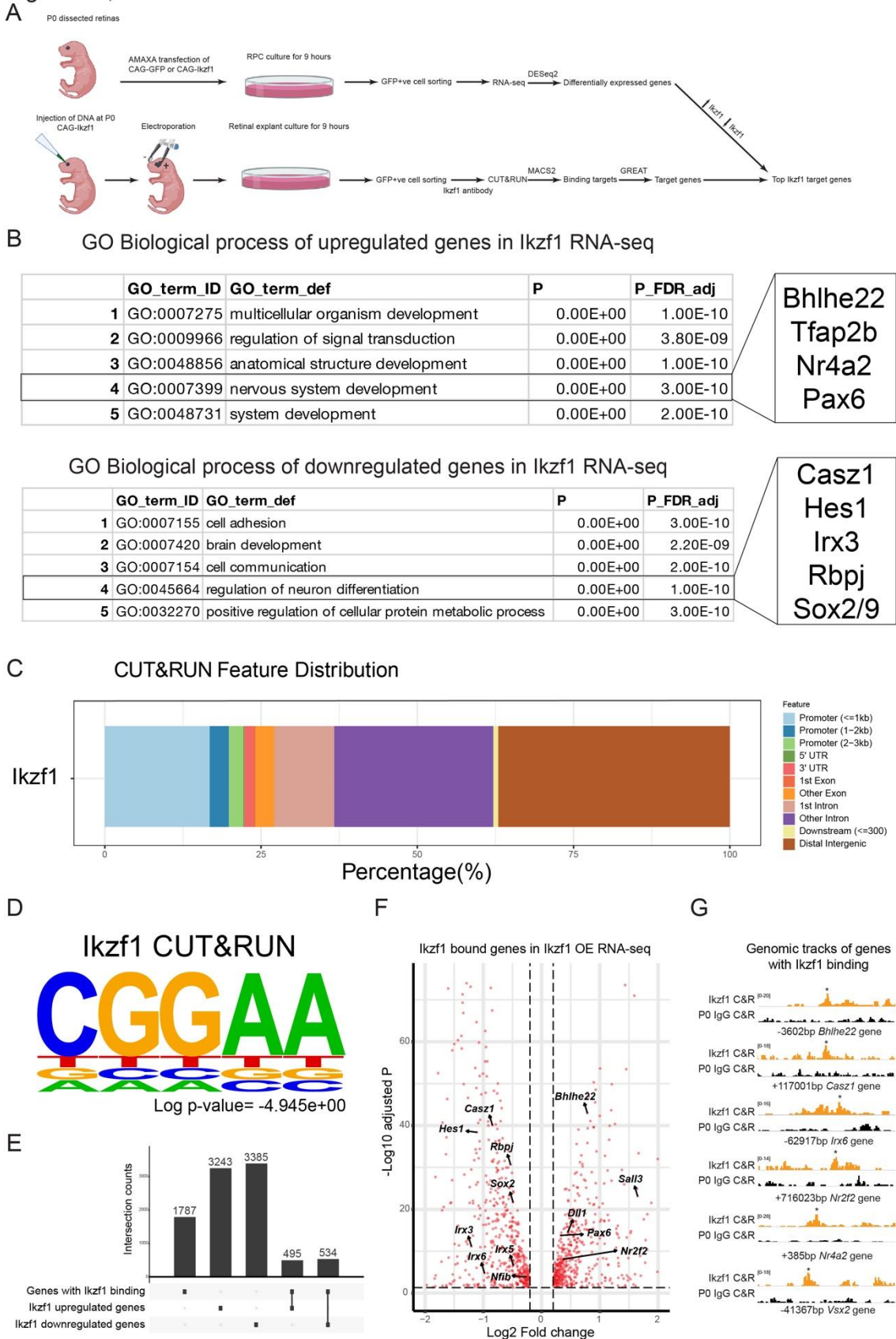Genomic tracks of genes with Ikzf1 binding

Fig. S5. Ikzf1 binds and upregulates early born cell type genes.

(A) Schematic showing the experimental design for Ikzf1 overexpression RNA-seq and CUT&RUN. (B) GONet analysis of top differentially regulated genes found in the RNA-seq dataset generated from Ikzf1 overexpressed RPCs. (C) ChIP-seeker genomic annotation of the Ikzf1 CUT&RUN peaks across the entire genome. (D) HOMER analysis showing canonical 'GGAA' motif enriched in Ikzf1 CUT&RUN peaks. (E) Upset plot representing the overlap between upregulated and downregulated genes from Ikzf1 overexpression RNA-seq and Ikzf1 CUT&RUN associated genes. (F) Volcano plot showing highlighted genes from (B) projected on the Ikzf1 RNA-seq data. (G) Genomic tracks of Ikzf1 CUT&RUN (orange) and P0 control IgG (black) on early and late genes found in (B). Asterisks indicate called peak.

Figure S6, Javed et al.
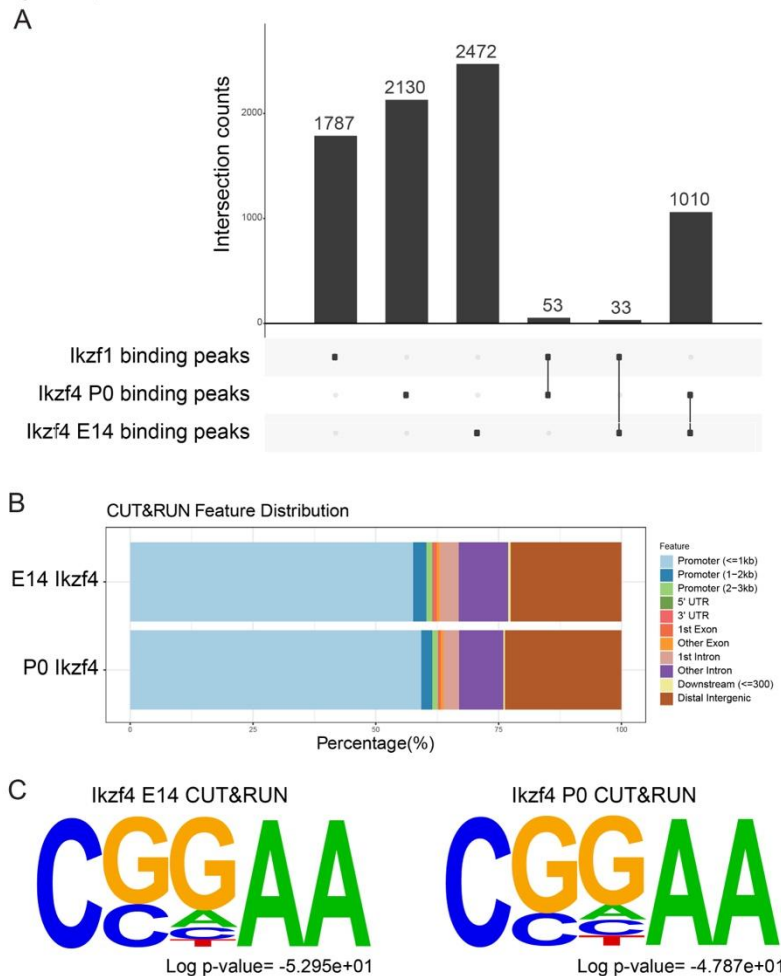
Fig. S6. Ikzf1 and Ikzf4 do not have a shared binding profile.

(A) Schematic showing the experimental design for Ikzf1 overexpression RNA-seq and CUT&RUN. (B) GONet analysis of top differentially regulated genes found in the RNA-seq dataset generated from Ikzf1 overexpressed RPCs. (C) ChIP-seeker genomic annotation of the Ikzf1 CUT&RUN peaks across the entire genome. (A) Upset plot representing the overlap between Ikzf1 CUT&RUN peaks and Ikzf4 CUT&RUN E14/P0 peaks. (B) ChIP-seeker genomic annotation of the Ikzf4 CUT&RUN E14/P0 peaks across the entire genome. (C) HOMER analysis showing canonical 'GGAA' motif enriched in Ikzf4 CUT&RUN E14/P0 peaks.
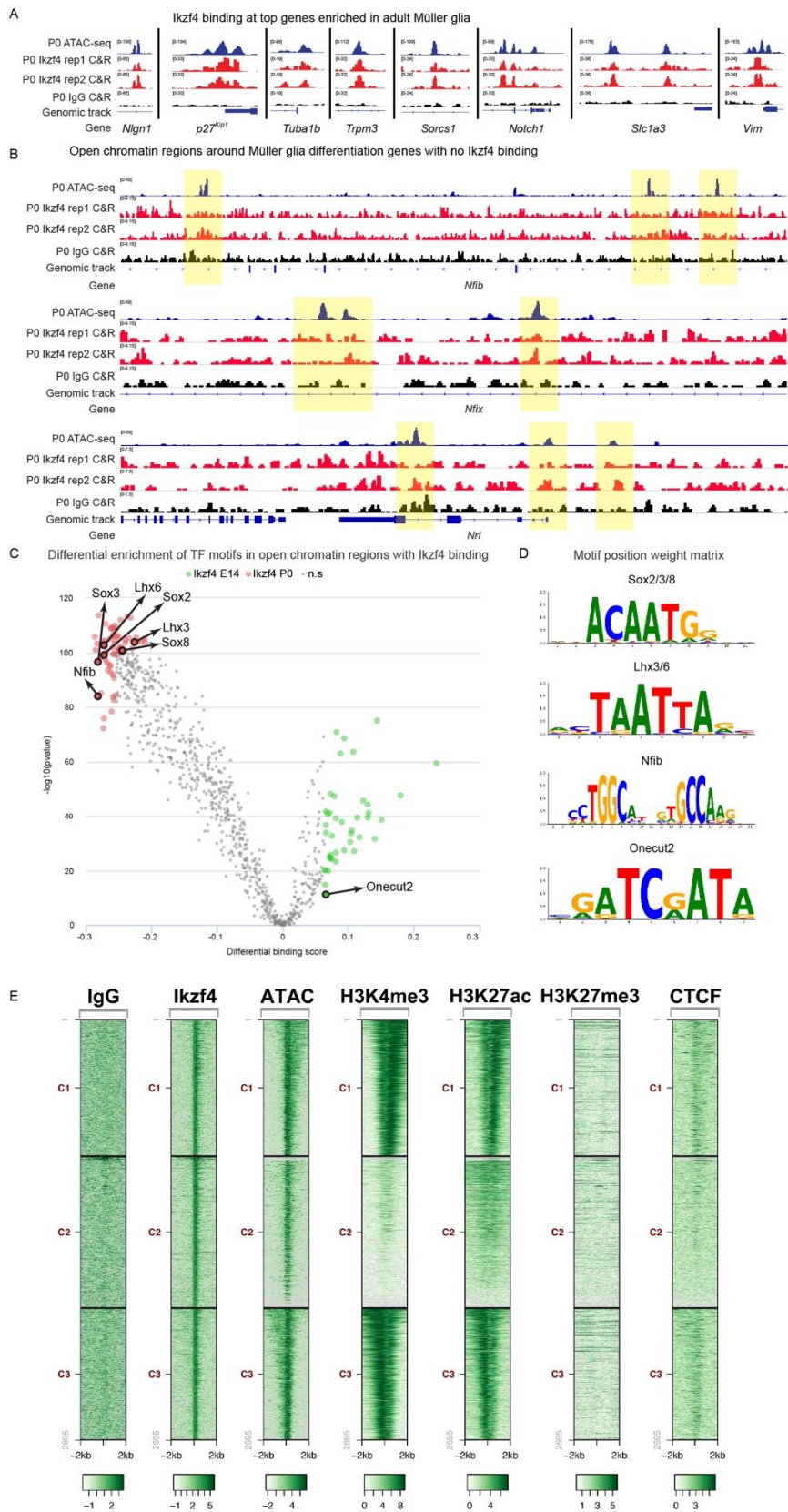
Figure S7, Javed et al.

A   Ikzf4 binding at top genes enriched in adult Müller glia

P0 ATAC-seq
P0 Ikzf4 rep1 C&R
P0 Ikzf4 rep2 C&R
P0 IgG C&R
Genomic track
Gene   *Nlgn1*   *p27^Kip1*   *Tuba1b*   *Trpm3*   *Sorcs1*   *Notch1*   *Slc1a3*   *Vim*

B   Open chromatin regions around Müller glia differentiation genes with no Ikzf4 binding

C Differential enrichment of TF motifs in open chromatin regions with Ikzf4 binding   **D** Motif position weight matrix

E

Fig. S7. Ikzf4 binds to cis regulatory regions important for Müller glia differentiation. (A) Genomic tracks of ATAC-seq at P0 in blue (Aldiri et al. 2017), two replicates of Ikzf4 CUT&RUN at P0 in red, or IgG Control CUT&RUN at P0 in black at genomic location of Müller glia genes enriched in scRNA-seq dataset of P14 mouse retinas (Clark et al. 2019). (B) Genomic tracks of ATAC-seq at P0 in blue (Aldiri et al. 2017), two replicates of Ikzf4 CUT&RUN at P0 in red, or IgG Control CUT&RUN at P0 in black at genomic location of *Nfib*, *Nfix* and *Nrl*. Yellow highlighted areas indicate regions with positive ATAC-seq signal but negative Ikzf4 CUT&RUN signal. (C) Volcano plot of TOBIAS BINDetect TF footprinting analysis on open chromatin regions bound by Ikzf4 at E14 and P0. Arrows indicate motif with TF name. (D) Representative motif position weight matrix for TFs listed in (C). (E) Seqplot heatmaps of P0 IgG C&R, P0 Ikzf4 C&R, ATAC-seq, H3K4me3, H3K27ac, H3K27me3 and CTCF ChIP-seq from (Aldiri et al. 2017). TF: Transcription factor.

Figure S8, Javed et al.
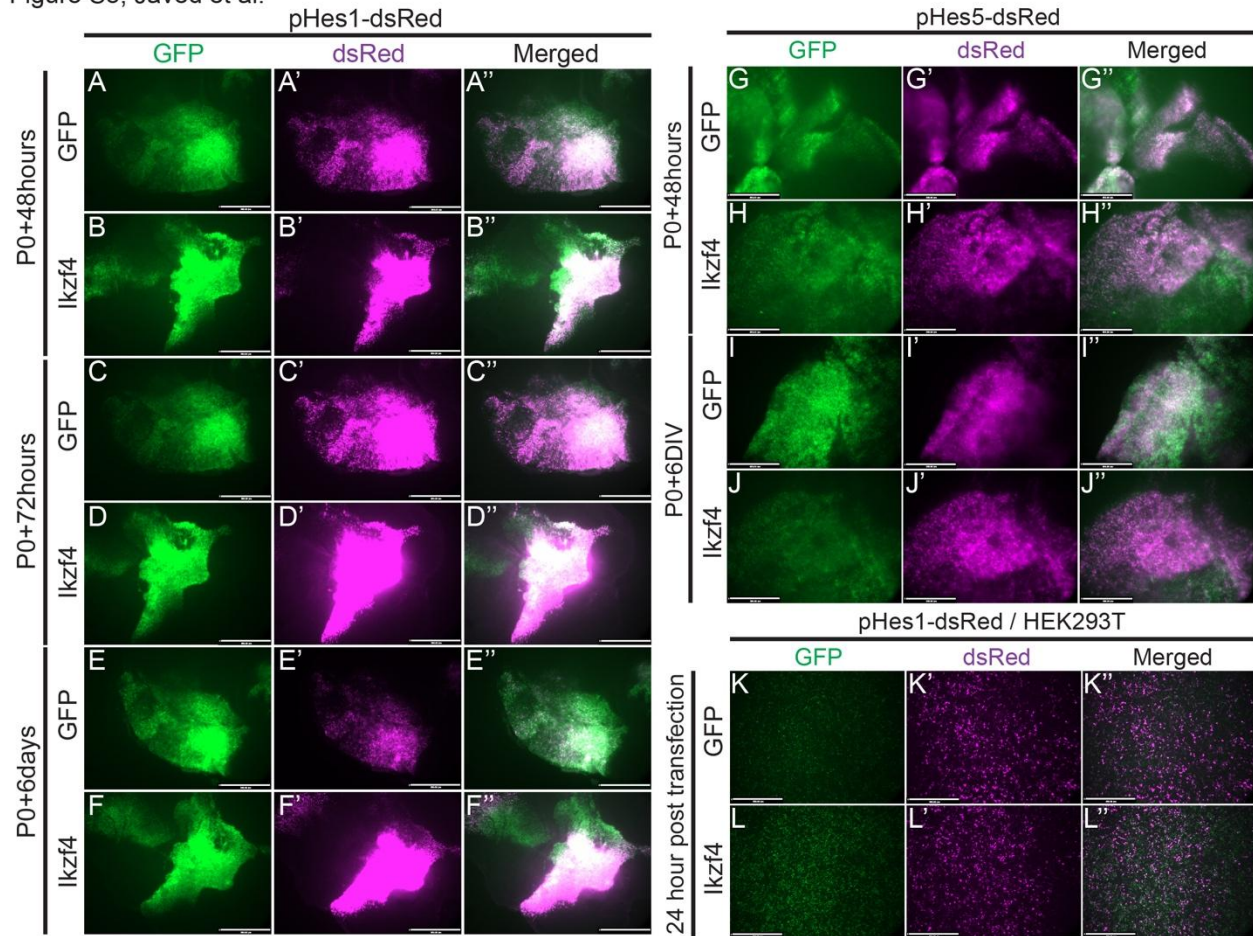
Fig. S8. Ikzf4 maintains Hes1 expression in post-mitotic cells during late retinogenesis.
(A-J'') Photomicrographs of retinal explants co-electroporated at P0 with Hes1-dsRed or Hes5-dsRed and either GFP (A-A'', C-C'', E-E'', G-G'', I-I'') or Ikzf4-IRES-GFP (B-B'', D-D'', F-F'', H-H'', J-J'') followed by imaging after either 48hours (A-B'', G-H''), 72hours (C-D''), or 6 days (E-F'', I-J''). (K-L'') Photomicrographs of HEK293T cells transfected with Hes1-dsRed and either GFP (K-K'') or Ikzf4-IRES-GFP (L-L'') imaged 24hours post-transfection. Scale bars: 360µm (A-L'').

Table S1. CSV file containing differentially expressed genes between P0+9hours RPC expressed CAG-GFP or CAG-Ikzf4-IRES-GFP.

Click here to download Table S1

Table S2. CSV file containing GONet analysis of Ikzf1 upregulated genes in P0+9hours RPCs.

Click here to download Table S2

Table S3. CSV file containing GONet analysis of Ikzf1 downregulated genes in P0+9hours RPCs.

Click here to download Table S3

Table S4. TXT file showing GREAT analysis of Ikzf1 P0+9hours CUT&RUN gene and peak location.

Click here to download Table S4

Table S5. CSV file containing differentially expressed genes between P0+9hours RPC expressed CAG-GFP or CAG-Ikzf4-IRES-GFP also associated with Ikzf1 binding.

Click here to download Table S5

Table S6. TXT file showing GREAT analysis of Ikzf4 E14 binding target gene and peak location.

Click here to download Table S6

Table S7. TXT file showing GREAT analysis of Ikzf4 P0 binding target gene and peak location.

Click here to download Table S7

Table S8. Excel spreadsheet containing the complete list of differential enriched motifs between Ikzf4 E14 and Ikzf4 P0 open chromatin regions using TOBIAS BINDetect.

Click here to download Table S8

Table S9: Sequences of primers

Click here to download Table S9

Table S10. Materials

Click here to download Table S10