The m⁶A reader YTHDF2 is a negative regulator for dendrite development and maintenance of retinal ganglion cells

Short title: YTHDF2 regulates dendrite development and maintenance of RGC

Fugui Niu^{1,2}, Peng Han¹, Jian Zhang¹, Yuanchu She¹, Lixin Yang¹, Jun Yu¹, Mengru Zhuang¹, Kezhen Tang³, Yuwei Shi¹, Baisheng Yang¹, Chunqiao Liu⁴, Bo Peng^{5,6*}, Sheng-Jian Ji^{1*}

¹School of Life Sciences, Department of Biology, Shenzhen Key Laboratory of Gene Regulation and Systems Biology, Brain Research Center, Southern University of Science and Technology, Shenzhen, Guangdong 518055, China.

²SUSTech-HIT Joint Graduate Program, Southern University of Science and Technology, Shenzhen, Guangdong 518055, China.

³Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, Guangdong 518055, China

⁴State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou 510060, China

⁵Department of Neurosurgery, Huashan Hospital, and Institute for Translational Brain Research, State Key Laboratory of Medical Neurobiology, MOE Frontier Center for Brain Science, Fudan University, Shanghai 200032, China.

⁶Co-innovation Center of Neuroregeneration, Nantong University, Nantong, Jiangsu 226001, China.

*For correspondence: peng@fudan.edu.cn (B.P.), jisi@SUSTech.edu.cn (S.-J.J.)

Abstract

The precise control of growth and maintenance of the retinal ganglion cell (RGC) dendrite arborization is critical for normal visual functions in mammals. However, the underlying mechanisms remain elusive. Here we find that the m⁶A reader YTHDF2 is highly expressed in the mouse RGCs. Conditional knockout (cKO) of *Ythdf2* in the retina leads to increased RGC dendrite branching, resulting in more synapses in the inner plexiform layer. Interestingly, the *Ythdf2* cKO mice show improved visual acuity compared with control mice. We further demonstrate that *Ythdf2* cKO in the retina protects RGCs from dendrite degeneration caused by the experimental acute glaucoma model. We identify the m⁶A-modified YTHDF2 target transcripts which mediate these effects. This study reveals mechanisms by which YTHDF2 restricts RGC dendrite development and maintenance. YTHDF2 and its target mRNAs might be valuable in developing new treatment approaches for glaucomatous eyes.

Impact statement

The m⁶A reader YTHDF2 negatively regulates RGC dendrite branching through destabilizing its m⁶A-modified target mRNAs encoding proteins controlling dendrite development and maintenance. *Ythdf2* cKO improves visual acuity and alleviates acute ocular hypertension-induced glaucoma in mice.

Introduction

The mammalian retina is an ideal model system to study neuronal development and neural circuit formation. The retinal ganglion cells (RGCs) are the final and only output neurons in the vertebrate retina and their dendrites collect the electrical information concerning the visual signal from all other cells preceding them. One of the major focuses of research in the retina is to understand how RGC dendrite arborization arises during development (**Prigge and Kay 2018**). Existing evidences supported that homotypic repulsion controls retinal dendrite patterning (**Lefebvre et al. 2015**). However, in mice which had most RGCs genetically eliminated, the dendrite size and shape of remaining RGCs appeared relatively normal (**Lin et al. 2004**). Thus, the fact that the dendrites of remaining RGC did not expand to neighboring areas by the remaining RGCs supports the existence of the intrinsic limit for RGC dendrite patterning, which cooperates with the homotypic repulsion to determine the dendrite size of RGCs (**Lefebvre et al. 2015**). However, such intrinsic limiting mechanisms remain elusive.

Glaucoma is one of the leading causes for blindness. The major risk factors for glaucoma include increased intraocular tension. Studies have shown that glaucoma causes pathological changes in RGC dendrites before axon degeneration and soma loss were detected in different model animals (**Weber et al. 1998; Shou et al. 2003; Morgan et al. 2006**). Thus, elucidation of mechanisms governing RGC dendrite arbor maintenance bears clinical significance.

N^6 -methyladenosine (m^6A) is the most widely distributed and extensively studied internal modification in mRNA (**Dominissini et al. 2012; Meyer et al. 2012; Nachtergaele and He 2018**). m^6A modification has been shown to regulate brain development and functions in the nervous system (**Livneh et al. 2020; Yu et al. 2021**). By effectors, most of these studies have focused on its demethylases (“ m^6A erasers”) and methyltransferases (“ m^6A writers”). Since the fate of m^6A -modified transcripts are decoded by the m^6A binding proteins (“ m^6A readers”), how the readers mediate these functions and what are their neural target mRNAs remain to be elucidated. In addition,

more precisely controlled spatial-temporal ablation of the m⁶A readers instead of null knockout is required to elucidate their functions and mechanisms in nervous system.

In this study, we identified an m⁶A-dependent intrinsic limiting mechanism for RGC dendrite arborization and maintenance. Conditional knockout of the m⁶A reader YTHDF2 in the developing mouse retina increases RGC dendrite branching and improves visual acuity. YTHDF2 also mediates acute ocular hypertension (AOH)-induced RGC degeneration, the experiment model for glaucoma, and *Ythdf2* cKO in the retina alleviates AOH-induced RGC dendrite shrinking and neuronal loss. The regulation of RGC dendrite development and maintenance by YTHDF2 is mediated by two distinct groups of m⁶A-modified target mRNAs which encode proteins that promote dendrite arborization during development and maintain dendrite tree during injury, respectively. Therefore, our study reveals mechanisms by which YTHDF2 restricts RGC dendrite development and maintenance, which sheds light on developing new treatment approaches for glaucomatous eyes.

Results

Knockdown of YTHDF2 leads to a robust increase of RGC dendrite branching

To examine whether m⁶A modification and its reader proteins play a role in the dendrite development, we utilized the retina as the model system. We first checked their expression patterns in the developing mouse retina. Immunostaining with a widely used m⁶A antibody demonstrated that RGCs had high m⁶A modification levels (*Figure 1—figure supplement 1A*). Consistent with the m⁶A distribution, the m⁶A reader YTHDF2 is highly expressed in RGCs (*Figure 1A; Figure 1—figure supplement 1A*). Conversely, the expression of YTHDF2 in other layers of the retina is much lower (*Figure 1A; Figure 1—figure supplement 1B*). Another two m⁶A readers YTHDF1 and YTHDF3 show similar expression patterns (*Figure 1—figure supplement 1C,D*). The strong expression of YTHDFs and high level of m⁶A modification in RGCs suggest that the m⁶A reader YTHDFs might play roles in RGC development. We dissected and dissociated the retinal cells and cultured in vitro. We generated lenti viral shRNAs against YTHDFs, which showed similarly efficient knockdown (KD) of YTHDFs in RGC

cultures in vitro (*Figure 1B; Figure 1—figure supplement 1E,F*). In these YTHDF-deficient RGC cultures, the first and most obvious phenotype that we observed is the robust increase of dendrite branching of cultured RGCs treated by *shYthdf2* (*Figure 1C,D; Figure 1—figure supplement 1G,H*). In contrast, the dendrite branching of RGCs with YTHDF1 KD using *shYthdf1* was not significantly different from control shRNA (*Figure 1—figure supplement 1I*), while YTHDF3 KD using *shYthdf3* caused a slight (statistically significant in several Sholl radii) decrease of RGC dendrite branching compared with control shRNA (*Figure 1—figure supplement 1J*). These results suggest that the m⁶A reader YTHDF2 might play an important role in controlling dendrite branching of RGCs.

Conditional knockout of *Ythdf2* in the retina increases RGC dendrite branching in vivo without disturbing sublamina targeting

To further explore whether YTHDF2 physiologically regulates RGC dendrite branching in vivo, we generated *Ythdf2* conditional knockout (*Ythdf2* cKO) mouse (*Figure 2A*). We used the *Six3-cre* mouse line (*Furuta et al. 2000*), which has been widely used in the field to generate retina-specific knockouts (*Lefebvre et al. 2012; Riccomagno et al. 2014; Sapkota et al. 2014; Krishnaswamy et al. 2015*). YTHDF2 expression is efficiently eliminated in the *Ythdf2* cKO retina compared with their littermate controls at E12.5 (*Figure 2—figure supplement 1A*) and E15.5 (*Figure 2B*). Retina progenitors, amacrine cells, bipolar cells, photoreceptors, or horizontal cells were not affected in *Ythdf2* cKO retina (*Figure 2—figure supplement 1B-L*), suggesting that YTHDF2 is not involved in the generation or development of these cells. This is in line with the low YTHDF2 expression in these cells. The RGC number or density was not affected in the *Ythdf2* cKO retina (*Figure 2C,D*), demonstrating that *Ythdf2* knockout does not disturb RGC neurogenesis. We then cultured RGCs from the *Ythdf2* cKO retina. The dendrite branching of *Ythdf2* cKO RGCs was significantly increased compared with littermate controls (*Figure 2E,F*). RGCs include over 40 subtypes (*Sanes and Masland 2015; Baden et al. 2016*). We thus examined the RGC dendrite branching within different subtypes. One of the RGC subgroups responds preferentially to movement in particular directions and is named the ON-OFF

directionally selective RGCs (ooDSGCs). Expression of CART (cocaine- and amphetamine-regulated transcript), a neuropeptide, distinguishes ooDSGCs from other RGCs (**Kay et al. 2011**). The dendrite branching of ooDSGCs marked by CART/Brn3a co-staining in *Ythdf2* cKO retinal cultures also increased compared with control (*Figure 2G,H*). These data further confirm that the m⁶A reader YTHDF2 regulates dendrite branching of RGCs.

Next, we wanted to confirm this phenotype in vivo by checking specific RGC subtypes. Intravitreal injection of an AAV reporter expressing ZsGreen visualized the dendrite morphology of ooDSGCs marked by CART immunostaining (*Figure 3A*). ooDSGCs showed dramatically increased dendrite branching in *Ythdf2* cKO retina compared with control retina by Sholl analysis (*Figure 3A,B*). The intrinsically photosensitive RGCs (ipRGCs) are unique and melanopsin-expressing cells, which exhibit an intrinsic sensitivity to light (**Hattar et al. 2002**). We analyzed the morphology of ipRGCs visualized by wholemount immunostaining of melanopsin and found that the dendrite branching of ipRGCs was significantly increased in the *Ythdf2* cKO retina (*Figure 3C,D; Figure 3—figure supplement 1A-E*). A similar trend was observed in the SMI-32⁺ α RGCs (*Figure 3E,F*). These results strongly indicate that the m⁶A reader YTHDF2 negatively regulates RGC dendrite branching in vivo and *Ythdf2* cKO promotes RGC dendrite arborization.

In the retina, RGCs target their dendrites in different sublaminae of the inner plexiform layer (IPL). Since the IPL sublamina targeting of RGC dendrites is critical for normal visual functions, we wondered whether the increased dendrite branching caused by *Ythdf2* cKO was also accompanied by altered sublamina patterning of RGC dendrites. We used a *Thy1-GFP* reporter (line O) which labels a few RGCs (**Feng et al. 2000**). As shown in *Figure 3—figure supplement 1F,G*, GFP intensity is generally higher in IPL of the *Ythdf2* cKO retina compared with their littermate controls, which further proves the increased RGC dendrite branching and density. However, the sublamina pattern of GFP signals looks similar between cKO and littermate control (*Figure 3—figure supplement 1F,G*). Sublamina dendrite patterning of the ipRGC subtype visualized by immunostaining of melanopsin also demonstrated the similar phenotype (*Figure 3—figure supplement 1H,I*). These data suggest that

YTHDF2 has a general control of RGC dendrite branching but has no striking effect on the sublamina targeting of RGC dendrite. These results are consistent with the previous findings that the RGC dendrite targeting is determined genetically and several transcription factors controlling laminar choice have been identified in RGCs and amacrine cells (*Cherry et al. 2011; Kay et al. 2011; Lefebvre et al. 2015; Liu et al. 2018*).

IPL of *Ythdf2* cKO retina is thicker and has more synapses

The increased dendrite branching of RGCs further prompted us to check whether *Ythdf2* cKO changes IPL development. Immunostaining of P6 retina vertical sections using a MAP2 antibody demonstrated that IPL thickness significantly increased in *Ythdf2* cKO retina (*Figure 4A,B*). As a control, the thicknesses of other retinal layers showed no difference between the *Ythdf2* cKO and control mice (*Figure 4—figure supplement 1A-D*). Quantification of MAP2 IF intensity in IPL suggested that the IPL of *Ythdf2* cKO retina became denser with dendrites (*Figure 4A,C*). These results suggest that the increased dendrite branching results in a thicker and denser IPL in the *Ythdf2* cKO retina.

The inner plexiform layer (IPL) of retina is concentrated with synaptic connections, which contain synapses among and between bipolar-amacrine-ganglion cells. The increased RGC dendrite branching and denser IPL in the *Ythdf2* cKO retina prompted us to wonder whether there are changes in synaptic connections in IPL. We used co-staining of the presynaptic marker Bassoon and the postsynaptic marker PSD-95 to count the colocalization puncta of Bassoon⁺/PSD-95⁺. We found that the numbers of Bassoon⁺/PSD-95⁺ excitatory synapses in IPL of *Ythdf2* cKO retina are significantly larger than that of control retina (*Figure 4D,E*). As a control, the numbers of the excitatory ribbon synapses marked by the colocalization of Bassoon⁺/PSD-95⁺ in OPL (outer plexiform layer) show no difference between *Ythdf2* cKO and control retinas (*Figure 4—figure supplement 1E,F*).

All these data verify that the IPL of *Ythdf2* cKO retina is thicker and has more synapses.

Visual acuity is improved for the *Ythdf2* cKO mice

The features of RGC dendrites, including their size, shape, arborization pattern and localization, influence the amount and type of synaptic inputs that RGCs receive, which in turn determine how RGCs respond to specific visual stimuli such as the direction of motion (**Liu and Sanes 2017**). The increased dendrite branching, the thicker and denser IPL, and the more synapses in the IPL inspired us to further explore whether the visual responses of the *Ythdf2* cKO mice were changed or not. *Ythdf2* cKO mice looked normal and had similar body weight and size compared with control mice for either sex (male in *Figure 5A,B*; female in *Figure 5C,D*). The generally normal development of *Ythdf2* cKO mice is consistent with the specific and limited expression of *Six3-cre* in retina (*Figure 5—figure supplement 1A*), and only sparse spots in ventral forebrain (*Figure 5—figure supplement 1B*) (**Furuta et al. 2000**). We used an optomotor response (OMR)-based assay (**Prusky et al. 2004; Umino et al. 2008; Shi et al. 2018**) to monitor visual functions of *Ythdf2* cKO mice (*Figure 5E*). Surprisingly, the *Ythdf2* cKO mice showed modestly improved visual acuity compared with the control mice, measuring spatial frequency threshold as 0.45 ± 0.0043 c/deg (cycle per degree) and 0.43 ± 0.0085 c/deg, respectively (*Figure 5F*, male mice). Similar phenotype was observed in female mice (*Figure 5G*). These results suggest that the visual acuity is modestly improved in the *Ythdf2* cKO mice.

This phenotype is most likely attributed to the increased RGC dendrite branching and thicker and denser IPL with more synapses because all other parts and processes of retina are not affected except RGC dendrite in the *Ythdf2* cKO mediated by *Six3-cre* (*Figure 2—figure supplement 1 and Figure 4—figure supplement 1*). The eyes and optic fibers also showed no difference between *Ythdf2* cKO and control mice (*Figure 5—figure supplement 1C-E*). We further checked the targeting of optic nerves to the brain by anterograde labeling with cholera toxin subunit B (CTB) and found no difference of retinogeniculate or retinocollicular projections between *Ythdf2* cKO and control mice (*Figure 5—figure supplement 1F,G*), suggesting the guidance and central targeting of RGC axons are not affected in the *Ythdf2* cKO.

YTHDF2 target mRNA were identified with transcriptomic and proteomic analysis

Next, we continued to explore the underlying molecular mechanisms of the effects on dendrite branching caused by *Ythdf2* cKO in the retina. First, we wanted to know what transcripts YTHDF2 recognizes and binds. We carried out anti-YTHDF2 RNA immunoprecipitation (RIP) in the retina followed by RNA sequencing of the elute (RIP-Seq). Two biological replicates of anti-YTHDF2 RIP-Seq identified 1638 transcripts (*Supplementary file 1*). Functional annotation of YTHDF2 RIP targets revealed significant enrichment in Cellular Component terms such as neuron part and neuron projection, and Biological Process terms such as cellular component organization and neuron projection development. We further zoomed in to check neural terms in Cellular Component (*Figure 6A*) and Biological Process (*Figure 6B*). We found that substantial numbers of YTHDF2 target transcripts are involved in cytoskeleton, dendrite and their organization and development (*Figure 6A,B*), which is consistent with the dendrite branching phenotype observed in the *Ythdf2* cKO retina.

The working model for YTHDF2 is that it binds and destabilizes its m⁶A-modified target transcripts (**Wang et al. 2014**). Since the destabilization of mRNAs will eventually decrease their protein levels, we carried out proteome analysis using mass spectrometry (MS) in acute *shYthdf2*-mediated knockdown of cultured RGCs, in order to identify directly affected targets. Three biological replicates of YTHDF2 knockdown (KD) followed by MS (YTHDF2 KD/MS) identified 114 proteins which were upregulated by YTHDF2 KD (*Supplementary file 2*). Functional annotation of these proteins revealed significant enrichment in neuron development- and cytoskeleton-related terms (*Figure 6C*), which is similar to anti-YTHDF2 RIP-Seq results.

By overlapping the two gene lists screened from anti-YTHDF2 RIP-Seq (*Supplementary file 1*) and YTHDF2 KD/MS_upregulation (*Supplementary file 2*), we identified a group of potential YTHDF2 target mRNAs in RGCs (*Supplementary file 3*), including *Kalrn*, *Strn* and *Ubr4*. m⁶A modification of these mRNAs were verified by anti m⁶A pull down (*Figure 6D*). *Kalrn* (*Kalirin*) gene generates three alternative splicing isoforms *Kalrn7*, *Kalrn9*, and *Kalrn12* encoding guanine-nucleotide exchange factors (GEFs) for Rho GTPases (Rho-GEF), which have been shown to regulate hippocampal and cortical dendritic branching (**Xie et al. 2010; Yan et al. 2015**), and are required for normal brain

functions (**Penzes et al. 2001; Xie et al. 2007; Cahill et al. 2009; Russell et al. 2014; Lu et al. 2015; Herring and Nicoll 2016**). Strn (Striatin) was first identified in striatum, and functions as a B subunit of the serine/threonine phosphatase PP2A and is also a core component of a multiprotein complex called STRIPAK (striatin-interacting phosphatase and kinase complex) (**Benoist et al. 2006; Li et al. 2018**). Strn was reported to regulate dendritic arborization only in striatal neurons but not in cortical neurons (**Li et al. 2018**). However, whether and how Kalrn and Strn work in the retina was still unknown. Ubr4 (ubiquitin protein ligase E3 component N-recogin 4) is also known as p600 and has been shown to play roles in neurogenesis, neuronal migration, neuronal signaling and survival (**Parsons et al. 2015**). However, whether Ubr4 regulates dendrite development remains elusive.

YTHDF2 controls the stability of its target mRNAs which encode proteins regulating RGC dendrite branching

MS analysis after YTHDF2 KD has shown that the protein levels of these target mRNAs were upregulated (*Supplementary file 2*). IF using antibodies against Strn and Ubr4 detected specific signals in the IPL which were increased in *Ythdf2* cKO retina compared with control retina (*Figure 7—figure supplement 1A*). Enrichment of these proteins in IPL implies that these proteins might function locally in RGC dendrites to regulate dendrite development.

We next wanted to know whether YTHDF2 controlled the protein levels of these m⁶A-modified target mRNAs through regulation of translation or transcript stability. As shown in *Figure 7—figure supplement 1B-D*, the mRNA levels of *Kalrn7*, *Kalrn9*, *Kalrn12*, *Strn* and *Ubr4* were dramatically increased after KD of YTHDF2 or METTL14, supporting that YTHDF2 might regulate stability of these target mRNAs. We further evaluated potential changes in the stability of these target mRNAs in an m⁶A-dependent manner. We further verified this by directly measuring the stability of these target mRNAs. As shown in *Figure 7A*, all the target mRNAs showed significantly increased stability in the *Ythdf2* cKO retina compared with controls. These results suggest that YTHDF2 controlled the protein levels of its m⁶A-modified target mRNAs by decreasing their stability.

Next we explored the functions of these YTHDF2 target mRNAs in RGC dendrite development.

We first generated siRNAs against these transcripts (*Figure 7—figure supplement 1E*). We then checked the effects on RGC dendrite branching after KD of these target mRNAs by siRNAs in cultured RGCs. As shown in *Figure 7B*, knockdown of *Kalrn7*, *Kalrn9*, *Kalrn12*, *Strn* or *Ubr4* led to significant decreases of RGC dendrite branching. Interestingly, the *siCocktail* against all these target mRNAs further significantly reduced the RGC dendrite branching compared with each individual siRNA (*Figure 7—figure supplement 1F*), suggesting that these targets may work in different pathways to regulate the RGC dendrite morphology. We further examined whether these target mRNAs mediate YTHDF2-regulated RGC dendrite branching. As shown in *Figure 2E-H*, and *Figure 3*, cKO of *Ythdf2* led to increased dendrite branching of RGCs both in vitro and in vivo. Transfection of siRNAs against these target mRNAs rescued dendrite branching increases in cultured *Ythdf2* cKO RGCs (*Figure 7C*). We continued to generate and performed intravitreal injection of AAV viral *shKalrn12* and *shUbr4*, which significantly rescued dendrite branching increases of CART⁺ oodSGCs and SMI-32⁺ αRGCs in *Ythdf2* cKO retina in vivo (*Figure 7D*).

Taken together, we identified a group of YTHDF2 target mRNAs that encode proteins regulating RGC dendrite branching, which mediate YTHDF2-controlled RGC dendrite branching.

***Ythdf2* cKO retina is more resistant to acute ocular hypertension (AOH)**

The glaucomatous eyes are symptomatized with progressive neurodegeneration and vision loss (*Agostinone and Di Polo 2015*). High intraocular pressure is a major risk factor in glaucoma and has been shown to cause pathological changes in RGC dendrites before axon degeneration or soma loss is detected in different model animals (*Weber et al. 1998; Shou et al. 2003; Morgan et al. 2006*). Our findings that *Ythdf2* cKO in retina promotes RGC dendrite branching during development inspired us to wonder whether YTHDF2 also regulates RGC dendrite maintenance in the acute glaucoma model caused by acute ocular hypertension (AOH). We utilized the AOH model made with control and *Ythdf2* cKO mice to check whether *Ythdf2* cKO in the retina could alter the pathology in the

glaucomatous eyes. RGC dendrite branching is significantly decreased after AOH operation compared with non-AOH in either genotype (*Figure 8—figure supplement 1A,B*). Interestingly, the *Ythdf2* cKO retina with AOH operation maintains significantly higher dendrite complexity compared with the glaucomatous eyes of *Ythdf2^{fl/fl}* control mice (*Figure 8A,B*). In addition, there are significant RGC neuron losses in both genotypes after AOH (*Figure 8C,D*). However, the reduction of RGC number in the *Ythdf2* cKO retina is less than control retina (*Figure 8C,D*). These results support that *Ythdf2* cKO protects retina from RGC dendrite degeneration and soma loss caused by AOH.

Next we wanted to know whether and how YTHDF2 target mRNAs mediate these effects in the AOH models. We first checked the expression of YTHDF2 target mRNAs identified in the developing retina (*Supplementary file 3*) in the adult *Ythdf2* cKO and control retina. We found that two target mRNAs *Hspa12a* and *Islr2* show upregulation in the adult *Ythdf2* cKO retina compared with control (*Figure 8—figure supplement 1C*). m⁶A modification of *Hspa12a* and *Islr2* mRNAs was further verified by anti-m⁶A pulldown (*Figure 8—figure supplement 1D*). *Hspa12a* encodes heat shock protein A12A which is an atypical member of the heat shock protein 70 family and has been shown to be downregulated in diseases such as ischemic stroke, schizophrenia, and renal cell carcinoma (**Pongrac et al. 2004; Mao et al. 2018; Min et al. 2020**). *Islr2* encodes immunoglobulin superfamily containing leucine-rich repeat protein 2 and is poorly studied. Here, we found that *Hspa12a* and *Islr2* are downregulated in the retina after AOH operation (*Figure 8—figure supplement 1E*), which is likely caused by upregulation of YTHDF2 in the AOH-treated retina (*Figure 8—figure supplement 1F-H*). We therefore hypothesized that AOH upregulates YTHDF2 which in turn downregulates its targets *Hspa12a* and *Islr2*, thus causing RGC dendrite degeneration and soma loss. If this is the case, overexpression of *Hspa12a* and *Islr2* might protect RGC dendrite from AOH-triggered degeneration. We thus generated AAV harboring overexpression constructs of *Hspa12a* and *Islr2* which were intravitreally injected to wild type retinas. After the AOH induction, the retinas overexpressing *Hspa12a* and *Islr2* maintain significantly more complex RGC dendrite arbor compared with control AAV (*Figure 8E,F*).

These data verify that loss-of-function of YTHDF2 and gain-of-function of its targets *Hspa12a* and *Islr2* have neuroprotective roles in the glaucomatous retina.

Discussion

Functions and mechanisms of mRNA m⁶A modification in the dendrite development were not known. Here, we revealed a critical role of the m⁶A reader YTHDF2 in RGC dendrite development and maintenance. YTHDF2 have two phases of function to control RGC dendrite development first and then maintenance through regulating two sets of target mRNAs. In early postnatal stages, the target mRNAs *Kalrn7*, *Kalrn9*, *Kalrn12*, *Strn* and *Ubr4* mediate YTHDF2 functions to regulate RGC dendrite development. In the adult mice, another set of target mRNAs *Hspa12a* and *Islr2* mediate YTHDF2 function to regulate RGC dendrite maintenance.

Positive and negative regulators for dendrite development

The general principle for dendrite arborization is that the dendrite arbor cannot be either too big or too small in order to precisely sample a presynaptic target area during neural circuit formation (*Lefebvre et al. 2015*). Numerous extrinsic and intrinsic mechanisms have been found to regulate dendritic arbor patterning, which involves both positive and negative factors to achieve balanced control of dendritic growth (*Jan and Jan 2010; Dong et al. 2015; Ledda and Paratcha 2017*). For the secreted and diffusible cues, BDNF promotes dendrite branching and complexity (*Cheung et al. 2007*); the non-canonical Wnt7b/PCP pathway is a positive regulator of dendrite growth and branching (*Rosso et al. 2005*); the non-canonical Wnt receptor Ryk works as a negative regulator by limiting the extent of dendritic branching (*Lanoue et al. 2017*). For the contact-mediated signals, the cadherins *Celsr2* and *Celsr3* regulate dendrite growth in an opposite manner in cortical pyramidal and Purkinje neurons, and hippocampal neurons, respectively (*Shima et al. 2004; Shima et al. 2007*). For the transcription factors, studies have shown that manipulation of *Cux1* and *Cux2* levels has distinct effects on apical and basal arbors of cortical dendrites (*Cubelos et al. 2015*); interestingly, the

functions of Sp4 in dendrite development are dependent on the cellular context of its expression, e.g. Sp4 promotes dendrite growth and branching in hippocampal dentate granule cells but limits dendrite branching in cerebellar granule cells (*Ramos et al. 2007; Zhou et al. 2007*). Here we identified another negative regulator YTHDF2 which works posttranscriptionally, and loss-of-function of YTHDF2 increased dendrite complexity during development and protected RGC degeneration from AOH.

Posttranscriptional regulation of dendrite development

It is well established that mRNAs can be transported and targeted to specific neuronal compartments such as axons and dendrites. Local translation of these mRNAs enables exquisite and rapid control of local proteome in specific subcellular compartments (*Ledda and Paratcha 2017*). Local translation is known to play roles in controlling dendrite arborization (*Chihara et al. 2007*), and is regulated by specific RNA-binding proteins (*Jan and Jan 2010*). In *Drosophila*, the RNA binding proteins Pumilio (Pum), Nanos (Nos), Glorund (Glo) and Smaug (Smg) regulate morphogenesis and branching of specific classes of dendritic arborization (da) neurons through controlling translation of their target mRNAs including *nanos* mRNA itself (*Ye et al. 2004; Brechbiel and Gavis 2008*). The mouse homologue of another RNA-binding protein Stauf, Stau1, regulates dendritic targeting of ribonucleoprotein particles and dendrite branching (*Vessey et al. 2008*). Here we found that the m⁶A reader and RNA-binding protein YTHDF2 controls stability of its target mRNAs and regulates dendrite branching in RGCs. It would be interesting to see whether these target mRNAs are localized into dendrites and whether YTHDF2 works in dendrites to control their stability and translation. Actually, *Strn4* mRNA has been shown to be present in dendrites and locally translated (*Lin et al. 2017*). In addition, how the proteins encoded by these target mRNAs regulate RGC dendrite branching during development and maintenance remains to be explored and will be important future directions.

Neuroprotective genes in retinal injuries and degeneration

Transcriptome analyses have revealed differentially expressed genes after retinal injuries such as AOH-induced glaucoma and optic nerve crush (ONC), and the up-regulated genes are of importance for discovering new treatment approaches (**Jakobs 2014; Tran et al. 2019**). One of the previous studies has identified *Mettl3*, encoding the m⁶A writer, as an up-regulated gene after ONC (**Agudo et al. 2008**). Here we found *Ythdf2*, encoding an m⁶A reader, was also up-regulated in the retina after AOH. We further found that *Hspa12a* and *Islr2*, two targets of YTHDF2 in adult retina, were downregulated in glaucomatous retinas. Overexpression of *Hspa12a* and *Islr2* protected retina from AOH-caused RGC dendrite degeneration. Our findings in this study suggest that YTHDF2 and its neuroprotective target mRNAs might be valuable in developing novel therapeutic approaches to treat neurodegeneration caused by glaucoma and other retinal injuries.

Materials and methods

Key resources table

Reagent type (species)or resource	Source or reference	Identifiers
Antibodies		
Chicken polyclonal anti-GFP	Abcam	Cat#: ab13970, RRID: AB_300798
Chicken polyclonal anti-MAP2	Abcam	Cat#: ab5392, RRID: AB_2138153
Guinea pig polyclonal anti-RBPMS	PhosphoSolutions	Cat#: 1832-RBPMS, RRID: AB_2492226
Goat polyclonal anti-VACHT	Millipore	Cat#: ABN100, RRID: AB_2630394
Mouse monoclonal anti-β Actin	Abcam	Cat#: ab6276, RRID: AB_2223210
Mouse monoclonal anti-β Actin	ABclonal	Cat#: AC004, RRID: AB_2737399
Mouse monoclonal anti-AP2α	DSHB	Cat#: 3B5, RRID: AB_2313947
Mouse monoclonal anti-Bassoon	Enzo Life Sciences	Cat#: ADI-VAM-PS003, RRID: AB_10618753
Mouse monoclonal anti-Brn3a	Millipore	Cat#: MAB1585, RRID: AB_94166
Mouse monoclonal anti-Calbindin-D-28K	Sigma-Aldrich	Cat#: C9848, RRID: AB_476894
Mouse monoclonal anti-PKCα	Santa Cruz Biotechnology	Cat#: sc-8393, RRID: AB_628142
Mouse monoclonal anti-SMI-32	BioLegend	Cat#: 801701, RRID: AB_2564642
Mouse monoclonal anti-Strn (Striatin)	BD Biosciences	Cat#: 610838, RRID: AB_398157
Rabbit polyclonal anti-CART	Phoenix Pharmaceuticals	Cat#: H-003-62, RRID: AB_2313614
Rabbit polyclonal anti m ⁶ A	Synaptic Systems	Cat# 202003, RRID: AB_2279214
Rabbit polyclonal anti-Melanopsin	Thermo Fisher Scientific	Cat#: PA1-780, RRID: AB_2267547
Rabbit polyclonal anti-PKCα	Cell Signaling	Cat#: CST-2056

Mouse monoclonal anti-PSD-95	Abcam	Cat#: ab2723, RRID: AB_303248
Rabbit polyclonal anti-Recoverin	Millipore	Cat#: AB5585, RRID: AB_2253622
Rabbit polyclonal anti-YTHDF2	Proteintech	Cat#: 24744-1-AP, RRID: AB_2687435
Rabbit polyclonal anti-YTHDF1	Proteintech	Cat#: 17479-1-AP, RRID: AB_2217473
Rabbit polyclonal anti-YTHDF3	Abcam	Cat#: ab103328, RRID: AB_10710895
Rabbit polyclonal anti-Ubr4	Abcam	Cat#: ab86738, RRID: AB_1952666
Sheep polyclonal anti-Chx10	Exalpha	Cat#: X1179P
Alexa 488 donkey anti-chicken IgY	Jackson Immunoresearch	Cat#: 703-545-155, RRID: AB_2340375
Alexa 488 donkey anti-g. pig IgG	Jackson Immunoresearch	Cat#: 706-545-148, RRID: AB_2340472
Alexa 488 donkey anti-mouse IgG	Thermo Fisher Scientific	Cat#: A-21202, RRID: AB_141607
Alexa 488 donkey anti-rabbit IgG	Thermo Fisher Scientific	Cat#: A-21206, RRID: AB_141708
Alexa 555 donkey anti-goat IgG	Thermo Fisher Scientific	Cat#: A-21432, RRID: AB_2535853
Alexa 555 donkey anti-mouse IgG	Thermo Fisher Scientific	Cat#: A-31570, RRID: AB_2536180
Alexa 555 donkey anti-rabbit IgG	Thermo Fisher Scientific	Cat#: A-31572, RRID: AB_162543
Alexa 555 donkey anti-sheep IgG	Thermo Fisher Scientific	Cat# A-21436, RRID: AB_2535857
Alexa 555 goat anti-chicken IgY	Thermo Fisher Scientific	Cat#: A-21437, RRID: AB_2535858
Alexa 647 donkey anti-mouse IgG	Thermo Fisher Scientific	Cat#: A-31571, RRID: AB_162542
HRP donkey anti-mouse IgG	Abcam	Cat#: ab97030, RRID: AB_10680919
HRP donkey anti-rabbit IgG	Abcam	Cat#: ab16284, RRID: AB_955387
HRP VHH anti-mouse IgG	AlpaLife	Cat#: KTSM1321
HRP VHH anti-rabbit IgG	AlpaLife	Cat#: KTSM1322

Chemicals, Peptides, and
Recombinant Proteins

TRIzol Reagent	Life	Cat#: 15596018
PrimeScript™ RT Master Mix	Takara	Cat#: RR036B
2× ChamQ™ Universal SYBR qPCR Master Mix	Vazyme	Cat#: Q711-02
DMEM, high glucose	Gibco	Cat#: 11965-092
Dulbecco's Modified Eagle's Medium, 10×, low glucose	Sigma	Cat#: D2429
DMEM, high glucose	Hyclone	Cat#: SH30022.01
Fetal Bovine Serum (FBS)	Gibco	Cat#: 10270-106
Dulbecco's Phosphate-Buffered Saline, 1× without calcium and magnesium (DPBS)	Corning	Cat#: 21-031-CVR
poly-D-lysine, Cultrex	Trevigen	Cat#: 3439-100-01
Laminin (mouse), Culrex	Trevigen	Cat#: 3400-010-01
DMEM/F-12, GlutaMAX™	Gibco	Cat#: 10565-018
Neurobasal™ Medium, minus phenol red	Gibco	Cat#: 12348-017
Penicillin-Streptomycin	Life	Cat#: 15140-122
B27 serum-free supplement, 50×	Life	Cat#: 17504044
N-2 Supplement, 100×	Gibco	Cat#: 17502-048
Insulin	Sigma	Cat#: I6634
cpt-cAMP, 8-(4-Chlorophenylthio) Adenosine 3':5'-CY	Sigma	Cat#: C3912

N-acetyl-L-cysteine (NAC)	Sigma	Cat#: A8199
Forskolin	Sigma	Cat#: F6886
Recombinant Human/Murine/Rat BDNF	PeproTech	Cat#: 450-02
Recombinant Human NT-3	PeproTech	Cat#: 450-03
Recombinant Murine EGF	PeproTech	Cat#: 315-09
Recombinant Human FGF-basic	PeproTech	Cat#: 100-18B
Puromycin	Thermo Fisher Scientific	Cat#: A11138-03
Puromycin	Sigma	Cat#: P8833
Paraformaldehyde	Vetec	Cat#: V900894-100G
O.C.T. Compound and Cryomolds, Tissue-Tek	SAKURA	Cat#: 4583
ChemiBLOCKER	Millipore	Cat#: 2170
Triton x-100	Sigma	Cat#: V900502
CTB (Cholera Toxin Subunit B) conjugated by Alexa Fluor™ 555	Invitrogen	Cat#: C34776
VECTASHIELD Antifade Mounting Medium with DAPI	Vector Laboratory	Cat#: H-1200
Mounting Medium, antifading (with DAPI)	Solarbio	Cat#: S2110
Normal Goat Serum	Novus	Cat#: NBP2-23475
Critical Commercial Assays		
Pierce™ BCA Protein Assay Kit	Thermo Fisher Scientific	Cat#: 23227
GeneSilencer® Transfection Reagent	Genlantis	Cat#: T500750
Magna MeRIP™ m ⁶ A Kit	Millipore	Cat#: 17-10499
EZ-Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit	Millipore	Cat#: 17-701
Experimental Models: Cell Lines		
HEK293T	ATCC	Cat#: CRL-11268, RRID: CVCL_1926
Experimental Models: Organisms/Strains		
Mouse: Ythdf2 ^{fl/fl}	(Yu et al. 2021)	N/A
Mouse: Tg(Six3-cre)69FrtY/GcoJ	Jackson Laboratory	Cat#: JAX_019755
Mouse: B6.Cg-Tg(Thy1-EGFP)OJrs/GfngJ	Jackson Laboratory	Cat#: JAX_007919
Mouse: B6.129X1-Gt(ROSA)26Sor ^{tm1(EYFP)Cos} /J	Jackson Laboratory	Cat#: JAX_006148
Oligonucleotides		
shRNA targeting sequence of negative control: GCATCAAGGTGAACTTCAAGA	This paper	N/A
shRNA targeting sequence of mouse Ythdf2: GGACGTTCCCAATAGCCAACT	(Yu et al. 2018)	N/A
shRNA targeting sequence of mouse Ythdf1: GGACATTGGTACTTGGGATAA	This paper	N/A
shRNA targeting sequence of mouse Mettl14#6: GCTGGACCTGGGATGATATTA	This paper	N/A

shRNA targeting sequence of mouse <i>Mettl14</i> #7: CCCAGCTTGACTTTGCTTTA	This paper	N/A
shRNA targeting sequence of negative control (AAV): TTCTCCGAACGTGTCACGTAA	This paper	N/A
shRNA targeting sequence of mouse <i>Kalrn12</i> : TGATGAGCTGATGGAAGAA	This paper	N/A
shRNA targeting sequence of mouse <i>Ubr4</i> : AATGATGAGCAGTCATCTC	This paper	N/A
shRNA targeting sequence of mouse <i>Ythdf3</i> : GGATTGGCAATGATACTTTG	This paper	N/A
siRNA targeting sequence of negative control: UUCUCCGAACGUGUCACGUTT	(Yu et al. 2018)	N/A
siRNA targeting sequence of mouse <i>Kalrn7</i> : AGUACAAUCCUGGCCAUGUTT	(Xie et al. 2007)	N/A
siRNA targeting sequence of mouse <i>Kalrn9</i> : ACUGGACUGGACUUCUAUUTT	(Yan et al. 2015)	N/A
siRNA targeting sequence of mouse <i>Kalrn12</i> : CGAUGAGCUGAUGGAAGAATT	(Yan et al. 2015)	N/A
siRNA targeting sequence of mouse <i>Strn</i> : GGUGAAGAUCGAGAUACAATT	(Breitman et al. 2008)	N/A
siRNA targeting sequence of mouse <i>Ubr4</i> : AAUGAUGAGCAGUCAUCUATT	(Shim et al. 2008)	N/A
qPCR primers of mouse <i>18s</i> Fwd: GCTTAATTTGACTCAACACGGGA Rev: AGCTATCAATCTGTCAATCCTGTC	(Wang et al. 2018)	N/A
qPCR primers of mouse <i>Gapdh</i> Fwd: TTGTCAGCAATGCATCCTGCACCACC Rev: CTGAGTGGCAGTGATGGCATGGAC	(Mains et al. 2011)	N/A
qPCR primers of mouse <i>Ythdf2</i> Fwd: GAGCAGAGACCAAAAGGTCAAG Rev: CTGTGGGCTCAAGTAAGGTTT	This paper	N/A
qPCR primers of mouse <i>Kalrn7</i> Fwd: GATACCATATCCATTGCCTCCAGGACC Rev: CCAGGCTGCGCGCTAAACGTAAG	(Mains et al. 2011)	N/A
qPCR primers of mouse <i>Kalrn9</i> Fwd: GCCCCTCGCCAAAGCCACAGC Rev: CCAGTGAGTCCCGTGGTGGGC	(Mains et al. 2011)	N/A

qPCR primers of mouse <i>Kalrn12</i> Fwd: CAGCAGCCACGTGCCTGCAGC Rev: TCTTGACATTGGGAATGGGCCGCAC	(Mains et al. 2011)	N/A
qPCR primers of mouse <i>Strn</i> Fwd: TGAAGCCTGGAATGTGGACC Rev: CTATTGGGCCTCTTCACCCC	This paper	N/A
qPCR primers of mouse <i>Ubr4</i> Fwd: TGAGTGAGGACAAGGGCAAC Rev: GGGTTGGATCGAACGAAGGT	This paper	N/A
qPCR Primer for mouse <i>Hspa12a</i> Fwd: GGGTTTGACAGGCTAAGGA Rev: TCTGATGGACGGTCAGGTCT	This paper	N/A
qPCR Primer for mouse <i>Islr2</i> Fwd: GAAGCTCCCTTAGACTGTCACC Rev: CCCCATCGTGACTCCTGCTG	This paper	N/A
PCR Primer for mouse <i>Hspa12a</i> CDS Fwd: ATGGCGGACAAGGAAGCTGG Rev: GTAATTTAAGAAGTCGATCCCC	This paper	N/A
PCR Primer for mouse <i>Islr2</i> CDS Fwd: ATGGGGCCCTTTGGAGC Rev: GCCCGCTGTCTGCCTGTAG	This paper	N/A
Mouse genotyping primers for <i>Ythdf2</i> loxp site 1: GCTTGTAGTTATGTTGTGTACCAC and GCAGCTCTGACTATTCTAAACCTCC	This paper	N/A
Mouse genotyping primers for <i>Ythdf2</i> loxp site 2: CTCATAACATCCATAGCCACAGG and CCAAGAGATAGCTTTCCTAATG	This paper	N/A
Mouse genotyping primers for <i>Six3-cre</i> : CCTTCCTCCCTCTCTATGTG and GAACGAACCTGGTCGAAATC	Chunqiao Liu's lab	N/A
Mouse genotyping primers for <i>Thy1-GFP</i> : CGGTGGTGCAGATGAACTT and ACAGACACACACCCAGGACA	The Jackson Laboratory website	N/A
Mouse genotyping primers for Rosa-YFP mutant site: AGGGCGAGGAGCTGTTCA and TGAAGTCGATGCCCTTCAG	The Jackson Laboratory website	N/A
Mouse genotyping primers for Rosa-YFP wildtype site: CTGGCTTCTGAGGACCG and CAGGACAACGCCACACA	The Jackson Laboratory website	N/A
Recombinant DNA		
Plasmid: pLKO.1-TRC	Addgene	Addgene plasmid #10878, RRID: Addgene_10878
Software and Algorithms		
GraphPad Prism 7.0	GraphPad	https://www.graphpad.com , RRID: SCR_002798

STAR v2.5	(<i>Dobin et al. 2013</i>)	https://github.com/alexdobin/STAR/
HTSeq	(<i>Anders et al. 2015</i>)	https://pypi.org/project/HTSeq/
ImageJ (Fiji)	(<i>Schindelin et al. 2012</i>)	http://fiji.sc , RRID:SCR_002285
Matlab	Matlab	https://ww2.mathworks.cn

Animals and generation of the *Ythdf2* cKO mice

Ythdf2^{f/f} mice were reported previously (*Yu et al. 2021*). *Six3-cre* (*Furuta et al. 2000*), *Thy1-GFP* (*Feng et al. 2000*) and *Rosa26-eYFP* (*Srinivas et al. 2001*) mice were from Jackson Laboratory. For timed pregnancy, embryos were identified as E0.5 when a copulatory plug was observed. Genotyping primers are as following: the first *Ythdf2-loxP* site, 5'-GCTTGTAGTTATGTTGTGTACCAC-3' and 5'-GCAGCTCTGACTATTCTAAACCTCC-3'; the second *Ythdf2-loxP* site, 5'-CTCATAACATCCATAGCCACAGG-3' and 5'-CCAAGAGATAGCTTTCCTAATG-3'. *Six3-cre* site, 5'-CCTCCTCCCTCTCTATGTG-3' and 5'-GAACGAACCTGGTCGAAATC-3'. *Rosa26-eYFP* wild type site, 5'-CTGGCTTCTGAGGACCG-3' and 5'-CAGGACAACGCCACACA-3'; the mutant site, 5'-AGGGCGAGGAGCTGTTC-3' and 5'-TGAAGTCGATGCCCTTCAG-3'. All experiments using mice were carried out following the animal protocols approved by the Laboratory Animal Welfare and Ethics Committee of Southern University of Science and Technology.

Retinal neuronal culture

Retinal neurons were dissociated from E14.5-15.5 mouse embryos by papain in DPBS (1× Dulbecco's Phosphate-Buffered Saline, Corning) following the previously described methods (*Kechad et al. 2012*), and neuronal suspension was plated on acid-washed glass coverslips pre-coated with poly-D-lysine (Trevigen, 100 µg/ml) for 1 hr and laminin (Trevigen, 5 µg/ml) overnight at 37°C. Culture medium was made up of half DMEM/F12 medium (Gibco) and half neurobasal medium (Gibco), supplemented with B27 supplement (Life, 0.5×), penicillin-streptomycin (Life, 1×), N-2 supplement (Gibco, 0.5×), N-acetyl-L-cysteine (Sigma, NAC 0.6 mg/ml), cpt-cAMP (Sigma, 100 µM), forskolin (Sigma, 10 µM), and insulin (Sigma, 25 µg/ml). EGF (PeproTech, 50 ng/ml), BDNF (PeproTech, 50 ng/ml), NT-3 (PeproTech, 25 ng/ml), and FGF-basic (PeproTech, 10 ng/ml) were freshly added before using.

Knockdown using lentiviral shRNA, siRNA or AAV shRNA, and overexpression using AAV system

Lentiviral knockdown plasmids encoding shRNA (*shCtrl*: 5'-GCATCAAGGTGAACTTCAAGA-3'; *shYthdf2*: 5'-GGACGTTCCCAATAGCCAACT-3'; *shYthdf1*: 5'-GGACATTGGTACTTGGGATAA-3'; *shYthdf3*: 5'-GGATTGGCAATGATACTTTG-3'; *shMettl14#6*: 5'-GCTGGACCTGGGATGATATTA-3'; *shMettl14#7*: 5'-CCCAGCTTGACTTTGCTTTA-3') were generated from pLKO.1-TRC and lentivirus preparation process was described previously (Yu et al. 2018). All siRNA were chosen from previous studies and the target sequences of siRNA are as following: *siCtrl* (RNAi negative control): 5'-UUCUCCGAACGUGUCACGUTT-3' (Yu et al. 2018); *siKalrn7*: 5'-AGUACAAUCCUGGCCAUGUTT-3' (Xie et al. 2007); *siKalrn9*: 5'-ACUGGACUGGACUUCUAUUTT-3' (Yan et al. 2015); *siKalrn12*: 5'-CGAUGAGCUGAUGGAAGAATT-3' (Yan et al. 2015); *siStrn*: 5'-GGUGAAGAUCGAGAUACAATT-3' (Breitman et al. 2008); *siUbr4*: 5'-AAUGAUGAGCAGUCAUCUATT-3' (Shim et al. 2008). AAV knockdown plasmids encoding shRNA (*shCtrl*: 5'-TTCTCCGAACGTGTCACGTAA-3'; *shKalrn12*: 5'-TGATGAGCTGATGGAAGAA-3'; *shUbr4*: 5'-AATGATGAGCAGTCATCTC-3') were generated using pHBAAV-U6-MCS-CMV-EGFP and packaged in serotype-9 by Hanbio (1.5×10¹² genomic copies per ml). AAV overexpression plasmids of *Hspa12a* (NM_175199.3; PCR primer for mouse *Hspa12a*: 5'-ATGGCGGACAAGGAAGCTGG-3' and 5'-GTAATTTAAGAAGTCGATCCCC-3') and *Islr2* (NM_001161541.1; PCR Primer for mouse *Islr2*: 5'-ATGGGGCCCTTTGGAGC-3' and 5'-GCCCCGCTGTCTGCCTGTAG-3') were generated from pHBAAV-CMV-MCS-3flag-T2A-ZsGreen and packaged serotype-9 by Hanbio (1.2×10¹² genomic copies per ml).

GeneSilencer® Transfection Reagent (Genlantis) was used in siRNA transfection following the manufacturer's protocols. Culture medium was changed after 1 day of lentiviral shRNA infection or siRNA transfection. For lentiviral shRNA assay, puromycin (Thermo or Sigma, 1 µg/ml) was added after 2 days of infection. Immunofluorescence, RNA or protein preparation was performed after shRNA or siRNA worked for 3 days. For AAV intravitreal injection, P0-P1 mouse pups were anesthetized in ice and then eyes were pierced at the edge of corneal by 30G × 1/2 needle (BD,

305106) under stereomicroscope. Then 1 μ L AAV was intravitreally injected with 10 μ L Syringe (Hamilton, 80330) following the pinhole. P15 or adult mice were anesthetized with 2.5% Avertin and then eyes were pierced at the side of corneal and the outer segment of sclera by 30G $\times$ 1/2 needle successively. 2 μ L AAV was intravitreally injected with 10 μ L Syringe following the pinhole on the sclera. All subsequent experiments such as acute ocular hypertension operation and immunostaining were carried out after at least 3 weeks (10 days for ZsGreen/CART labeling of ooDSGCs in *Ythdf2* cKO and control mice in *Figure 3A*).

RT-qPCR

Total RNA was extracted from cells or tissues with TRIzol Reagent (Life) and then used for reverse transcription by PrimeScriptTM RT Master Mix (TaKaRa). Synthesized cDNA was used for qPCR by 2 $\times$ ChamQTM Universal SYBR qPCR Master Mix (Vazyme) on StepOnePlusTM Real-Time PCR System (ABI) or BioRad CFX96 Touch Real-Time PCR system. Primers used for qPCR are as following: mouse *Gapdh*: 5'-TTGTCAGCAATGCATCCTGCACCACC-3' and 5'-CTGAGTGGCAGTGATGGCATGGAC-3' (*Mains et al. 2011*); mouse *Kalrn7*: 5'- GATACCATATCCATTGCCTCCAGGACC-3' and 5'- CCAGGCTGCGCGCTAAACGTAAG-3' (*Mains et al. 2011*); mouse *Kalrn9*: 5'- GCCCCTCGCCAAAGCCACAGC-3' and 5'-CCAGTGAGTCCCGTGGTGGGC-3' (*Mains et al. 2011*); mouse *Kalrn12*: 5'- CAGCAGCCACGTGCCTGCAGC-3' and 5'-TCTTGACATTGGGAATGGGCCGCAC-3' (*Mains et al. 2011*); mouse *Strn*: 5'-TGAAGCCTGGAATGTGGACC-3' and 5'-CTATTGGCCTCTTCACCCC-3'; mouse *Ubr4*: 5'- TGAGTGAGGACAAGGGCAAC-3' and 5'-GGGTTGGATCGAACGAAGGT-3'; mouse *Ythdf2*: 5'- GAGCAGAGACCAAAAGGTCAAG-3' and 5'-CTGTGGGCTCAAGTAAGGTTC-3'; 18s: 5'- GCTTAATTTGACTCAACACGGGA-3' and 5'-AGCTATCAATCTGTCAATCCTGTC-3' (*Wang et al. 2018*); mouse *Hspa12a*: 5'-GGGTTTGCACAGGCTAAGGA-3' and 5'-TCTGATGGACGGTCAGGTCT-3'; mouse *Islr2*: 5'-GAAGCTCCCTTAGACTGTCACC-3' and 5'-CCCCATCGTGACTCCTGCTG-3'.

Immunofluorescence and immunostaining

447 For tissue sections, mouse embryonic eyes were fixed with 4% PFA (Sigma) in 0.1 M Phosphate Buffer
 448 (PB) for 30-45 min at room temperature (RT); eyes of mouse pups (< P10) were pre-fixed briefly and
 449 then eyecups were dissected and fixed for 45 min-1 hr at RT; for P20-30 or adult mice, eyecups were
 450 dissected after myocardial perfusion with 0.9% NaCl, followed by fixation for 1 hr. After PBS (3 × 5
 451 min) washing, tissues were dehydrated with 30% sucrose in 0.1 M PB overnight at 4°C, then
 452 embedded with O.C.T. (SAKURA) and cryosectioned at 12 μm (20 μm for Thy1-GFP section analysis)
 453 with Leica CM1950 Cryostat. Tissue sections were permeabilized and blocked with 10%
 454 ChemiBLOCKER (Millipore) and 0.5% Triton x-100 (Sigma) in PBS (PBST) for 1 hr at RT and incubated
 455 in PBST overnight at 4°C with following primary antibodies: chicken anti-GFP (1:1000, Abcam
 456 ab13970), chicken anti-MAP2 (1:10000, Abcam ab5392), goat anti-VACHT (1:1000, Millipore ABN100),
 457 guinea pig anti-RBPMS (1:1000, PhosphoSolutions 1832-RBPMS), mouse anti-AP2α (1:1000, DSHB
 458 3B5), mouse anti-Bassoon (1:2500, Enzo Life Sciences ADI-VAM-PS003), mouse anti-Brn3a (1:300,
 459 Millipore MAB1585), mouse anti-Calbindin-D-28K (1:200, Sigma C9848), mouse anti-PKCα (1:500,
 460 Santa Cruz sc-8393), rabbit anti-Strn (Striatin) (1:500, BD Biosciences 610838), rabbit anti-CART
 461 (1:2000, Phoenix Pharmaceuticals H-003-62), rabbit anti-m⁶A (1:200, Synaptic Systems 202003),
 462 rabbit anti-melanopsin (1:1000, Thermo PA1-780), rabbit anti-PKCα (1:1000, Cell Signaling CST-2056),
 463 rabbit anti-PSD95 (1:1000, Abcam ab18258), rabbit anti-Recoverin (1:1000, Millipore AB5585), rabbit
 464 anti-YTHDF2 (1:1000, Proteintech 24744-1-AP), rabbit anti-YTHDF1 (1:1000, Proteintech 17479-1-AP),
 465 rabbit anti-YTHDF3 (1:1000, Abcam ab103328), rabbit anti-Ubr4 (1:300, Abcam ab86738), sheep anti-
 466 Chx10 (1:1000, Exalpha X1179P). After three times of PBS washing, sections were incubated in PBST
 467 for 1 hr at RT with secondary antibodies: Alexa 488 donkey anti-chicken (1:500, Jackson 703-545-155),
 468 Alexa 488 donkey anti guinea pig (1:500, Jackson 706-545-148), Alexa 488 donkey anti-mouse (1:500,
 469 Thermo A21202), Alexa 488 donkey anti-rabbit (1:500, Thermo A21206), Alexa 555 donkey anti-goat
 470 (1:1000, Thermo A21432), Alexa 555 donkey anti-mouse (1:1000, Thermo A31570), Alexa 555 donkey
 471 anti-rabbit (1:1000, Thermo A31572), Alexa 555 donkey anti-sheep (1:1000, Thermo A21436), Alexa
 472 555 goat anti-chicken (1:1000, Thermo A21437), or Alexa 647 donkey anti-mouse (1:200, Thermo

A31571) and then mounted with the VECTASHIELD Antifade Mounting Medium with DAPI (Vector Laboratory).

For cultured neurons, after twice of PBS washing, cells were fixed for 15 min with 4% PFA in 0.1 M PB at RT, then washed with PBS three times and blocked in PBST for 20 min at RT. Antibody incubation conditions are the same as tissue sections.

For wholemount immunostaining of retina, eyes were dissected after myocardial perfusion with 0.9% NaCl. Then retinas were separated from sclera and fixed with 4% PFA in 0.1M PB for 1 hr at RT. Then retinas were blocked with 5% normal goat serum (Novus), 0.4% Triton x-100 in PBS overnight at 4°C. Primary antibodies such as chicken anti-GFP (1:1000, Abcam ab13970), mouse anti-Brn3a (1:300, Millipore MAB1585), mouse anti-SMI-32 (1:200, BioLegend 801701), or rabbit anti-Melanopsin (1:1000, Thermo PA1-780), rabbit anti-CART (1:2000, Phoenix Pharmaceuticals H-003-62) were diluted in 5% normal goat serum, 0.4% Triton x-100 in PBS and incubated overnight at 4°C. Then retinas were incubated with Alexa 488 donkey anti-chicken (1:500, Jackson 703-545-155), Alexa 488 donkey anti-mouse (1:500, Thermo A21202), Alexa 555 donkey anti-mouse (1:1000, Thermo A-31570) and Alexa 555 donkey anti-rabbit (1:1000, Thermo A31572) second antibodies in 5% normal goat serum (Novus), 0.4% Triton x-100 in PBS and finally mounted with the VECTASHIELD Antifade Mounting Medium with DAPI.

All images were captured on Nikon A1R confocal microscope or Zeiss LSM 800 confocal microscope with identical settings for each group in the same experiment. A region of interest (ROI), length or thickness in immunofluorescence experiments were obtained with ImageJ. The number of neurons in specific area was counted blindly and manually. To quantify RGC dendrite lamination in IPL with Thy1-GFP, z-stack and maximum projection were performed during the analysis. GFP intensity values across IPL depth were measured by ImageJ/Analyze/Plot Profile function (*Liu et al. 2018*). To quantify the numbers of Bassoon⁺/PSD-95⁺ excitatory synapses in IPL, the colocalization puncta was measured by ImageJ/Analyze/Puncta Analyzer as described previously (*Ippolito and Eroglu 2010*).

499

500 **Sholl analysis**

501 For confocal images of cultured RGCs, MAP2 signals in original format were analyzed with simple
502 neurite tracer and then quantified with Sholl analysis (5 μm per distance from soma center) which
503 was a widely used method in neurobiology to quantify the complexity of dendritic arbors using
504 ImageJ (*Schindelin et al. 2012; Binley et al. 2014*). Retina wholemount data were captured in z-stack
505 mode (0.5-1 μm per slide) with confocal microscopes. ZsGreen, eGFP and SMI-32 signals were
506 directly analyzed with simple neurite tracer and then z projection of all tracers was quantified with
507 Sholl analysis (10 μm per distance from soma center), while melanopsin signals were maximum-
508 projected before tracing.

509

510 **Optomotor response assay.**

511 *Ythdf2* cKO and control mice aged about 6 weeks were dark-adapted overnight before experiment
512 and used in the optomotor response assay following the previously reported protocols (*Douglas et al.*
513 *2005; Sergeeva et al. 2018*). Using the Matlab program, 0.2 cycle/degree (15 sec per direction of
514 rotation) was first used for mice to adapt this experiment, and 0.3, 0.35, 0.4, 0.43, 0.45, 0.47, 0.5 and
515 0.55 c/deg (30 sec per direction of rotation) were used in the following recordings. Mouse behaviors
516 were analyzed in real time during the experiment and re-checked with video recordings. Finally, data
517 for each mouse were determined by the minimal spatial frequency between left and right optomotor
518 response.

519

520 **CTB Labelling of Optic Nerve**

521 To label RGC axon terminals in mouse brain, RGC axons were anterogradely labeled by CTB (Cholera
522 Toxin Subunit B) conjugated with Alexa Fluor™ 555 (Invitrogen, C34776) through intravitreal injection
523 48 hr before sacrifice. After PFA perfusion, the brains were fixed with 4% PFA in 0.1 M PB overnight,
524 dehydrated with 15% sucrose and 30% sucrose in 0.1 M PB overnight at 4°C sequentially, embedded

with O.C.T. for coronal section, and cryosectioned at 12 μ m with Leica CM1950 Cryostat. After PBS washing, the sections were mounted with VECTASHIELD Antifade Mounting Medium with DAPI (Vector Laboratory). The images were captured on Tissue Genostics with identical settings for each group in the same experiment with the TissueFAXS 7.0 software.

RNA immunoprecipitation and sequencing (RIP-Seq)

For RNA Immunoprecipitation (RIP) experiment, we used the EZ-Magna RIPTM RNA-Binding Protein Immunoprecipitation Kit (Millipore) following the manual with minor modifications. Briefly, 1×10^7 retina neurons were subjected to each 100 μ l lysis buffer. The amount of YTHDF2 antibody (Proteintech, 24744-1-AP) and control IgG used for immunoprecipitation is 5 μ g, respectively. Incubation was done overnight at 4 °C. After quality control monitoring using Agilent 2100, 100 ng RNA of input and elutes after RIP were used to generate the library using the TruSeq Stranded RNA Sample Preparation Kit (Illumina) and sequenced on the Illumina HiSeq 3000 platform (Jingneng, Shanghai, China). The filtered reads were mapped to the mouse reference genome (GRCm38) using STAR v2.5 (*Dobin et al. 2013*) with default parameters. The resulting bam files were fed to HTSeq tool (*Anders et al. 2015*) to count the number of RNA-seq reads, which was further normalized to calculate FPKM. To determine which gene is enriched, we computed the FPKM from RIP elute to input and any fold change greater than 2 (p value less than 0.05) was considered enriched. All enriched genes were used to do the Gene Ontology (GO) analyses. GO enrichment analysis was implemented by the Goseq R package, in which gene length bias was corrected. GO terms with corrected p value less than 0.05 were considered significantly enriched.

MS analysis

E15.5 retinal neurons were cultured and infected with lenti viral *shYthdf2* or *shCtrl*. After puromycin (Sigma) selection, cells were washed with ice-cold PBS and then lysed with freshly prepared lysis buffer composed of 8 M urea (Sigma), 0.1 M HEPES (pH 7.4, Invitrogen), and protease inhibitors

(Roche). The cell lysates were then ultrasonicated on ice and centrifuged at $10,000 \times g$ for 10 min at 4 °C to discard the cell debris. Protein concentration was measured using the BCA Protein Assay Kit (Thermo Scientific). 100 µg of total protein for each group were reduced with 5 mM dithiothreitol (Sigma) for 30 min at 56 °C and then alkylated with 11 mM iodoacetamide (Sigma) for 15 min at RT in dark. After using 100 mM TEAB (Sigma) to dilute the urea concentration to less than 2 M in each sample, trypsin (Promega) was then added to digest the proteins overnight at 37 °C. Peptides were further desalted by Strata X C18 SPE column (Phenomenex) and labelled with TMT10plex Mass Tag Labelling kit (Thermo Scientific) according to the manufacturer's instructions. Finally, the labeled peptides were subjected to HPLC fractionation and LC-MS/MS analysis. Proteins with fold changes greater than 1.3 and p values less than 0.05 were considered to be regulated by YTHDF2 KD with statistical significance.

Anti-m⁶A Immunoprecipitation

Total retinal RNA was extracted from P0 WT mouse pups. Immunoprecipitation of m⁶A-modified transcripts was carried out with Magna MeRIP™ m⁶A Kit (Merck-Millipore, 17-10499) following the manual. m⁶A antibody (Synaptic Systems, 202003) and corresponding control IgG were used in this experiment. The RNA samples pulled down from the experiment were used for RT-qPCR.

Acute ocular hypertension (AOH) model

Mice were anesthetized with 5% chloral hydrate in normal saline (10 µl/g) based on body weight and the Compound Tropicamide Eye Drops were used to scatter pupil. The anterior chamber was penetrated using the 32G × 1/2'' needles (TSK) and filled with the BBS Sterile Irrigating Solution (Alcon) which was hung at a high position to provide proper pressure. Intraocular pressure was measured with the Tonolab tonometer (icare) for every 10 min and maintained at 85-90 mmHg for 1 hr. Levofloxacin hydrochloride was used after the operation and mice were revived in a 37°C environment. Retinas were analyzed for gene expression of YTHDF2 1 day after AOH, gene

expression of *Hspa12a* and *Islr2* 3 days after AOH, dendritic complexity and RGC number 3-7 days after AOH.

Statistical analysis

All experiments were conducted at a minimum of three independent biological replicates (two biological replicates for the RIP assay) or three mice/pups for each genotype/condition in the lab. Data are mean $\pm$ SEM. Statistical analysis was performed using GraphPad Prism 7.0. When comparing the means of two groups, an unpaired or paired *t* test was performed on the basis of experimental design. The settings for all box and whisker plots are: 25th-75th percentiles (boxes), minimum and maximum (whiskers), and medians (horizontal lines). A *p* value less than 0.05 was considered as statistically significant: **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001.

Acknowledgements

We thank Ke Wang and Kwok-Fai So (Jinan University) for help on the optomotor response assay. We thank Mengqing Xiang and Suo Qiu (Zhongshan Ophthalmic Center, Sun Yat-sen University) for help on AAV experiments. We thank other members of Ji laboratory for technical support, helpful discussions and comments on the manuscript. This work was supported by National Natural Science Foundation of China (31871038 and 32170955 to S.-J.J.; 31922027 and 32170958 to B.P.), Shenzhen-Hong Kong Institute of Brain Science-Shenzhen Fundamental Research Institutions (2021SHIBS0002, 2019SHIBS0002), High-Level University Construction Fund for Department of Biology (internal grant no. G02226301), Science and Technology Innovation Commission of Shenzhen Municipal Government (ZDSYS20200811144002008), Program of Shanghai Subject Chief Scientist (21XD1420400), and the Innovative Research Team of High-Level Local University in Shanghai (B.P.).

Author contributions

S.-J.J., F.N. and B.P. formulated the idea and designed the experiments; F.N. performed and analyzed most of the experiments; P.H., J.Z. and Y.S. carried out plasmid construction, AAV injection and imaging; L.Y. performed the RIP-seq experiment; J.Y. performed the MS experiment; M.Z., Y.S., B.Y. and C.L. provided technical help and helped with data analysis; F.N. and K.T. performed the AOH experiments under the supervision of B.P.; the optomotor code was written by B.P.; S.-J.J., F.N., L.Y., and J.Y. wrote the manuscript with inputs from other authors.

Ethics

All experiments using mice were carried out following the animal protocols approved by the Laboratory Animal Welfare and Ethics Committee of Southern University of Science and Technology (approval numbers: SUSTC-JY2017004, SUSTC-JY2019081).

Competing interests

The authors have declared that no competing interests exist.

Data availability statement

The RIP-seq data have been deposited to the Gene Expression Omnibus (GEO) with accession number GSE145390. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD017775.

References

- Agostinone J, Di Polo A. 2015. Retinal ganglion cell dendrite pathology and synapse loss: Implications for glaucoma. *Prog Brain Res* **220**:199-216. DOI: <https://doi.org/10.1016/bs.pbr.2015.04.012>, PMID: 26497792
- Agudo M, Perez-Marin MC, Lonngren U, Sobrado P, Conesa A, Canovas I, Salinas-Navarro M, Miralles-Imperial J, Hallbook F, Vidal-Sanz M. 2008. Time course profiling of the retinal transcriptome after optic nerve transection and optic nerve crush. *Mol Vis* **14**:1050-1063. PMID: 18552980

- Anders S, Pyl PT, Huber W. 2015. HTSeq--a Python framework to work with high-throughput sequencing data. *Bioinformatics* **31**:166-169. DOI: <https://doi.org/10.1093/bioinformatics/btu638>, PMID: 25260700
- Baden T, Berens P, Franke K, Roman Roson M, Bethge M, Euler T. 2016. The functional diversity of retinal ganglion cells in the mouse. *Nature* **529**:345-350. DOI: <https://doi.org/10.1038/nature16468>, PMID: 26735013
- Benoist M, Gaillard S, Castets F. 2006. The striatin family: a new signaling platform in dendritic spines. *J Physiol Paris* **99**:146-153. DOI: <https://doi.org/10.1016/j.jphysparis.2005.12.006>, PMID: 16460920
- Binley KE, Ng WS, Tribble JR, Song B, Morgan JE. 2014. Sholl analysis: a quantitative comparison of semi-automated methods. *J Neurosci Methods* **225**:65-70. DOI: <https://doi.org/10.1016/j.jneumeth.2014.01.017>, PMID: 24485871
- Brechbiel JL, Gavis ER. 2008. Spatial regulation of nanos is required for its function in dendrite morphogenesis. *Curr Biol* **18**:745-750. DOI: <https://doi.org/10.1016/j.cub.2008.04.033>, PMID: 18472422
- Breitman M, Zilberberg A, Caspi M, Rosin-Arbesfeld R. 2008. The armadillo repeat domain of the APC tumor suppressor protein interacts with Striatin family members. *Biochim Biophys Acta* **1783**:1792-1802. DOI: <https://doi.org/10.1016/j.bbamcr.2008.04.017>, PMID: 18502210
- Cahill ME, Xie Z, Day M, Photowala H, Barbolina MV, Miller CA, Weiss C, Radulovic J, Sweatt JD, Disterhoft JF, Surmeier DJ, Penzes P. 2009. Kalirin regulates cortical spine morphogenesis and disease-related behavioral phenotypes. *Proc Natl Acad Sci U S A* **106**:13058-13063. DOI: <https://doi.org/10.1073/pnas.0904636106>, PMID: 19625617
- Cherry TJ, Wang S, Bormuth I, Schwab M, Olson J, Cepko CL. 2011. NeuroD factors regulate cell fate and neurite stratification in the developing retina. *J Neurosci* **31**:7365-7379. DOI: <https://doi.org/10.1523/jneurosci.2555-10.2011>, PMID: 21593321
- Cheung ZH, Chin WH, Chen Y, Ng YP, Ip NY. 2007. Cdk5 is involved in BDNF-stimulated dendritic growth in hippocampal neurons. *PLoS Biol* **5**:e63. DOI: <https://doi.org/10.1371/journal.pbio.0050063>, PMID: 17341134
- Chihara T, Luginbuhl D, Luo L. 2007. Cytoplasmic and mitochondrial protein translation in axonal and dendritic terminal arborization. *Nat Neurosci* **10**:828-837. DOI: <https://doi.org/10.1038/nn1910>, PMID: 17529987
- Cubelos B, Briz CG, Esteban-Ortega GM, Nieto M. 2015. Cux1 and Cux2 selectively target basal and apical dendritic compartments of layer II-III cortical neurons. *Dev Neurobiol* **75**:163-172. DOI: <https://doi.org/10.1002/dneu.22215>, PMID: 25059644
- Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. 2013. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**:15-21. DOI: <https://doi.org/10.1093/bioinformatics/bts635>, PMID: 23104886
- Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, Cesarkas K, Jacob-Hirsch J, Amariglio N, Kupiec M, Sorek R, Rechavi G. 2012. Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. *Nature* **485**:201-206. DOI: <https://doi.org/10.1038/nature11112>, PMID: 22575960
- Dong X, Shen K, Bulow HE. 2015. Intrinsic and extrinsic mechanisms of dendritic morphogenesis. *Annu Rev Physiol* **77**:271-300. DOI: <https://doi.org/10.1146/annurev-physiol-021014-071746>, PMID: 25386991
- Douglas RM, Alam NM, Silver BD, McGill TJ, Tschetter WW, Prusky GT. 2005. Independent visual threshold measurements in the two eyes of freely moving rats and mice using a virtual-reality optokinetic system. *Vis Neurosci* **22**:677-684. DOI: <https://doi.org/10.1017/s0952523805225166>, PMID: 16332278
- Feng G, Mellor RH, Bernstein M, Keller-Peck C, Nguyen QT, Wallace M, Nerbonne JM, Lichtman JW, Sanes JR. 2000. Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. *Neuron* **28**:41-51. PMID: 11086982

- 681 Furuta Y, Lagutin O, Hogan BL, Oliver GC. 2000. Retina- and ventral forebrain-specific Cre
682 recombinase activity in transgenic mice. *Genesis* **26**:130-132. PMID: 10686607
- 683 Hattar S, Liao HW, Takao M, Berson DM, Yau KW. 2002. Melanopsin-containing retinal ganglion cells:
684 architecture, projections, and intrinsic photosensitivity. *Science* **295**:1065-1070. DOI:
685 <https://doi.org/10.1126/science.1069609>, PMID: 11834834
- 686 Herring BE, Nicoll RA. 2016. Kalirin and Trio proteins serve critical roles in excitatory synaptic
687 transmission and LTP. *Proc Natl Acad Sci U S A* **113**:2264-2269. DOI:
688 <https://doi.org/10.1073/pnas.1600179113>, PMID: 26858404
- 689 Ippolito DM, Eroglu C. 2010. Quantifying synapses: an immunocytochemistry-based assay to quantify
690 synapse number. *J Vis Exp*. DOI: <https://doi.org/10.3791/2270>, PMID: 21113117
- 691 Jakobs TC. 2014. Differential gene expression in glaucoma. *Cold Spring Harb Perspect Med* **4**:a020636.
692 DOI: <https://doi.org/10.1101/cshperspect.a020636>, PMID: 24985133
- 693 Jan YN, Jan LY. 2010. Branching out: mechanisms of dendritic arborization. *Nat Rev Neurosci* **11**:316-
694 328. DOI: <https://doi.org/10.1038/nrn2836>, PMID: 20404840
- 695 Kay JN, De la Huerta I, Kim IJ, Zhang Y, Yamagata M, Chu MW, Meister M, Sanes JR. 2011. Retinal
696 ganglion cells with distinct directional preferences differ in molecular identity, structure, and
697 central projections. *J Neurosci* **31**:7753-7762. DOI: [https://doi.org/10.1523/jneurosci.0907-](https://doi.org/10.1523/jneurosci.0907-11.2011)
698 [11.2011](https://doi.org/10.1523/jneurosci.0907-11.2011), PMID: 21613488
- 699 Kay JN, Voinescu PE, Chu MW, Sanes JR. 2011. Neurod6 expression defines new retinal amacrine cell
700 subtypes and regulates their fate. *Nat Neurosci* **14**:965-972. DOI: <https://doi.org/10.1038/nn.2859>,
701 PMID: 21743471
- 702 Kechad A, Jolicoeur C, Tufford A, Mattar P, Chow RWY, Harris WA, Cayouette M. 2012. Numb is
703 required for the production of terminal asymmetric cell divisions in the developing mouse retina. *J*
704 *Neurosci* **32**:17197-17210. DOI: <https://doi.org/10.1523/jneurosci.4127-12.2012>, PMID: 23197712
- 705 Krishnaswamy A, Yamagata M, Duan X, Hong YK, Sanes JR. 2015. Sidekick 2 directs formation of a
706 retinal circuit that detects differential motion. *Nature* **524**:466-470. DOI:
707 <https://doi.org/10.1038/nature14682>, PMID: 26287463
- 708 Lanoue V, Langford M, White A, Sempert K, Fogg L, Cooper HM. 2017. The Wnt receptor Ryk is a
709 negative regulator of mammalian dendrite morphogenesis. *Sci Rep* **7**:5965. DOI:
710 <https://doi.org/10.1038/s41598-017-06140-z>, PMID: 28729735
- 711 Ledda F, Paratcha G. 2017. Mechanisms regulating dendritic arbor patterning. *Cell Mol Life Sci*
712 **74**:4511-4537. DOI: <https://doi.org/10.1007/s00018-017-2588-8>, PMID: 28735442
- 713 Lefebvre JL, Kostadinov D, Chen WV, Maniatis T, Sanes JR. 2012. Protocadherins mediate dendritic
714 self-avoidance in the mammalian nervous system. *Nature* **488**:517-521. DOI:
715 <https://doi.org/10.1038/nature11305>, PMID: 22842903
- 716 Lefebvre JL, Sanes JR, Kay JN. 2015. Development of dendritic form and function. *Annu Rev Cell Dev*
717 *Biol* **31**:741-777. DOI: <https://doi.org/10.1146/annurev-cellbio-100913-013020>, PMID: 26422333
- 718 Li D, Musante V, Zhou W, Picciotto MR, Nairn AC. 2018. Striatin-1 is a B subunit of protein
719 phosphatase PP2A that regulates dendritic arborization and spine development in striatal neurons.
720 *J Biol Chem* **293**:11179-11194. DOI: <https://doi.org/10.1074/jbc.RA117.001519>, PMID: 29802198
- 721 Lin B, Wang SW, Masland RH. 2004. Retinal ganglion cell type, size, and spacing can be specified
722 independent of homotypic dendritic contacts. *Neuron* **43**:475-485. DOI:
723 <https://doi.org/10.1016/j.neuron.2004.08.002>, PMID: 15312647
- 724 Lin L, Lo LH, Lyu Q, Lai KO. 2017. Determination of dendritic spine morphology by the striatin scaffold
725 protein STRN4 through interaction with the phosphatase PP2A. *J Biol Chem* **292**:9451-9464. DOI:
726 <https://doi.org/10.1074/jbc.M116.772442>, PMID: 28442576
- 727 Liu J, Reggiani JDS, Laboulaye MA, Pandey S, Chen B, Rubenstein JLR, Krishnaswamy A, Sanes JR. 2018.
728 Tbr1 instructs laminar patterning of retinal ganglion cell dendrites. *Nat Neurosci* **21**:659-670. DOI:
729 <https://doi.org/10.1038/s41593-018-0127-z>, PMID: 29632360
- 730 Liu J, Sanes JR. 2017. Cellular and Molecular Analysis of Dendritic Morphogenesis in a Retinal Cell
731 Type That Senses Color Contrast and Ventral Motion. *J Neurosci* **37**:12247-12262. DOI:
732 <https://doi.org/10.1523/jneurosci.2098-17.2017>, PMID: 29114073

- 733 Livneh I, Moshitch-Moshkovitz S, Amariglio N, Rechavi G, Dominissini D. 2020. The m(6)A
734 epitranscriptome: transcriptome plasticity in brain development and function. *Nat Rev Neurosci*
735 **21**:36-51. DOI: <https://doi.org/10.1038/s41583-019-0244-z>, PMID: 31804615
- 736 Lu J, Luo C, Bali KK, Xie RG, Mains RE, Eipper BA, Kuner R. 2015. A role for Kalirin-7 in nociceptive
737 sensitization via activity-dependent modulation of spinal synapses. *Nat Commun* **6**:6820. DOI:
738 <https://doi.org/10.1038/ncomms7820>, PMID: 25865668
- 739 Mains RE, Kiraly DD, Eipper-Mains JE, Ma XM, Eipper BA. 2011. Kalrn promoter usage and isoform
740 expression respond to chronic cocaine exposure. *BMC neuroscience* **12**:20. DOI:
741 <https://doi.org/10.1186/1471-2202-12-20>, PMID: 21329509
- 742 Mao Y, Kong Q, Li R, Zhang X, Gui Y, Li Y, Li C, Zhao Y, Liu L, Ding Z. 2018. Heat shock protein A12A
743 encodes a novel prosurvival pathway during ischaemic stroke. *Biochim Biophys Acta Mol Basis Dis*
744 **1864**:1862-1872. DOI: <https://doi.org/10.1016/j.bbadis.2018.03.006>, PMID: 29530582
- 745 Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE, Jaffrey SR. 2012. Comprehensive analysis of
746 mRNA methylation reveals enrichment in 3' UTRs and near stop codons. *Cell* **149**:1635-1646. DOI:
747 <https://doi.org/10.1016/j.cell.2012.05.003>, PMID: 22608085
- 748 Min X, Zhang X, Li Y, Cao X, Cheng H, Li Y, Li C, Kong Q, Mao Q, Peng P, Ni Y, Li J, Duan Y, Liu L, Ding Z.
749 2020. HSPA12A unstabilizes CD147 to inhibit lactate export and migration in human renal cell
750 carcinoma. *Theranostics* **10**:8573-8590. DOI: <https://doi.org/10.7150/thno.44321>, PMID:
751 32754264
- 752 Morgan JE, Datta AV, Erichsen JT, Albon J, Boulton ME. 2006. Retinal ganglion cell remodelling in
753 experimental glaucoma. *Adv Exp Med Biol* **572**:397-402. DOI: [https://doi.org/10.1007/0-387-](https://doi.org/10.1007/0-387-32442-9_56)
754 [32442-9](https://doi.org/10.1007/0-387-32442-9_56) 56, PMID: 17249602
- 755 Nachtergaele S, He C. 2018. Chemical Modifications in the Life of an mRNA Transcript. *Annu Rev*
756 *Genet* **52**:349-372. DOI: <https://doi.org/10.1146/annurev-genet-120417-031522>, PMID: 30230927
- 757 Parsons K, Nakatani Y, Nguyen MD. 2015. p600/UBR4 in the central nervous system. *Cell Mol Life Sci*
758 **72**:1149-1160. DOI: <https://doi.org/10.1007/s00018-014-1788-8>, PMID: 25424645
- 759 Penzes P, Johnson RC, Sattler R, Zhang X, Huganir RL, Kambampati V, Mains RE, Eipper BA. 2001. The
760 neuronal Rho-GEF Kalirin-7 interacts with PDZ domain-containing proteins and regulates dendritic
761 morphogenesis. *Neuron* **29**:229-242. DOI: [https://doi.org/10.1016/s0896-6273\(01\)00193-3](https://doi.org/10.1016/s0896-6273(01)00193-3), PMID:
762 11182094
- 763 Pongrac JL, Middleton FA, Peng L, Lewis DA, Levitt P, Mirnics K. 2004. Heat shock protein 12A shows
764 reduced expression in the prefrontal cortex of subjects with schizophrenia. *Biol Psychiatry* **56**:943-
765 950. DOI: <https://doi.org/10.1016/j.biopsych.2004.09.005>, PMID: 15601604
- 766 Prigge CL, Kay JN. 2018. Dendrite morphogenesis from birth to adulthood. *Curr Opin Neurobiol*
767 **53**:139-145. DOI: <https://doi.org/10.1016/j.conb.2018.07.007>, PMID: 30092409
- 768 Prusky GT, Alam NM, Beekman S, Douglas RM. 2004. Rapid quantification of adult and developing
769 mouse spatial vision using a virtual optomotor system. *Invest Ophthalmol Vis Sci* **45**:4611-4616.
770 DOI: <https://doi.org/10.1167/iovs.04-0541>, PMID: 15557474
- 771 Ramos B, Gaudilliere B, Bonni A, Gill G. 2007. Transcription factor Sp4 regulates dendritic patterning
772 during cerebellar maturation. *Proc Natl Acad Sci U S A* **104**:9882-9887. DOI:
773 <https://doi.org/10.1073/pnas.0701946104>, PMID: 17535924
- 774 Riccomagno MM, Sun LO, Brady CM, Alexandropoulos K, Seo S, Kurokawa M, Kolodkin AL. 2014. Cas
775 adaptor proteins organize the retinal ganglion cell layer downstream of integrin signaling. *Neuron*
776 **81**:779-786. DOI: <https://doi.org/10.1016/j.neuron.2014.01.036>, PMID: 24559672
- 777 Rosso SB, Sussman D, Wynshaw-Boris A, Salinas PC. 2005. Wnt signaling through Dishevelled, Rac
778 and JNK regulates dendritic development. *Nat Neurosci* **8**:34-42. DOI:
779 <https://doi.org/10.1038/nn1374>, PMID: 15608632
- 780 Russell TA, Blizinsky KD, Cobia DJ, Cahill ME, Xie Z, Sweet RA, Duan J, Gejman PV, Wang L, Csernansky
781 JG, Penzes P. 2014. A sequence variant in human KALRN impairs protein function and coincides
782 with reduced cortical thickness. *Nat Commun* **5**:4858. DOI: <https://doi.org/10.1038/ncomms5858>,
783 PMID: 25224588

- Sanes JR, Masland RH. 2015. The types of retinal ganglion cells: current status and implications for neuronal classification. *Annu Rev Neurosci* **38**:221-246. DOI: <https://doi.org/10.1146/annurev-neuro-071714-034120>, PMID: 25897874
- Sapkota D, Chintala H, Wu F, Fliesler SJ, Hu Z, Mu X. 2014. Onecut1 and Onecut2 redundantly regulate early retinal cell fates during development. *Proc Natl Acad Sci U S A* **111**:E4086-4095. DOI: <https://doi.org/10.1073/pnas.1405354111>, PMID: 25228773
- Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez JY, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A. 2012. Fiji: an open-source platform for biological-image analysis. *Nat Methods* **9**:676-682. DOI: <https://doi.org/10.1038/nmeth.2019>, PMID: 22743772
- Sergeeva EG, Espinosa-Garcia C, Atif F, Pardue MT, Stein DG. 2018. Neurosteroid allopregnanolone reduces ipsilateral visual cortex potentiation following unilateral optic nerve injury. *Exp Neurol* **306**:138-148. DOI: <https://doi.org/10.1016/j.expneurol.2018.05.005>, PMID: 29729249
- Shi C, Yuan X, Chang K, Cho KS, Xie XS, Chen DF, Luo G. 2018. Optimization of Optomotor Response-based Visual Function Assessment in Mice. *Sci Rep* **8**:9708. DOI: <https://doi.org/10.1038/s41598-018-27329-w>, PMID: 29946119
- Shim SY, Wang J, Asada N, Neumayer G, Tran HC, Ishiguro K, Sanada K, Nakatani Y, Nguyen MD. 2008. Protein 600 is a microtubule/endoplasmic reticulum-associated protein in CNS neurons. *J Neurosci* **28**:3604-3614. DOI: <https://doi.org/10.1523/jneurosci.5278-07.2008>, PMID: 18385319
- Shima Y, Kawaguchi SY, Kosaka K, Nakayama M, Hoshino M, Nabeshima Y, Hirano T, Uemura T. 2007. Opposing roles in neurite growth control by two seven-pass transmembrane cadherins. *Nat Neurosci* **10**:963-969. DOI: <https://doi.org/10.1038/nn1933>, PMID: 17618280
- Shima Y, Kengaku M, Hirano T, Takeichi M, Uemura T. 2004. Regulation of dendritic maintenance and growth by a mammalian 7-pass transmembrane cadherin. *Dev Cell* **7**:205-216. DOI: <https://doi.org/10.1016/j.devcel.2004.07.007>, PMID: 15296717
- Shou T, Liu J, Wang W, Zhou Y, Zhao K. 2003. Differential dendritic shrinkage of alpha and beta retinal ganglion cells in cats with chronic glaucoma. *Invest Ophthalmol Vis Sci* **44**:3005-3010. DOI: <https://doi.org/10.1167/iovs.02-0620>, PMID: 12824245
- Srinivas S, Watanabe T, Lin CS, William CM, Tanabe Y, Jessell TM, Costantini F. 2001. Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC Dev Biol* **1**:4. PMID: 11299042
- Tran NM, Shekhar K, Whitney IE, Jacobi A, Benhar I, Hong G, Yan W, Adiconis X, Arnold ME, Lee JM, Levin JZ, Lin D, Wang C, Lieber CM, Regev A, He Z, Sanes JR. 2019. Single-Cell Profiles of Retinal Ganglion Cells Differing in Resilience to Injury Reveal Neuroprotective Genes. *Neuron*. DOI: <https://doi.org/10.1016/j.neuron.2019.11.006>, PMID: 31784286
- Umino Y, Solessio E, Barlow RB. 2008. Speed, spatial, and temporal tuning of rod and cone vision in mouse. *J Neurosci* **28**:189-198. DOI: <https://doi.org/10.1523/jneurosci.3551-07.2008>, PMID: 18171936
- Vessey JP, Macchi P, Stein JM, Mikl M, Hawker KN, Vogelsang P, Wieczorek K, Vendra G, Riefler J, Tubing F, Aparicio SA, Abel T, Kiebler MA. 2008. A loss of function allele for murine Stauf1 leads to impairment of dendritic Stauf1-RNP delivery and dendritic spine morphogenesis. *Proc Natl Acad Sci U S A* **105**:16374-16379. DOI: <https://doi.org/10.1073/pnas.0804583105>, PMID: 18922781
- Wang X, Lu Z, Gomez A, Hon GC, Yue Y, Han D, Fu Y, Parisien M, Dai Q, Jia G, Ren B, Pan T, He C. 2014. N6-methyladenosine-dependent regulation of messenger RNA stability. *Nature* **505**:117-120. DOI: <https://doi.org/10.1038/nature12730>, PMID: 24284625
- Wang Y, Li Y, Yue M, Wang J, Kumar S, Wechsler-Reya RJ, Zhang Z, Ogawa Y, Kellis M, Duester G, Zhao JC. 2018. N(6)-methyladenosine RNA modification regulates embryonic neural stem cell self-renewal through histone modifications. *Nat Neurosci* **21**:195-206. DOI: <https://doi.org/10.1038/s41593-017-0057-1>, PMID: 29335608
- Weber AJ, Kaufman PL, Hubbard WC. 1998. Morphology of single ganglion cells in the glaucomatous primate retina. *Invest Ophthalmol Vis Sci* **39**:2304-2320. PMID: 9804139

Xie Z, Cahill ME, Penzes P. 2010. Kalirin loss results in cortical morphological alterations. *Mol Cell Neurosci* **43**:81-89. DOI: <https://doi.org/10.1016/j.mcn.2009.09.006>, PMID: 19800004

Xie Z, Srivastava DP, Photowala H, Kai L, Cahill ME, Woolfrey KM, Shum CY, Surmeier DJ, Penzes P. 2007. Kalirin-7 controls activity-dependent structural and functional plasticity of dendritic spines. *Neuron* **56**:640-656. DOI: <https://doi.org/10.1016/j.neuron.2007.10.005>, PMID: 18031682

Yan Y, Eipper BA, Mains RE. 2015. Kalirin-9 and Kalirin-12 Play Essential Roles in Dendritic Outgrowth and Branching. *Cereb Cortex* **25**:3487-3501. DOI: <https://doi.org/10.1093/cercor/bhu182>, PMID: 25146373

Ye B, Petritsch C, Clark IE, Gavis ER, Jan LY, Jan YN. 2004. Nanos and Pumilio are essential for dendrite morphogenesis in Drosophila peripheral neurons. *Curr Biol* **14**:314-321. DOI: <https://doi.org/10.1016/j.cub.2004.01.052>, PMID: 14972682

Yu J, Chen M, Huang H, Zhu J, Song H, Zhu J, Park J, Ji SJ. 2018. Dynamic m6A modification regulates local translation of mRNA in axons. *Nucleic acids research* **46**:1412-1423. DOI: <https://doi.org/10.1093/nar/gkx1182>, PMID: 29186567

Yu J, She Y, Ji SJ. 2021. m(6)A Modification in Mammalian Nervous System Development, Functions, Disorders, and Injuries. *Front Cell Dev Biol* **9**:679662. DOI: <https://doi.org/10.3389/fcell.2021.679662>, PMID: 34113622

Yu J, She Y, Yang L, Zhuang M, Han P, Liu J, Lin X, Wang N, Chen M, Jiang C, Zhang Y, Yuan Y, Ji SJ. 2021. The m(6) A Readers YTHDF1 and YTHDF2 Synergistically Control Cerebellar Parallel Fiber Growth by Regulating Local Translation of the Key Wnt5a Signaling Components in Axons. *Adv Sci (Weinh)* **8**:e2101329. DOI: <https://doi.org/10.1002/advs.202101329>, PMID: 34643063

Zhou X, Qyang Y, Kelsoe JR, Masliah E, Geyer MA. 2007. Impaired postnatal development of hippocampal dentate gyrus in Sp4 null mutant mice. *Genes Brain Behav* **6**:269-276. DOI: <https://doi.org/10.1111/j.1601-183X.2006.00256.x>, PMID: 16899055

(A-D) Representative confocal images showing high levels of m⁶A modification **(A)**, and strong expressions of YTHDF2 **(B)**, YTHDF1 **(C)**, and YTHDF3 **(D)** in RGCs (marked by Brn3a) in P0 retina. Scale bars: 20 μ m.

(E, F) Western blotting (WB) confirming efficient knockdown (KD) of YTHDF1 and YTHDF3 in cultured RGCs using *shYthdf1* and *shYthdf3*, respectively.

(G, H) Quantification of total length **(G)** and length of maximum branch **(H)** of RGC dendrites after YTHDF2 KD. Data are represented as box and whisker plots: $n = 36$ RGCs for *shCtrl*, $n = 32$ RGCs for *shYthdf2*; *** $p = 0.00062$ for **G**; $p = 0.22$ for **H**; ns, not significant; by unpaired Student's t test.

(I, J) Quantification of dendrite branching using Sholl analysis after YTHDF1 KD **(I)** and YTHDF3 KD **(J)**. Data are mean $\pm$ SEM. In **I**, $n = 29$ RGCs for *shCtrl*, $n = 32$ RGCs for *shYthdf1*, all not significant; in **J**, $n = 32$ RGCs for *shCtrl*, $n = 27$ RGCs for *shYthdf3*, ** $p = 0.0028$ (20 μ m), ** $p = 0.0028$ (30 μ m), ** $p = 0.0052$ (35 μ m); by unpaired Student's t test.

Figure 2. Dendrite branching is dramatically increased in cultured RGCs from *Ythdf2* cKO.

899 **(A)** Schematic drawings of the genetic deletion strategy for *Ythdf2*. Exon 4 which contains YTH
900 domain-coding sequence is deleted after Cre-mediated recombination.

901 **(B)** Depletion of YTHDF2 protein in retina of *Six3-cre^{+/-};Ythdf2^{fl/fl}* cKO mice. Anti-YTHDF2
902 immunostaining of E15.5 retina vertical sections confirmed cKO of YTHDF2 protein, compared with
903 *Ythdf2^{fl/fl}* littermate controls. Scale bar: 20 μ m.

904 **(C, D)** RGC neurogenesis not affected in the *Ythdf2* cKO retina. Wholemount immunostaining using a
905 Brn3a antibody was carried out in P20 retina **(C)**. Numbers of Brn3a⁺ RGC per 10000 μ m² of retina
906 were quantified and showed no difference between the *Ythdf2* cKO and their littermate controls **(D)**.
907 $n = 12$ confocal fields for each genotype. Data are represented as box and whisker plots: ns, not
908 significant ($p = 0.79$); by unpaired Student's t test. Scale bar: 25 μ m.

909 **(E)** Examination of RGC dendrite development in *Ythdf2* cKO RGCs. As shown, knockout of YTHDF2
910 was confirmed by YTHDF2 IF (green). Significantly increased branching of dendrites marked by MAP2
911 IF (red) was observed in cultured RGCs from the *Ythdf2* cKO retina compared with their littermate
912 controls. Dendrite traces were drawn for the corresponding RGCs. Scale bar: 10 μ m.

913 **(F)** Quantification of RGC dendrite branching **(E)** using Sholl analysis. Data are mean $\pm$ SEM. Numbers
914 of interactions are significantly greater in *Six3-cre^{+/-};Ythdf2^{fl/fl}* groups ($n = 68$ RGCs) than *Ythdf2^{fl/fl}*
915 groups ($n = 42$ RGCs) in Sholl radii between 10-30 μ m: *** $p = 0.00030$ (10 μ m), **** $p = 1.19E-05$ (15
916 μ m), *** $p = 0.00018$ (20 μ m), * $p = 0.021$ (25 μ m), ** $p = 0.0022$ (30 μ m), by unpaired Student's t test.

917 **(G)** Examination of CART⁺ RGC dendrite development in *Ythdf2* cKO RGCs. Cultured CART⁺ RGCs from
918 the *Ythdf2* cKO retina have significantly increased branching of dendrites marked by MAP2 IF (red)
919 compared with their littermate controls. Dendrite traces were drawn for the corresponding RGCs.
920 Scale bar: 10 μ m.

921 **(H)** Quantification of CART⁺ RGC dendrite branching **(G)** using Sholl analysis. Data are mean $\pm$ SEM.
922 Numbers of interactions are significantly greater in *Six3-cre^{+/-};Ythdf2^{fl/fl}* groups ($n = 77$ RGCs) than
923 *Ythdf2^{fl/fl}* groups ($n = 90$ RGCs) in Sholl radii between 10-30 μ m: **** $p = 3.17E-05$ (10 μ m), **** $p =$

924 6.50E-11 (15 μm), **** $p = 5.14\text{E-}12$ (20 μm), **** $p = 5.00\text{E-}07$ (25 μm), *** $p = 0.00020$ (30 μm), by
 925 unpaired Student's t test.
 926
 927

Figure 2—figure supplement 1. *Ythdf2* cKO does not change numbers of retinal progenitors, amacrine cells, bipolar cells, photoreceptors, or horizontal cells.

931 **(A)** YTHDF2 protein was efficiently knocked out in the retinas of Six3-Cre-mediated *Ythdf2* cKO mice
 932 at E12.5.

933 **(B, C)** Retinal progenitors not affected in *Ythdf2* cKO retina. CHX10 IF was used to label retinal
 934 progenitors at E15.5 **(B)**. Thickness of CHX10⁺ retinal layer was quantified and showed no difference
 935 between the *Ythdf2* cKO retina ($n = 10$ sections) and their littermate controls ($n = 19$ sections) **(C)**.

936 **(D, E)** Amacrine cells not affected in the *Ythdf2* cKO retina. AP2 α IF was used to mark amacrine cells
 937 in P6 retina **(D)**. Numbers of AP2 α ⁺ amacrine cells per 100 μm of layer width in retina vertical
 938 sections were quantified and showed no difference between the *Ythdf2* cKO ($n = 26$ sections) and
 939 littermate controls ($n = 26$ sections) **(E)**. ONL, outer nuclear layer; INL, inner nuclear layer; IPL, inner
 940 plexiform layer.

941 **(F-H)** Bipolar cells not changed in the *Ythdf2* cKO retina. PKC α and CHX10 IF were used to label
 942 different bipolar cells in P15 retina **(F)**. Numbers of PKC α ⁺ and CHX10⁺ bipolar cells per 100 μm of
 943 layer width in retina vertical sections were quantified and showed no difference between the *Ythdf2*
 944 cKO ($n = 18$ sections for PKC α ⁺ in **G**, $n = 19$ sections for CHX10⁺ in **H**) and littermate controls ($n = 18$
 945 sections for PKC α ⁺ in **G**, $n = 19$ sections for CHX10⁺ in **H**).

946 **(I, J)** Photoreceptors not changed in the *Ythdf2* cKO retina. Recoverin IF was used to label
 947 photoreceptors in P20 retina **(I)**. Thickness of Recoverin⁺ photoreceptor layer (e.g. ONL) in the retinal
 948 vertical sections was quantified and showed no difference between the *Ythdf2* cKO ($n = 34$ confocal
 949 fields) and littermate controls ($n = 30$ confocal fields) **(J)**.

950 **(K, L)** Horizontal cells not affected in the *Ythdf2* cKO retina. Calbindin IF was used to mark horizontal
 951 cells in P20 retina (arrowheads in **K**). Numbers of Calbindin⁺ horizontal cells per 100 μm of layer
 952 width in retina vertical sections were quantified and showed no difference between the *Ythdf2* cKO
 953 ($n = 17$ sections) and littermate controls ($n = 17$ sections) **(L)**.

954 All quantification data are represented as box and whisker plots: ns, not significant; $p = 0.41$ for **C**, $p =$
 955 0.16 for **E**, $p = 0.82$ for **G**, $p = 0.97$ for **H**, $p = 0.89$ for **J**, $p = 0.19$ for **L**; by unpaired Student's t test.

956 Scale bars: 20 μm .

Figure 3. Dendrite branching of specific RGC subtypes increases in *Ythdf2* cKO in vivo.

(A) Co-labeling of ooDSGCs by AAV-ZsGreen and CART IF in vivo. Intravitreal injection of AAV expressing ZsGreen reporter was performed at P17 and retinas were collected at P27. The white arrowheads indicate ooDSGCs co-labeled by ZsGreen and CART IF, which show dramatically increased

dendrite branching in *Ythdf2* cKO compared with control. Dendrite traces were drawn for the corresponding RGCs shown. Scale bar: 20 μ m.

(B) Quantification of dendrite branching of ZsGreen⁺/CART⁺ ooDSGCs **(A)** using Sholl analysis. Data are mean $\pm$ SEM. Numbers of interactions are significantly greater in *Six3-cre*^{+/-}, *Ythdf2*^{fl/fl} groups (n = 15 RGCs) than *Ythdf2*^{fl/fl} groups (n = 18 RGCs) in Sholl radii between 50-120 μ m: ** p = 0.0041 (50 μ m), ** p = 0.0059 (60 μ m), *** p = 0.00036 (70 μ m), ** p = 0.0058 (80 μ m), ** p = 0.0018 (90 μ m), ** p = 0.0064 (100 μ m), ** p = 0.0045 (110 μ m), * p = 0.040 (120 μ m), by unpaired Student's t test.

(C) Dendrites of ipRGCs visualized by wholemount immunostaining of P20 retina using a melanopsin antibody in vivo. Dendrite traces were drawn for the corresponding RGCs shown. Scale bar: 50 μ m.

(D) Quantification of dendrite branching of melanopsin⁺ ipRGCs **(C)** using Sholl analysis. Data are mean $\pm$ SEM. Numbers of interactions are significantly greater in *Six3-cre*^{+/-}, *Ythdf2*^{fl/fl} groups (n = 18 RGCs) than *Ythdf2*^{fl/fl} groups (n = 21 RGCs) in Sholl radii between 20-100 μ m: ** p = 0.0083 (20 μ m), * p = 0.018 (40 μ m), *** p = 0.00068 (50 μ m), *** p = 0.00027 (60 μ m), * p = 0.048 (70 μ m), ** p = 0.0048 (80 μ m), ** p = 0.0023 (100 μ m), by unpaired Student's t test.

(E) Dendrites of α RGCs visualized by wholemount immunostaining of P20 retina using a SMI-32 antibody in vivo. Dendrite traces were drawn for the corresponding RGCs shown. Scale bar: 20 μ m.

(F) Quantification of dendrite branching of SMI-32⁺ α RGCs **(E)** using Sholl analysis. Data are mean $\pm$ SEM. Numbers of interactions are significantly greater in *Six3-cre*^{+/-}, *Ythdf2*^{fl/fl} groups (n = 14 RGCs) than *Ythdf2*^{fl/fl} groups (n = 22 RGCs) in Sholl radii between 40-140 μ m: ** p = 0.0044 (40 μ m), ** p = 0.0035 (50 μ m), *** p = 0.00021 (60 μ m), **** p = 2.63E-05 (70 μ m), **** p = 2.38E-06 (80 μ m), **** p = 1.68E-06 (90 μ m), **** p = 6.76E-06 (100 μ m), **** p = 5.72E-05 (110 μ m), ** p = 0.0011 (120 μ m), ** p = 0.0032 (130 μ m), * p = 0.047 (140 μ m), by unpaired Student's t test.

985

Figure 3—figure supplement 1. General dendrite density in IPL is increased without affecting sublaminal targeting.

(A-E) Quantification of total length **(A)**, length of maximum branch **(B)**, branch numbers **(C)**, number of total segments **(D)**, and numbers of segments on each branch order **(E)** of melanopsin⁺ ipRGCs dendrites visualized by wholemount immunostaining of P20 retina using a melanopsin antibody in vivo (shown in *Figure 3C*). Data are represented as box and whisker plots in **A-D**: $n = 58$ RGCs for *Ythdf2*^{fl/fl}, $n = 51$ RGCs for *Six3-cre*^{+/-}, *Ythdf2*^{fl/fl}; * $p = 0.040$ for **A**; $p = 0.12$ for **B**; **** $p = 1.39E-06$ for **C**; * $p = 7.89E-08$ for **D**; ns, not significant. Data are mean $\pm$ SEM in **E**: * $p = 0.038$ (branch order 1), * $p = 0.039$ (branch order 2), *** $p = 0.00045$ (branch order 4), * $p = 0.026$ (branch order 5), * $p = 0.029$ (branch order 6). All by unpaired Student's *t* test.

(F) Cross-sections of the IPL showing dendritic sublaminal patterning of Thy1-GFP⁺ RGCs in P20 control and *Ythdf2* cKO retina. ON and OFF refer to the ON-OFF bipartite divisions of the IPL marked by VACHT. Scale bar: 20 μ m.

(G) Quantification and distribution of GFP intensities from Thy1-GFP⁺ RGC dendrites through the depth of IPL shown in **(F)**. GFP IF intensities are increased for the 30-95% depth of IPL in the *Ythdf2* cKO retina compared with their littermate controls, but the general patterning is similar between the two genotypes. Data are mean $\pm$ SEM ($n = 13$ sections for each genotype): ** $p = 0.0039$ (95%), ** $p = 0.0014$ (90%), *** $p = 0.00049$ (85%), *** $p = 0.00020$ (80%), *** $p = 0.00018$ (75%), *** $p = 0.00036$ (70%), ** $p = 0.0018$ (65%), ** $p = 0.0067$ (60%), ** $p = 0.0040$ (55%), *** $p = 0.00057$ (50%), **** $p = 3.48E-05$ (45%), **** $p = 4.76E-05$ (40%), **** $p = 6.85E-05$ (35%), ** $p = 0.0034$ (30%), by unpaired Student's *t* test. Arrows indicate peaks of VACHT signals.

(H) Cross-sections of the IPL showing dendritic sublaminal patterning of melanopsin⁺ ipRGCs in P20 control and *Ythdf2* cKO retina. Scale bar: 20 μ m.

(I) Quantification and distribution of melanopsin IF intensities from melanopsin⁺ ipRGC dendrites through the depth of IPL shown in **(H)**. Melanopsin IF intensities are increased in the *Ythdf2* cKO retina compared with their littermate controls, but the general patterning is similar between the two

genotypes. Data are mean $\pm$ SEM ($n = 11$ neurons for control, $n = 8$ neurons for *Ythdf2* cKO): * $p = 0.049$ (95%), * $p = 0.010$ (90%), * $p = 0.039$ (75%), * $p = 0.022$ (70%), * $p = 0.019$ (65%), * $p = 0.018$ (60%), * $p = 0.016$ (55%), * $p = 0.013$ (50%), ** $p = 0.0095$ (45%), ** $p = 0.0044$ (40%), ** $p = 0.0053$ (35%), ** $p = 0.0091$ (30%), * $p = 0.014$ (25%), ** $p = 0.0074$ (20%), ** $p = 0.0071$ (15%), ** $p = 0.0053$ (10%), ** $p = 0.0025$ (5%), ** $p = 0.0029$ (0%), by unpaired Student's t test.

Figure 4. IPL of the *Ythdf2* cKO retina is thicker and has more synapses.

(A) Cross-sections of P6 *Six3-cre*^{+/+}, *Ythdf2*^{fl/fl} retina showing increased IPL thickness by MAP2 staining compared with littermate control. ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, granule cell layer. Scale bar: 20 μm .

(B, C) Quantification showing increased IPL thickness and MAP2 IF intensity per area in IPL of the *Ythdf2* cKO retina (A). Quantification data are represented as box and whisker plots: *** $p = 1.28E-07$ for B ($n = 12$ sections for each genotype), by unpaired Student's t test; ** $p = 0.0045$ for C ($n = 12$ sections for each genotype), by paired Student's t test. (D, E) Representative confocal images showing the excitatory synapses labeled by co-localization of Bassoon (presynaptic) and PSD-95 (postsynaptic) in the IPL of P30 retina (D). There are significantly more synapses in the *Ythdf2* cKO IPL compared with control. Quantification data are represented as box and whisker plots (E): $n = 47$ confocal fields for *Ythdf2*^{fl/fl}, $n = 23$ confocal fields for *Six3-cre*^{+/-}, *Ythdf2*^{fl/fl}; ** $p = 1.63E-05$; by unpaired Student's t test. Scale bars: 10 μm (D) and 5 μm (inset in D).

Figure 4—figure supplement 1. Thickness or synapse numbers in OPL shows no difference between the *Ythdf2* cKO and control retinas.

(A-D) Quantification of the thickness of different layers by MAP2/DAPI IF in P6 *Ythdf2* cKO and control retinas as shown in Figure 4A. Data are represented as box and whisker plots: $n = 14$ sections

for each genotype; $p = 0.60$ for ONL in **A**, $p = 0.61$ for OPL in **B**, $p = 0.84$ for INL in **C**, $p = 0.62$ for GCL in **D**; ns, not significant; by unpaired Student's t test.

(**E**, **F**) Representative confocal images showing the excitatory ribbon synapses labeled by co-localization of Bassoon (presynaptic) and PSD-95 (postsynaptic) in the OPL of P30 retina (**E**), which shows no difference between *Ythdf2* cKO and control. Quantification data are represented as box and whisker plots (**F**): $n = 39$ confocal fields for *Ythdf2*^{fl/fl}, $n = 36$ confocal fields for *Six3-cre*^{+/-}, *Ythdf2*^{fl/fl}; $p = 0.66$; ns, not significant; by unpaired Student's t test. Scale bar: 5 μ m.

Figure 5. Visual acuity is improved for the *Ythdf2* cKO mice.

1048 **(A-D)** Six3-Cre-mediated *Ythdf2* cKO showing normal animal development and body weight (male in
 1049 **A**, female in **C**). Quantification data of body weight (**B, D**) are represented as box and whisker plots: p
 1050 = 0.41 in **B** (male, $n = 24$ for control, $n = 18$ for cKO); $p = 0.08$ in **D** (female, $n = 23$ for control, $n = 25$
 1051 for cKO); ns, not significant; by unpaired Student's t test.
 1052 **(E)** The setup of optomotor response assay is illustrated by schematic drawing.
 1053 **(F, G)** Optomotor response assay demonstrating improved visual acuity in the *Ythdf2* cKO mice.
 1054 Quantification data are mean $\pm$ SEM: $*p = 0.048$ in **F** (male, $n = 24$ control, $n = 16$ cKO); $*p = 0.015$ in
 1055 **G** (female, $n = 21$ control, $n = 25$ cKO); by unpaired Student's t test.
 1056

Figure 5—figure supplement 1. Guidance or central targeting of optic nerves is not affected in Six-
Cre-mediated *Ythdf2* cKO.

1060 **(A)** Cross-sections of E14.5 retina showing strong expression of Six3-Cre using an eYFP reporter. Scale
1061 bar: 100 μ m.

1062 **(B)** Sagittal sections of P10 brain showing negligible expression of Six3-Cre in the potential RGC target
1063 regions in the brain. SCN, suprachiasmatic nucleus; LGN, lateral geniculate nucleus; MTN, medial
1064 terminal nucleus; SC, superior colliculus. Scale bar: 1 mm.

1065 **(C-E)** Normal eye diameter and optic nerve length. Quantification data **(D, E)** are represented as box
1066 and whisker plots: $p = 0.80$ ($n = 31$ for each genotype in **D**); $p = 0.99$ ($n = 31$ for each genotype in **E**);
1067 ns, not significant; by unpaired Student's t test. Scale bar: 1 mm.

1068 **(F, G)** Representative images of coronal sections through the LGN **(F)** and SC **(G)** after unilateral
1069 injection of CTB-Alexa Fluor 555 at P37 in *Ythdf2* cKO and control mice. Projections to the
1070 contralateral (Contra), ipsilateral (Ipsi) LGN and contralateral (Contra) SC are visible, which shows no
1071 difference between *Ythdf2* cKO and control mice. Scale bars: 100 μ m **(F)** and 200 μ m **(G)**.
1072

Figure 6. YTHDF2 target mRNAs were identified with transcriptomic and proteomic analysis.

(A, B) Gene Ontology (GO) analysis of YTHDF2 target transcripts identified by anti-YTHDF2 RNA immunoprecipitation (RIP) in the retina followed by RNA sequencing (RIP-seq). Neural terms were picked out in Cellular Component (A) and Biological Process (B).

(C) GO analysis of proteins which are upregulated after YTHDF2 KD by MS.

(D) Verification of m⁶A modification of YTHDF2 target mRNAs by anti-m⁶A pulldown followed by RT-

qPCR. ND, not detected. Data are mean ± SEM and are represented as dot plots (*n* = 3 replicates):

p* = 0.0016 for *Kalrn7*; **p* = 1.40E-08 for *Kalrn9*; *****p* = 1.46E-06 for *Kalrn12*; *****p* = 5.46E-06

for *Strn*; *****p* = 4.90E-06 for *Ubr4*; by unpaired Student's *t* test.

Figure 7. YTHDF2 target mRNAs mediate YTHDF2-controlled RGC dendrite branching.

1086 (A) YTHDF2 target mRNAs showing increased stability in the *Ythdf2* cKO retina. RGCs dissected from
1087 E14.5 *Ythdf2* cKO and control embryos were cultured, treated with actinomycin D (ActD) and
1088 collected at different timepoints. Data are mean $\pm$ SEM ($n = 3$ replicates). For *Kalrn7*, $^{**}p = 0.0057$ (3
1089 hr), $^{*}p = 0.014$ (6 hr), $^{***}p = 0.00039$ (12 hr); for *Kalrn9*, $^{**}p = 0.0036$ (3 hr), $^{***}p = 0.00090$ (6 hr), $^{*}p$
1090 $= 0.032$ (12 hr); for *Kalrn12*, $^{**}p = 0.0012$ (3 hr), $^{*}p = 0.010$ (6 hr), $^{**}p = 0.0069$ (12 hr); for *Strn*, $^{*}p =$
1091 0.014 (3 hr), $^{*}p = 0.012$ (6 hr), $^{*}p = 0.016$ (12 hr); for *Ubr4*, $^{**}p = 0.0077$ (3 hr), $^{**}p = 0.0059$ (6 hr), $^{*}p$
1092 $= 0.041$ (12 hr); all by unpaired Student's *t* test.

1093 (B) KD of the target mRNAs causing decreased dendrite branching of cultured RGCs prepared from
1094 wild type (WT) E14.5 retina by Sholl analysis. Brn3a and MAP2 IF were used to mark RGCs and
1095 visualize dendrites. Data are mean $\pm$ SEM. For *Kalrn7* ($n = 59$ for *siCtrl*, $n = 56$ for *siKalrn7*), $^{***}p =$
1096 $2.33E-06$ (10 μ m), $^{***}p = 5.85E-06$ (15 μ m), $^{***}p = 8.67E-05$ (20 μ m), $^{**}p = 0.0045$ (25 μ m), $^{**}p =$
1097 0.0058 (30 μ m), $^{*}p = 0.010$ (35 μ m); for *Kalrn9* ($n = 59$ for *siCtrl*, $n = 46$ for *siKalrn9*), $^{***}p = 3.69E-$
1098 05 (10 μ m), $^{***}p = 5.53E-05$ (15 μ m), $^{***}p = 0.00020$ (20 μ m), $^{***}p = 3.09E-06$ (25 μ m), $^{***}p =$
1099 $4.63E-06$ (30 μ m), $^{***}p = 0.00059$ (35 μ m), $^{**}p = 0.0010$ (40 μ m), $^{*}p = 0.042$ (45 μ m); for *Kalrn12* (n
1100 $= 59$ for *siCtrl*, $n = 39$ for *siKalrn12*), $^{**}p = 0.0031$ (10 μ m), $^{***}p = 0.00017$ (15 μ m), $^{***}p = 6.56E-05$
1101 (20 μ m), $^{**}p = 0.0017$ (25 μ m), $^{*}p = 0.017$ (30 μ m); for *Strn* ($n = 51$ for *siCtrl*, $n = 57$ for *siStrn*), $^{***}p$
1102 $= 4.19E-05$ (10 μ m), $^{***}p = 0.00067$ (15 μ m), $^{**}p = 0.0079$ (20 μ m), $^{*}p = 0.015$ (30 μ m); for *Ubr4* ($n =$
1103 81 for *siCtrl*, $n = 81$ for *siUbr4*), $^{***}p = 1.26E-08$ (10 μ m), $^{***}p = 7.61E-10$ (15 μ m), $^{***}p = 2.35E-$
1104 08 (20 μ m), $^{***}p = 1.39E-05$ (25 μ m), $^{**}p = 0.0061$ (30 μ m); all by unpaired Student's *t* test.

1105 (C) Increased dendrite branching of cultured RGCs prepared from E14.5 *Ythdf2* cKO retina was
1106 rescued by KD of target mRNAs using siRNAs. Data are mean $\pm$ SEM. *Ctrl*, *Ythdf2*^{fl/fl}; *Y2 cKO*, *Six3-cre*^{+/-}
1107 *, Ythdf2*^{fl/fl}. In **C1**, "*Ctrl*, *siCtrl*" ($n = 35$ neurons) vs "*Y2 cKO*, *siCtrl*" ($n = 52$ neurons), $^{*}p = 0.038$ (15 μ m),
1108 $^{*}p = 0.045$ (20 μ m), $^{**}p = 0.0036$ (30 μ m); "*Y2 cKO*, *siKalrn7*" ($n = 55$ neurons) vs "*Y2 cKO*, *siCtrl*", $^{*}p$
1109 $= 0.020$ (15 μ m), $^{*}p = 0.025$ (20 μ m), $^{*}p = 0.031$ (25 μ m); "*Y2 cKO*, *siKalrn9*" ($n = 66$ neurons) vs "*Y2*
1110 *cKO*, *siCtrl*", $^{*}p = 0.020$ (10 μ m), $^{*}p = 0.013$ (15 μ m), $^{*}p = 0.031$ (20 μ m), $^{*}p = 0.017$ (25 μ m), $^{*}p =$
1111 0.031 (30 μ m), $^{*}p = 0.031$ (45 μ m); "*Y2 cKO*, *siKalrn12*" ($n = 80$ neurons) vs "*Y2 cKO*, *siCtrl*", $^{*}p =$

0.015 (10 μ m), ** p = 0.0018 (15 μ m), * p = 0.015 (20 μ m), * p = 0.027 (40 μ m). In **C2**, “Ctrl, siCtrl” (n = 50 neurons) vs “Y2 cKO, siCtrl” (n = 47 neurons), ** p = 0.0031 (10 μ m), ** p = 0.0013 (15 μ m), * p = 0.029 (20 μ m), ** p = 0.0015 (30 μ m), * p = 0.014 (35 μ m); “Y2 cKO, siStrn” (n = 45 neurons) vs “Y2 cKO, siCtrl”, *** p = 0.00016 (10 μ m), ** p = 0.0043 (15 μ m), * p = 0.010 (20 μ m), * p = 0.018 (30 μ m); “Y2 cKO, siUbr4” (n = 57 neurons) vs “Y2 cKO, siCtrl”, *** p = 0.00084 (10 μ m), **** p = 4.89E-05 (15 μ m), ** p = 0.0058 (20 μ m), ** p = 0.0045 (30 μ m). All by unpaired Student’s t test.

(D) Increased dendrite branching of RGC subtypes in *Ythdf2* cKO retina was rescued by KD of target mRNAs through intravitreal injection of AAV siRNAs in vivo. Data are mean $\pm$ SEM. Ctrl, *Ythdf2*^{fl/fl}; Y2 cKO, *Six3-cre*^{+/-}; *Ythdf2*^{fl/fl}. In **D1** (CART⁺/eGFP⁺ ooDSGCs), “Ctrl, shCtrl” (n = 10 neurons) vs “Y2 cKO, shCtrl” (n = 6 neurons), * p = 0.010 (10 μ m), *** p = 0.00049 (20 μ m), ** p = 0.0021 (30 μ m), ** p = 0.0047 (40 μ m), * p = 0.028 (50 μ m), * p = 0.011 (60 μ m), * p = 0.030 (90 μ m), * p = 0.042 (110 μ m); “Y2 cKO, shKalrn12” (n = 8 neurons) vs “Y2 cKO, shCtrl”, * p = 0.012 (20 μ m), * p = 0.014 (30 μ m); “Y2 cKO, shUbr4” (n = 6 neurons) vs “Y2 cKO, shCtrl”, * p = 0.011 (10 μ m), ** p = 0.0084 (20 μ m), * p = 0.029 (30 μ m). In **D2** (SMI-32⁺ α RGCs), “Ctrl, shCtrl” (n = 14 neurons) vs “Y2 cKO, shCtrl” (n = 14 neurons), * p = 0.032 (40 μ m), ** p = 0.0019 (50 μ m), ** p = 0.0014 (60 μ m), * p = 0.015 (70 μ m), * p = 0.044 (90 μ m); “Y2 cKO, shKalrn12” (n = 26 neurons) vs “Y2 cKO, shCtrl”, ** p = 0.0023 (20 μ m), *** p = 0.00076 (30 μ m), *** p = 0.00030 (40 μ m), *** p = 0.00020 (50 μ m), * p = 0.015 (60 μ m); “Y2 cKO, shUbr4” (n = 15 neurons) vs “Y2 cKO, shCtrl”, * p = 0.042 (30 μ m), * p = 0.024 (40 μ m), * p = 0.018 (50 μ m). All by unpaired Student’s t test.

1131

1132

Figure 7—figure supplement 1. YTHDF2 target mRNAs were characterized and validated.

1135 **(A)** Upregulation of target mRNAs-encoding proteins Strn and Ubr4 in *Ythdf2* cKO retina in vivo.

1136 Enrichment and higher levels of these proteins were detected in the IPL of P6 *Ythdf2* cKO retina

1137 compared with control by IF. Scale bar: 20 μ m.

1138 **(B)** Upregulation of target mRNA levels after YTHDF2 KD. RT-qPCR confirmed upregulation of the

1139 candidate target mRNAs after KD of YTHDF2 in cultured RGCs using *shYthdf2*. Data are mean $\pm$ SEM

1140 and are represented as dot plots ($n = 3$ replicates): *** $p = 0.00084$ for *Kalrn7*; ** $p = 0.0039$ for

1141 *Kalrn9*; ** $p = 0.0026$ for *Kalrn12*; **** $p = 1.11E-06$ for *Strn*; *** $p = 0.00011$ for *Ubr4*; by unpaired

1142 Student's t test.

1143 **(C)** Confirmation of METTL14 KD in cultured RGCs using *shMettl14* by RT-qPCR. Data are mean $\pm$ SEM

1144 and are represented as dot plots ($n = 3$ replicates): *** $p = 0.00012$ (*shMettl14#6* vs *shCtrl*); ** $p =$

1145 0.0079 (*shMettl14#7* vs *shCtrl*); by unpaired Student's t test.

1146 **(D)** Upregulation of target mRNA levels after METTL14 KD. RT-qPCR confirmed upregulation of the

1147 candidate target mRNAs after KD of METTL14 in cultured RGCs using *shMettl14*. Data are mean $\pm$

1148 SEM and are represented as dot plots ($n = 3$ replicates): for *Kalrn7*, ** $p = 0.0034$ (*shMettl14#6* vs

1149 *shCtrl*), * $p = 0.016$ (*shMettl14#7* vs *shCtrl*); for *Kalrn9*, ** $p = 0.0010$ (*shMettl14#6* vs *shCtrl*), ** $p =$

1150 0.0067 (*shMettl14#7* vs *shCtrl*); for *Kalrn12*, ** $p = 0.0079$ (*shMettl14#6* vs *shCtrl*), * $p = 0.026$

1151 (*shMettl14#7* vs *shCtrl*); for *Strn*, **** $p = 5.45E-06$ (*shMettl14#6* vs *shCtrl*), * $p = 0.025$ (*shMettl14#7*

1152 vs *shCtrl*); for *Ubr4*, ** $p = 0.0029$ (*shMettl14#6* vs *shCtrl*), ** $p = 0.0066$ (*shMettl14#7* vs *shCtrl*); by

1153 unpaired Student's t test.

1154 **(E)** Confirmation of KD by siRNAs against target mRNAs. Data are mean $\pm$ SEM and are represented as

1155 dot plots ($n = 3$ replicates): ** $p = 0.0020$ for *Kalrn7*; ** $p = 0.0025$ for *Kalrn9*; *** $p = 0.00020$ for

1156 *Kalrn12*; ** $p = 0.0021$ for *Strn*; ** $p = 0.0033$ for *Ubr4*; by unpaired Student's t test.

1157 **(F)** KD of target mRNAs all together using a siRNA cocktail causing further decrease of dendrite

1158 branching of cultured RGCs compared with single siRNA against each target mRNA. Data are mean $\pm$

1159 SEM: $n = 32$ RGCs for *siCtrl*, $n = 33$ RGCs for *siKalrn7*, $n = 32$ RGCs for *siKalrn9*, $n = 35$ RGCs for

1160 *siKalrn12*, $n = 35$ RGCs for *siStrn*, $n = 36$ RGCs for *siUbr4*, $n = 36$ RGCs for *siCocktail*. *siKalrn7* vs *siCtrl*:

1161 $*p = 0.031$ (10 μm), $*p = 0.046$ (15 μm); *siKalrn9* vs *siCtrl*: $**p = 0.0011$ (10 μm), $**p = 0.0090$ (15
1162 μm); *siKalrn12* vs *siCtrl*: $**p = 0.0061$ (10 μm), $**p = 0.0086$ (15 μm); *siStrn* vs *siCtrl*: $***p = 0.00056$
1163 (10 μm), $*p = 0.025$ (15 μm); *siUbr4* vs *siCtrl*: $**p = 0.0018$ (10 μm), $*p = 0.026$ (15 μm), $*p = 0.048$
1164 (20 μm), $*p = 0.011$ (25 μm); *siCocktail* vs *siCtrl*: $****p = 3.44\text{E-}06$ (10 μm), $****p = 4.07\text{E-}06$ (15 μm),
1165 $***p = 0.00077$ (20 μm), $**p = 0.0010$ (25 μm), $**p = 0.0049$ (30 μm), $**p = 0.0094$ (35 μm). *siKalrn7*
1166 vs *siCocktail*: $**p = 0.0092$ (10 μm), $**p = 0.0040$ (15 μm), $**p = 0.0028$ (20 μm), $**p = 0.0034$ (25
1167 μm); *siKalrn9* vs *siCocktail*: $**p = 0.0042$ (15 μm), $*p = 0.034$ (20 μm); *siKalrn12* vs *siCocktail*: $*p =$
1168 0.029 (10 μm), $*p = 0.019$ (15 μm), $**p = 0.0014$ (20 μm), $**p = 0.0091$ (25 μm), $**p = 0.0063$ (30
1169 μm); *siStrn* vs *siCocktail*: $*p = 0.043$ (10 μm), $**p = 0.0051$ (15 μm), $**p = 0.0045$ (20 μm), $***p =$
1170 $3.79\text{E-}06$ (25 μm), $***p = 0.00022$ (30 μm); *siUbr4* vs *siCocktail*: $*p = 0.049$ (10 μm), $*p = 0.011$ (15
1171 μm). All by unpaired Student's *t* test.

1172

Figure 8. *Ythdf2* cKO retina is more resistant to acute ocular hypertension (AOH).

(A, B) Better maintenance of RGC dendrite arborization in *Ythdf2* cKO retina after AOH operation.

AOH was performed using adult mice, and retinas were collected after AOH for wholemount

immunostaining of melanopsin and SMI-32 to visualize the dendrite arbors of corresponding RGC subtype, respectively. Dendrite traces were drawn as previously shown and quantification of dendrite branching was done using Sholl analysis. Data are mean $\pm$ SEM. Numbers of interactions are significantly greater in *Six3-cre^{+/+}, Ythdf2^{fl/fl}* retina than *Ythdf2^{fl/fl}* control retina in both RGC subtypes after AOH: for melanopsin⁺ ipRGCs in **A**, *Ythdf2^{fl/fl}*/AOH ($n = 51$ RGCs) vs cKO/AOH ($n = 64$ RGCs), *** $p = 0.00015$ (10 μ m), ** $p = 0.0017$ (20 μ m), * $p = 0.034$ (30 μ m), *** $p = 0.00035$ (40 μ m), **** $p = 3.02E-05$ (50 μ m), **** $p = 2.63E-05$ (60 μ m), ** $p = 0.0029$ (70 μ m), ** $p = 0.0028$ (80 μ m), *** $p = 0.00035$ (90 μ m), ** $p = 0.0032$ (100 μ m), ** $p = 0.0014$ (110 μ m), ** $p = 0.0043$ (120 μ m), ** $p = 0.0014$ (130 μ m), * $p = 0.023$ (140 μ m), * $p = 0.013$ (150 μ m); for SMI-32⁺ α RGCs in **B**, *Ythdf2^{fl/fl}*/AOH ($n = 21$ neurons) vs cKO/AOH ($n = 15$ neurons), ** $p = 0.0052$ (40 μ m), ** $p = 0.0057$ (50 μ m); all by unpaired Student's t test.

(C, D) *Ythdf2* cKO retina showing less severe RGC loss after AOH. AOH was performed using adult mice and retinas were collected after AOH for wholemount immunostaining using a Brn3a antibody **(C)**. Numbers of Brn3a⁺ RGCs per 10000 μ m² of retina were quantified for different genotypes and conditions (confocal fields for analysis: $n = 117$ for *Ythdf2^{fl/fl}*/Ctrl; $n = 98$ for *Ythdf2^{fl/fl}*/AOH; $n = 110$ for cKO/Ctrl; $n = 104$ for cKO/AOH). Data are represented as box and whisker plots **(D)**: ns, not significant ($p = 0.16$; *Ythdf2^{fl/fl}*/Ctrl vs cKO/Ctrl); ** $p = 0.0077$ (*Ythdf2^{fl/fl}*/AOH vs cKO/AOH); by unpaired Student's t test. Scale bar: 25 μ m.

(E, F) Overexpression (OE) of YTHDF2 targets *Hspa12a* and *Islr2* protecting retina from RGC dendrite degeneration. Wild type (WT) mice were intravitreally injected with AAV overexpressing *Hspa12a* or *Islr2* and then operated with AOH. Wholemount immunostaining of CART/ZsGreen and SMI-32/ZsGreen was carried out to visualize the dendrite arbors of corresponding RGC subtype, respectively. Dendrite traces were drawn as previously shown and quantification of dendrite branching was done using Sholl analysis. Data are mean $\pm$ SEM. Numbers of interactions are significantly greater in retina with OE of *Hspa12a* or *Islr2* than control retina in both RGC subtypes after AOH. For CART⁺ ooDSGCs in **E**: OE-Ctrl/AOH ($n = 11$ RGCs) vs OE-Hspa12a/AOH ($n = 11$ RGCs), * p

1203 = 0.014 (20 μ m), ** p = 0.0025 (30 μ m), * p = 0.018 (40 μ m); OE-Ctrl/AOH vs OE-Isir2/AOH (n = 6
1204 RGCs), * p = 0.024 (10 μ m), *** p = 0.00031 (20 μ m), ** p = 0.0038 (30 μ m), * p = 0.013 (40 μ m). For
1205 SMI-32⁺ α RGCs in F: OE-Ctrl/AOH (n = 49 neurons) vs OE-Hspa12a/AOH (n = 46 neurons), ** p =
1206 0.0023 (30 μ m), *** p = 0.00080 (40 μ m), ** p = 0.0059 (50 μ m), ** p = 0.0051 (60 μ m), ** p = 0.0036
1207 (70 μ m), *** p = 0.00070 (80 μ m), ** p = 0.0015 (90 μ m), * p = 0.016 (100 μ m), * p = 0.011 (110 μ m);
1208 OE-Ctrl/AOH vs OE-Isir2/AOH (n = 13 RGCs), * p = 0.010 (30 μ m), ** p = 0.0093 (40 μ m), ** p = 0.0019
1209 (50 μ m), *** p = 0.00085 (60 μ m), *** p = 0.00067 (70 μ m), **** p = 4.25E-05 (80 μ m), **** p = 2.54E-
1210 05 (90 μ m), *** p = 0.00020 (100 μ m), ** p = 0.0016 (110 μ m). All by unpaired Student's t test.
1211

Figure 8—figure supplement 1. *Hspa12a* and *Islr2* are two target mRNAs of YTHDF2 in adult retina.

(A, B) The curves from *Figure 3D,F* and *Figure 8A,B* were plotted together for easy comparison. The error bars and the asterisks were removed from these graphs for easy reading and these information can still be seen in *Figure 3D,F* and *Figure 8A,B*.

(C) Upregulation of YTHDF2 target mRNA *Hspa12a* and *Islr2* in adult *Ythdf2* cKO retina compared with control by RT-qPCR. Data are mean $\pm$ SEM and are represented as dot plots ($n = 3$ replicates): $**p = 0.0035$ for *Hspa12a*; $***p = 0.00012$ for *Islr2*; by unpaired Student's *t* test.

(D) Verification of m⁶A modification of *Hspa12a* and *Islr2* mRNAs by anti-m⁶A pulldown followed by RT-qPCR. ND, not detected. Data are mean $\pm$ SEM and are represented as dot plots ($n = 3$ replicates): $****p = 7.07E-06$ for *Islr2*; $****p = 1.55E-06$ for *Hspa12a*; by unpaired Student's *t* test.

(E) Downregulation of *Hspa12a* and *Islr2* mRNA levels in retina 3 days after AOH. Data are mean $\pm$ SEM and are represented as dot plots ($n = 3$ replicates): $**p = 0.0032$ for *Hspa12a*; $****p = 5.41E-07$ for *Islr2*; by unpaired Student's *t* test.

(F, G) Cross-sections of retina showing increased YTHDF2 expression in Brn3a⁺ RGCs by IF. AOH was performed using P60 mice, and retinas were collected 1 day after AOH for analysis. Quantification data of YTHDF2 IF were represented as box and whisker plots **(G)**: $****p = 1.13E-06$ ($n = 110$ RGCs for each condition); by unpaired Student's *t* test. Scale bar: 10 μ m.

(H) Upregulation of *Ythdf2* mRNA level after AOH. Data are mean $\pm$ SEM and are represented as dot plots ($n = 3$ replicates): $***p = 0.00066$; by unpaired Student's *t* test.

Supplementary file 1. List of YTHDF2 target mRNAs by anti YTHDF2 RIP-seq.

Supplementary file 2. Proteome of YTHDF2 knockdown vs control.

Supplementary file 3. Overlapping mRNA of Y2-RIP vs Y2-KD-MS.

1239

1240 **Figure 1-source data 1. Source data for Figure 1B.**

1241 (A) WB of anti YTHDF2 after KD of YTHDF2.

1242 (B) WB of anti β -actin after KD of YTHDF2.

1243

1244 **Figure 1-source data 2. Source data for Figure 1B.**

1245 Original file of the full raw unedited blot of anti YTHDF2 after KD of YTHDF2.

1246

1247 **Figure 1-source data 3. Source data for Figure 1B.**

1248 Original file of the full raw unedited blot of anti β -actin after KD of YTHDF2.

1249

1250 **Figure 1-figure supplement 1-source data 1. Source data for Figure 1-figure supplement 1E,F.**

1251 (A) WB of anti YTHDF1 after KD of YTHDF1.

1252 (B) WB of anti β -actin after KD of YTHDF1.

1253 (C) WB of anti YTHDF3 after KD of YTHDF3.

1254 (D) WB of anti β -actin after KD of YTHDF3.

1255

1256 **Figure 1-figure supplement 1-source data 2. Source data for Figure 1-figure supplement 1E.**

1257 Original file of the full raw unedited blot of anti YTHDF1 after KD of YTHDF1.

1258

1259 **Figure 1-figure supplement 1-source data 3. Source data for Figure 1-figure supplement 1E.**

1260 Original file of the full raw unedited blot of anti β -actin after KD of YTHDF1.

1261

1262 **Figure 1-figure supplement 1-source data 4. Source data for Figure 1-figure supplement 1F.**

1263 Original file of the full raw unedited blot of anti YTHDF3 after KD of YTHDF3.

1264

1265 **Figure 1-figure supplement 1-source data 5. Source data for Figure 1-figure supplement 1F.**

1266 Original file of the full raw unedited blot of anti β -actin after KD of YTHDF3.

1267

1268