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Supplementary Materials for

Robust reprogramming of glia into neurons by inhibition of Notch signaling and nuclear factor I (NFI) factors in adult mammalian retina

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

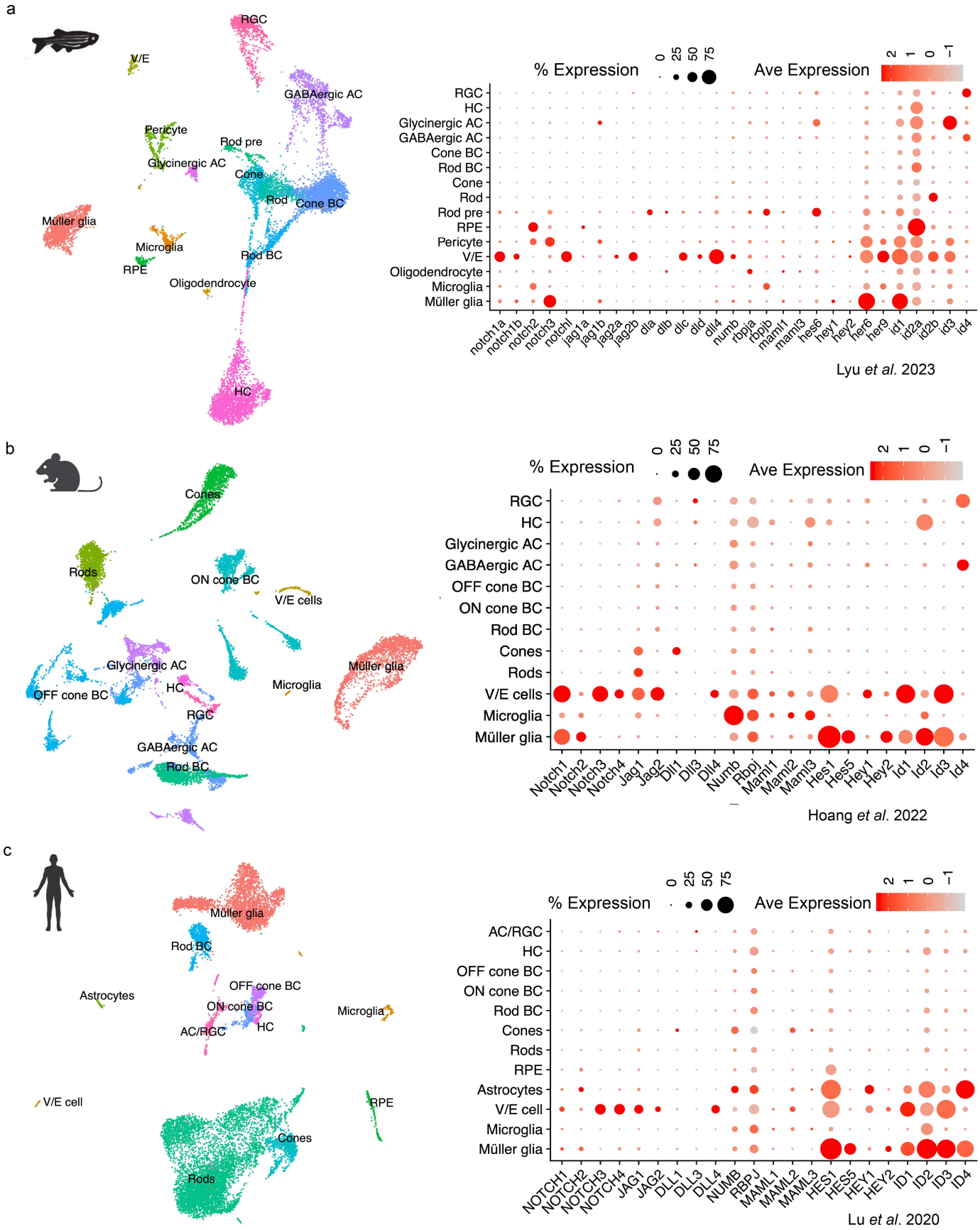

Figure S1: ScRNA expression of Notch signaling components in adult zebrafish, mouse and human retinas.
(a) UMAP plot of scRNA-Seq data from adult zebrafish retina from (23), and Dot Plot showing cellular expression levels of Notch pathway components in each major cell type. (b) UMAP plot of scRNA-Seq data from adult mouse retina from (22), and Dot Plot showing cellular expression levels of Notch pathway components in each major cell type. (c) UMAP of scRNA-Seq data from adult human retina from (21), and Dot Plot showing cellular expression levels of Notch pathway components in each major cell type.

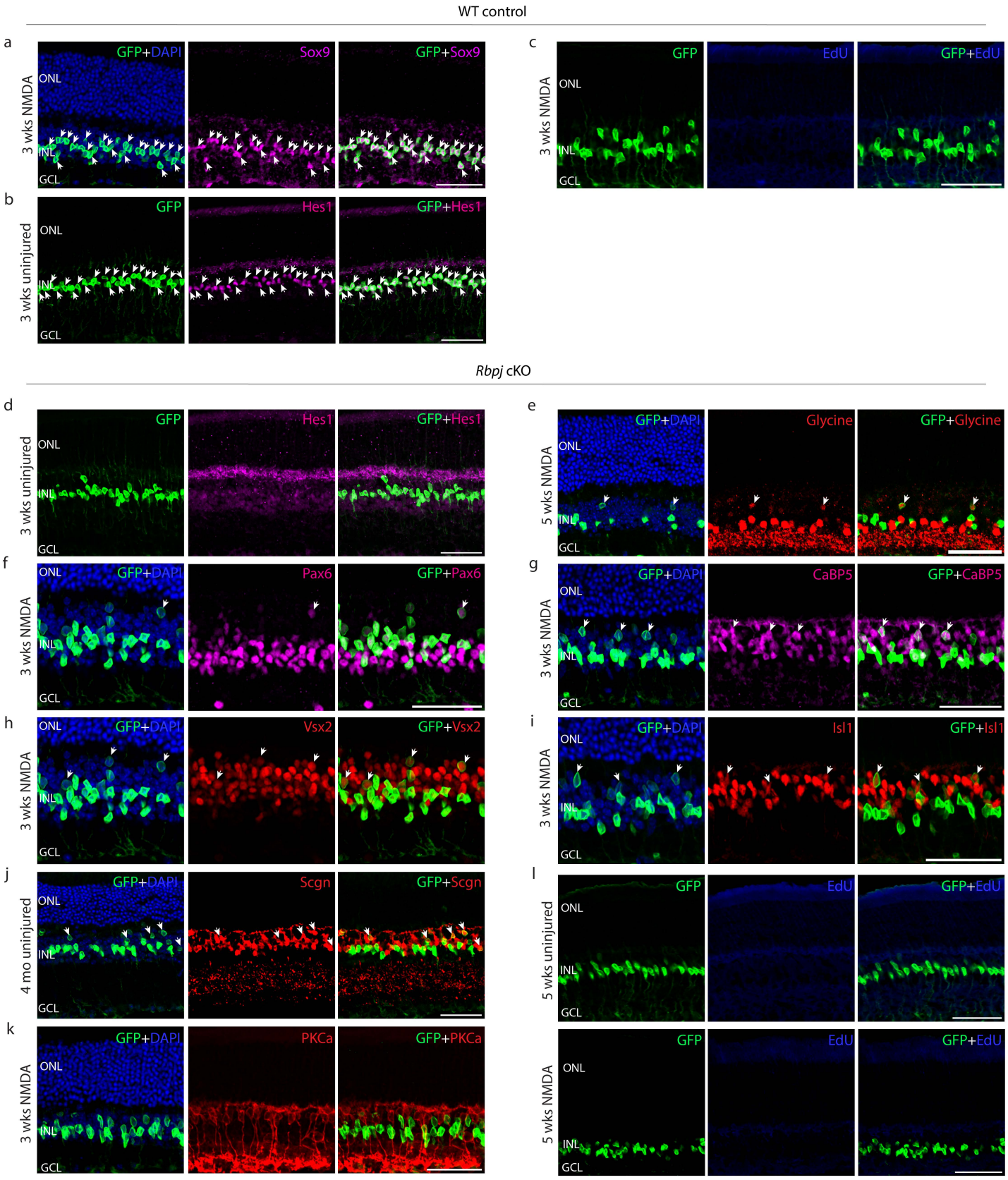

Figure S2: Immunohistochemical analysis of glial and neuronal markers and EdU incorporation in wildtype and *Rbpj*-deficient Müller glia.

(a-c) Representative images of control retina immunolabeled for GFP and (a) Sox9, (b) Hes1, (c) EdU. (d-h) Representative images of *Rbpj*-deficient retina immunolabeled for GFP and (d) Hes1, (e) Glycine, (f) Pax6, (g) CaBP5, (h) Vsx2, (i) Isl1, (j) Scgn, (k) PKCa, (l) EdU. White arrowheads indicate cells colabeled with both GFP and the relevant marker. ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. Scale bar = 50μm.

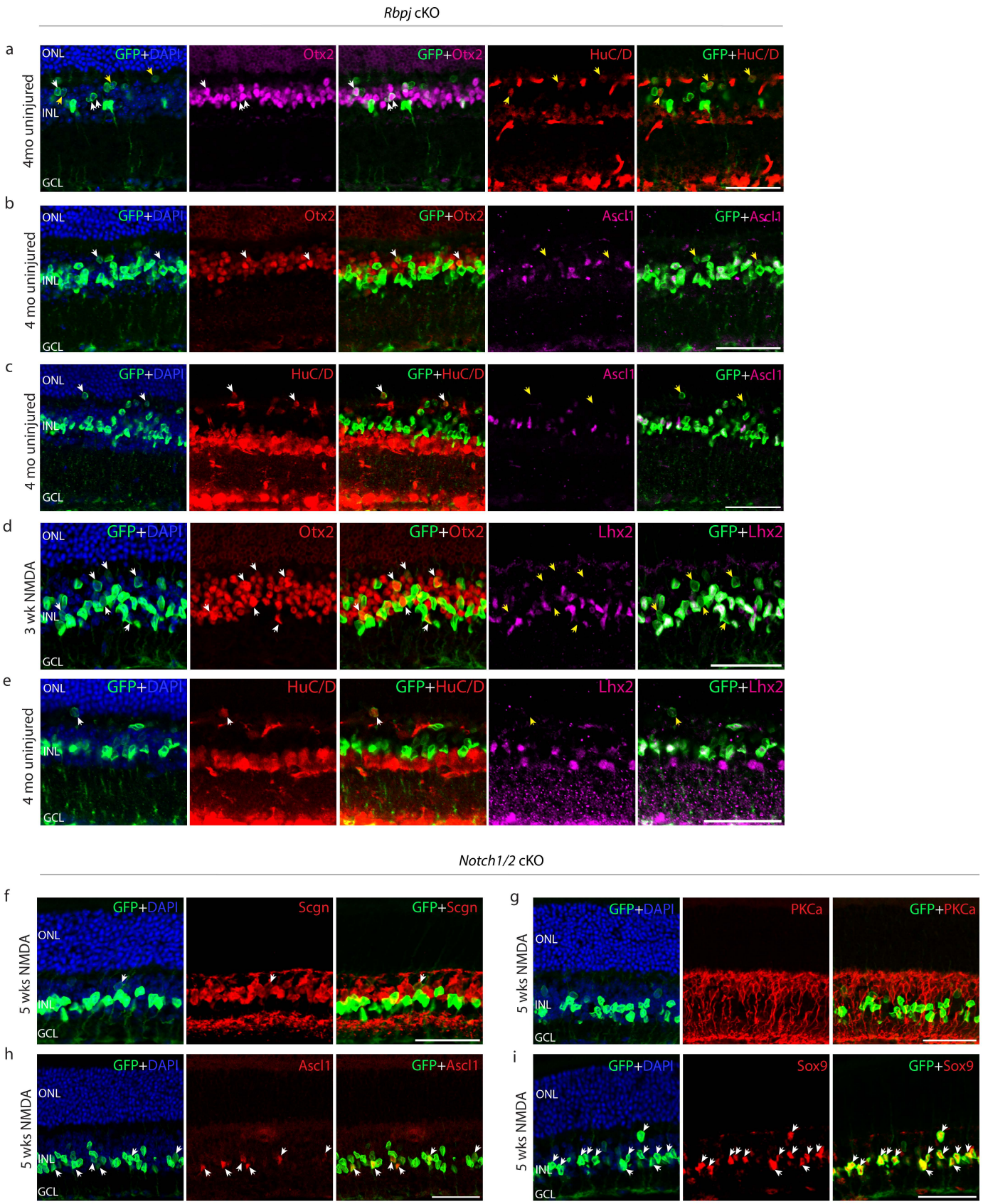

Figure S3: Immunohistochemical analysis of glial and neuronal markers following loss of function of *Rbpj* or *Notch1/2* in adult Müller glia. Representative images of Rbpj-deficient retinas co-immunolabeled for (a) GFP, Otx2, and HuC/D, (b) GFP, Otx2, and Ascl1, (c) GFP, HuC/D, and Ascl1, (d) GFP, Otx2, and Lhx2, (e) GFP, HuC/D, and Lhx2. Representative images of Notch1/2-deficient retina immunolabeled for GFP and (f) Scgn, (g) PKCa, (h) Ascl1, (i) Sox9. White arrowheads indicate cells colabeled with both GFP and the relevant marker. Yellow arrowheads indicate GFP+ cells that do not express the relevant marker. ONL, outer nuclear layer; INL, inner nuclear layer;

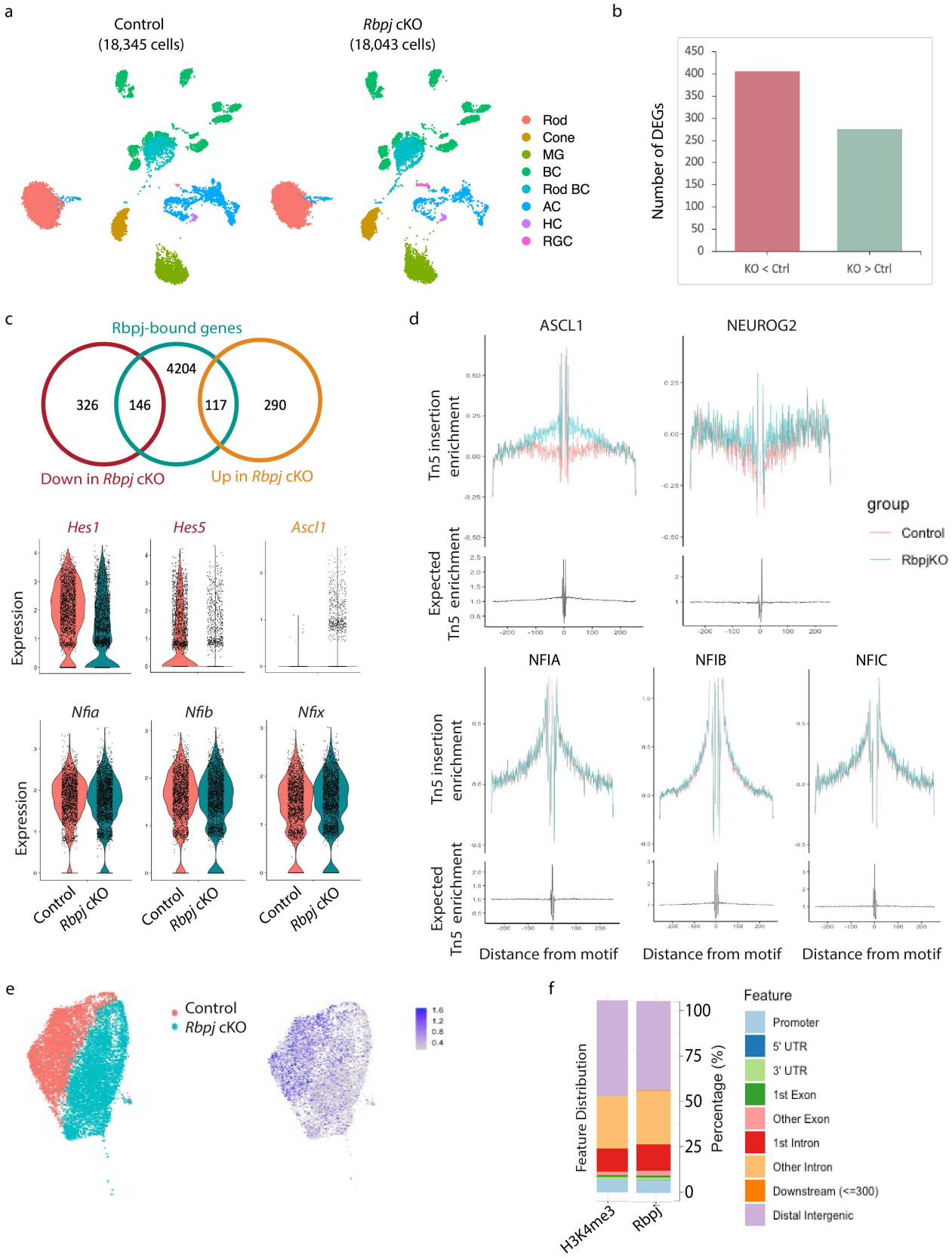

Figure S4: Integrated multiomic analysis to identify Rbpj target genes

(a) scRNA-Seq clustering of control and *Rbpj* cKO uninjured whole retinas at 1 week after tamoxifen injection. (b) Number of differentially expressed genes between control and *Rbpj*-deficient Müller glia. (c) Integrative analysis of CUT&Tag and scRNA-seq data to identify genes regulated by Rbpj. Violin plots showing gene expression levels of *Hes1/5*, *Ascl1*, *Nfia/b/x* in control and *Rbpj*-deficient MG. (d) TF footprint profiles for Ascl1, Neurog2, and Nfia/b/x. Tn5 insertion tracks are shown below. (e) Clustering of scATAC-seq data showing a reduction of Rbpj motif activity in the *Rbpj*-deficient Müller glia, as expected. (f) Distribution of Cut&Tag sequencing reads from H3K4me3 and Rbpj samples mapping to genomic features.

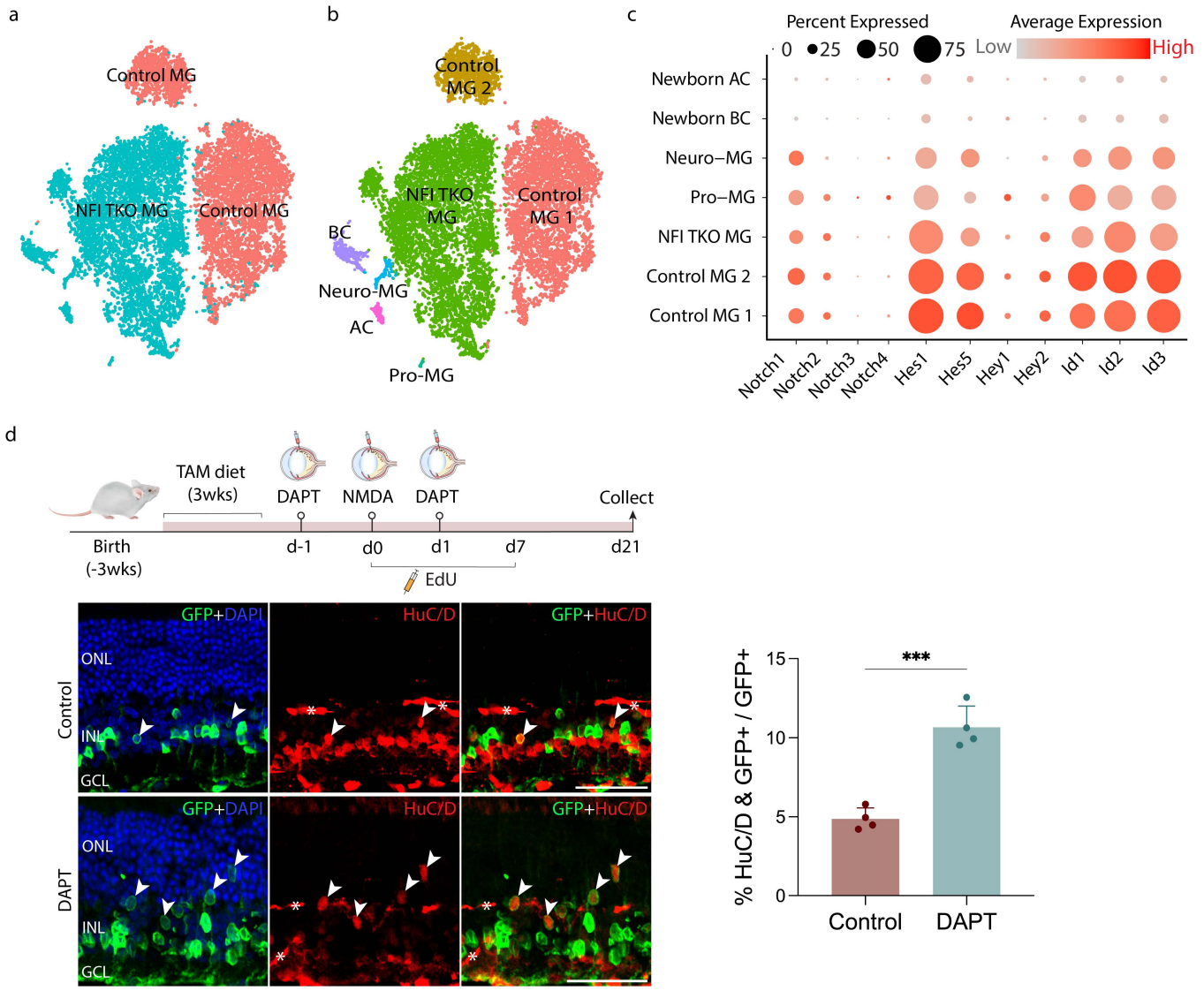

Figure S5: Notch signaling is retained in *Nfia/b/x*-deficient Müller glia, and DAPT-mediated Notch inhibition enhances reprogramming.

(a) Clustering of control and *Nfia/b/x*-deficient MG (b) Cell clusters determined by marker gene expression. (c) Dot plot showing gene expression and cell percentages for Notch pathway genes for different cell populations. (d) Representative IHC for GFP and HuC/D expression in control and DAPT-treated retinas. White arrowheads indicate colabeled GFP+/marker+ cell. *Asterisks indicate mouse-on-mouse vascular staining. Quantification of mean percentage ±SD HuC/D/GFP+ / GFP+ cells. Significance was determined via unpaired t test: ****$p < 0.0001$. n=4. ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. Scale bar = 50 µm.

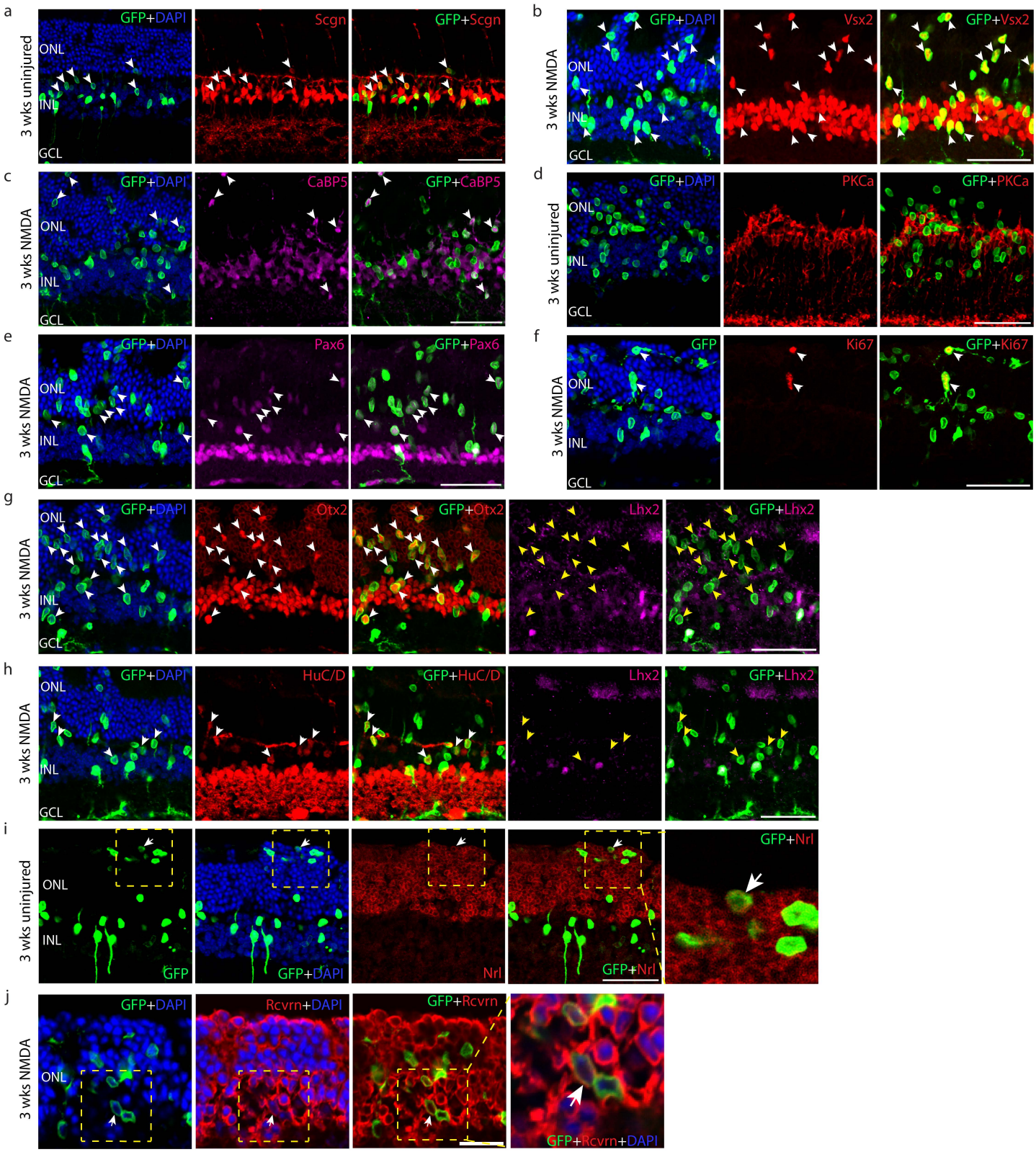

Figure S6: Immunohistochemical analysis of additional markers and EdU incorporation following loss of function of *Nfia/b/x* and *Rbpj* in adult Müller glia. Representative images of retinal sections immunolabeled for GFP and (a) Scgn, (b) Vsx2, (c) CaBP5 (d) PKCa, (e) Pax6, (f) Ki67, (g) Otx2 and Lhx2, (h) HuC/D and Lhx2, (i) Nrl, (j) Rcvrn. White arrowheads indicate cells colabeled with both GFP and the relevant marker. Yellow arrowheads indicate GFP+ cells that do not express the relevant marker. ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. Scale bar = 50μm (a-i) and 20μm (j).

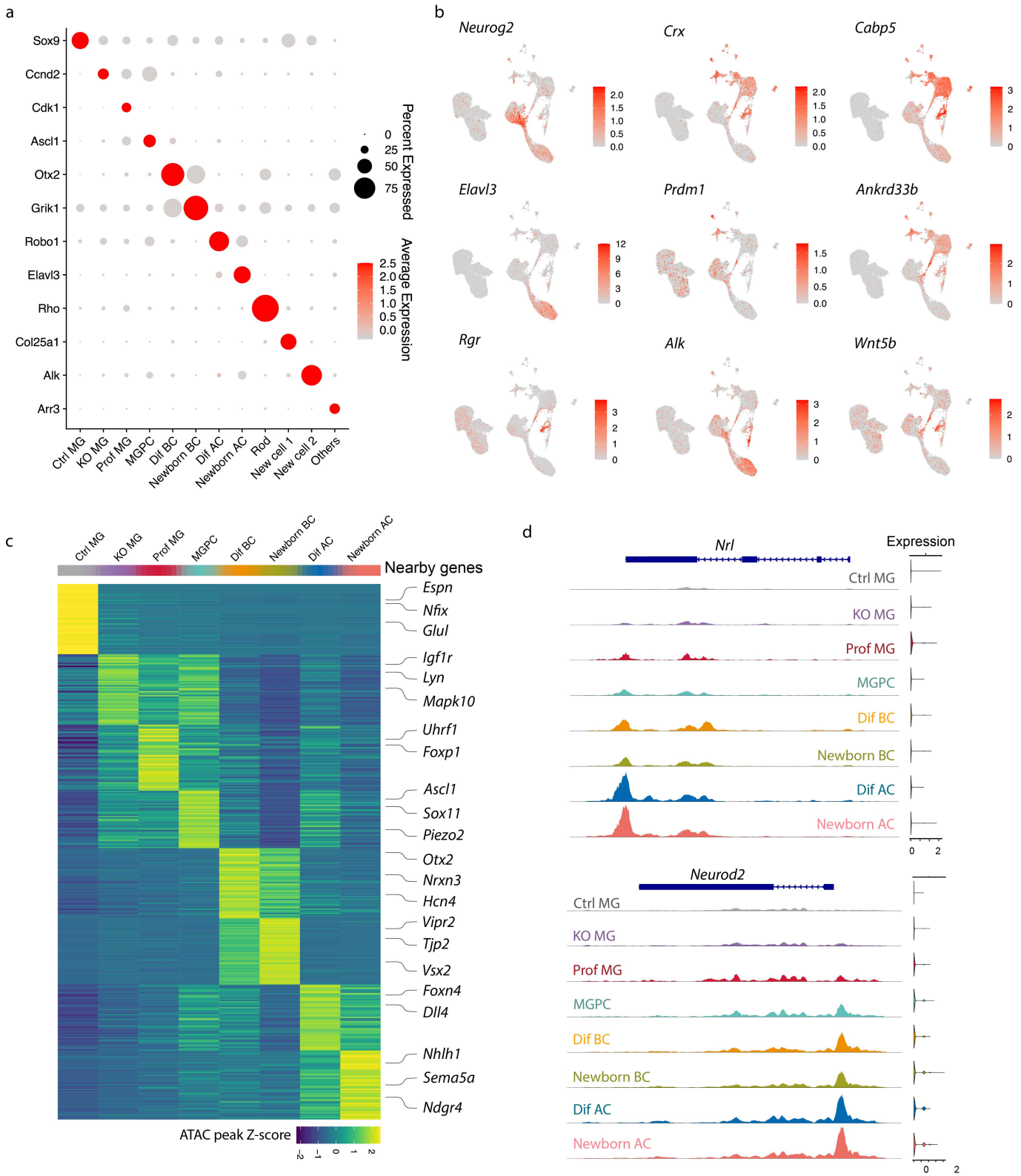

Figure S7: Additional analysis of multiomic data from wildtype control and *Nfia/b/x;Rbpj*-deficient retinas.
(a) Dot plot showing gene expression and cell percentages for Müller glia, neurogenic and neuronal marker genes for different cell populations. (b) Feature plots highlighting the cluster of MGPC (*Neurog2*), bipolar cell (*Cabp5*), amacrine cell (*Elavl3*), photoreceptors and their precursors (*Prdm1, Ankrd33b, Wnt5b, Arr3, Crx, Rgr*). (c) Heatmap of top 50 differential ATAC peaks for each cell population and selected nearby genes. (d) Representative ATAC peaks and RNA expression levels for *Nrl* and *Neurod2* across different cell clusters.

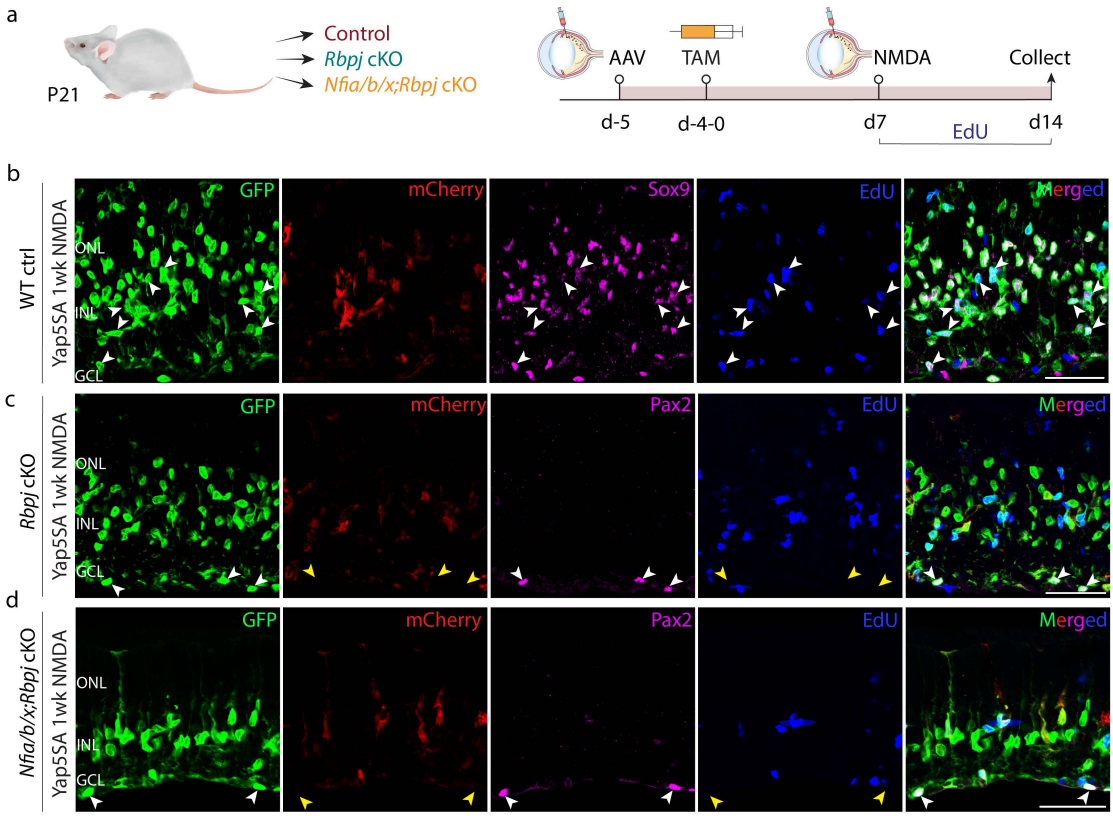

Figure S8: Immunohistochemical analysis of Müller glia and astrocyte markers following AAV-mediated overexpression of YAP5SA in wildtype, *Rbpj*- and *Nfia/b/x;Rbpj*-deficient retinas.
(a) A schematic of experimental pipeline. (b) Representative images of retinal sections immunolabeled for GFP, mCherry, Sox9 and EdU in wildtype control retinas. (c, d) Representative images of retinal sections immunolabeled for GFP, mCherry, astrocyte marker Pax2 and EdU in *Rbpj* cKO and *Nfia/b/x;Rbpj* cKO retinas. White arrowheads indicate co-labeled GFP/marker+ cells. Yellow arrowheads indicate GFP+ cells that do not co-express m Cherry and the relevant marker. ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. Scale bar = 50 μm.

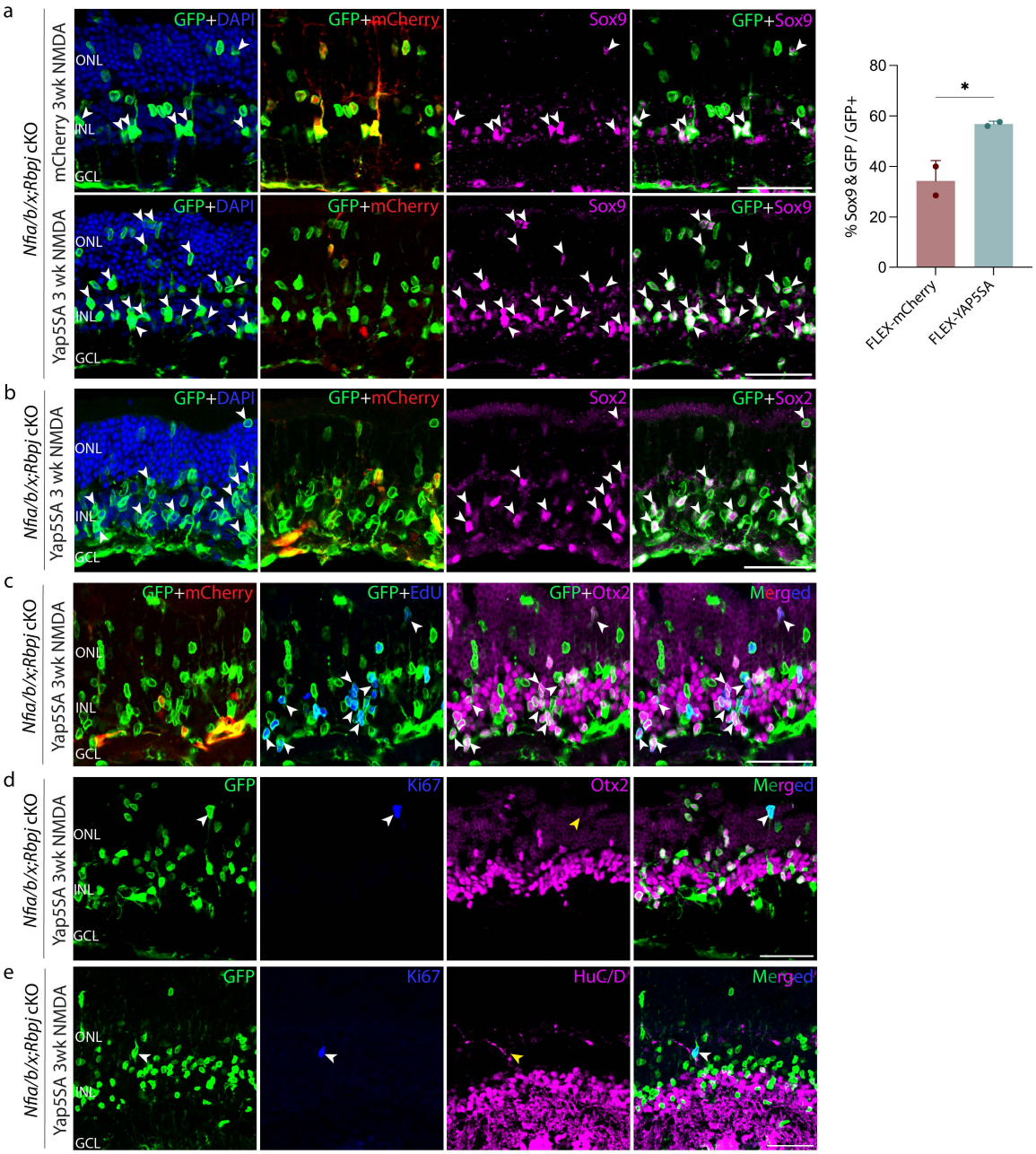

Figure S9: Immunohistochemical analysis of additional markers following AAV-mediated overexpression of YAP5SA in *Nfia/b/x;Rbpj*-deficient retinas.

(a) Representative images of retinal sections immunolabeled for GFP, mCherry and Sox9 in *Nfia/b/x;Rbpj* cKO retinas. Quantification of mean percentage ±SD Sox9/GFP+ cells. (b) Representative images of retinal sections immunolabeled for GFP, mCherry, and Sox2 in *Nfia/b/x;Rbpj* cKO retinas. (c) Representative images of retinal sections immunolabeled for GFP, mCherry, EdU, and Otx2 in *Nfia/b/x;Rbpj* cKO retinas. (d,e) Representative images of retinal sections immunolabeled for GFP, Ki67, and Otx2 or HuC/D in *Nfia/b/x;Rbpj* cKO retinas. White arrowheads indicate co-labeled GFP/marker+ cells. Yellow arrowheads indicate GFP+ cells that do not express the relevant marker. Significance was determined via unpaired t test: ****p < 0.0001. n=2. ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. Scale bar = 50 µm.

AAV-FLEX Constructs	Titre (gc/ml)	Injection volume (µl)
smCBA-DIO-mCherry	1.30E^13	2µl/eye
smCBA-DIO-Yap5SA-P2A-mCherry	4.20E^12	2.5µl/eye

Table S4: List of AAV constructs used in the study. Columns identify the AAV construct, titre, and injection volume.

KEY RESOURCE TABLE		
REAGENT or RESOURCE	**SOURCE**	**IDENTIFIER**
Antibodies		
Chicken anti-GFP	ThermoFisher	A10262, RRID:AB_2534023
Rabbit anti-RFP	Abcam	ab124754, RRID:AB_10971665
Goat anti-RFP	Rockland	200-101-379, RRID:AB_2744552
Goat anti-Otx2	R&D Systems	AF1979, RRID:AB_2157172
Mouse anti-HuC/D	ThermoFisher	A-21271, RRID:AB_221448
Mouse anti-NeuN	Sigma-Aldrich	MAB377, RRID:AB_2298772
Rabbit anti-Sox9	Sigma-Aldrich	AB5535, RRID:AB_2239761
Goat anti-Nrl	R&D Systems	AF2945, RRID:AB_2155098
Rabbit anti-Rcvrn	Millipore	AB5585, RRID:AB_2253622
Rabbit anti-Scgn	Biovendor Laboratory Medicine	RD181120100, RRID:AB_2034060
Mouse anti-PKCa	Santa Cruz	sc-8393, RRID:AB_628142
Rabbit anti-Ascl1	Abcam	ab211327, RRID:AB_2924270
Rabbit anti-CaBP5	SynapticSystems	475 002, RRID:AB_2924962
Rabbit anti-Pax2	BioLegend	901002, RRID:AB_2734656
Goat anti-Sox2	R&D Systems	AF2018, RRID:AB_355110
Rabbit anti-Lhx2	ThermoFisher	PA5-78287, RRID:AB_2736088
Rabbit anti-Pax6	BioLegend	901301, RRID:AB_2749901
Mouse anti-Isl1	DSHB	40.2D6, RRID:AB_528315
Sheep anti-Vsx2	Exalpha	X1180P, RRID:AB_2314191
Rabbit anti-Hes1	Cell Signaling Technology	11988, RRID:AB_2728766
Rat anti-Glycine	Immunosolution	IG1002, RRID:AB_10013222
Rabbit anti-Ki67	ThermoFisher	MA5-14520, RRID:AB_10979488
Rabbit anti-Rbpj	Abcam	ab25949, RRID:AB_778155
Rabbit anti-H3K27me3	EpiCypher	13-0055t
Rabbit anti-IgG	EpiCypher	13-0042t
Donkey anti-Rabbit 405	Abcam	ab175651, RRID:AB_2923541
Donkey anti-Chicken 488	Sigma-Aldrich	SAB4600031, RRID:AB_2721061
Donkey anti-Rabbit 568	ThermoFisher	A-10042, RRID:AB_2757564
Donkey anti-Goat 568	ThermoFisher	A11057, RRID:AB_2534104
Donkey anti-Mouse 568	ThermoFisher	A10037, RRID:AB_2534013
Donkey anti-Rat 568	ThermoFisher	A78946, RRID:AB_2910653
Donkey anti-Goat 633	ThermoFisher	A-21082, RRID:AB_2535739
Donkey anti-Rabbit 647	ThermoFisher	A-31573, RRID:AB_2536183
Donkey anti-Mouse 647	ThermoFisher	A-31571, RRID:AB_162542
Critical commercial assays		
10x scRNaseq 3′ v3.1	10X Genomics	1000268
10x scATACseq v1.1	10X Genomics	1000175
10x Multiome ATAC + GEX	10X Genomics	1000283
Click-iT EdU Alexa Fluor 647	ThermoFisher	C10340
Click-iT EdU Pacific Blue	ThermoFisher	C10418
CUTANA Cut&Tag kit	Epicypher	SKU: 14-1101

Table S5: List of antibodies used in the study. Columns identify the antibody, source, catalog number, and RRID identifier.

Table S1: List of genes, chromatin regions, and transcription factor motifs that are differentially regulated in *Rbpj*-deficient Müller glia+MGPCs (scRNA-Seq and scATAC-Seq).

Table S2: List of genes and genomic loci directly targeted by RBPJ in Müller glia.

Table S3: List of genes, chromatin regions, and transcription factor motifs that are differentially regulated in wildtype control, *Nfia/b/x+Rbpj*-deficient Müller glia and their progenies.