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

Short title: YTHDF2 regulates RGC dendrite development and maintenance

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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, and conditional knockout of *Ythdf2* in retina 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 (m⁶A) is the most widely distributed and extensively studied internal modification in mRNA (*Dominissini et al. 2012; Meyer et al. 2012; Nachtergaele and He 2018*). m⁶A 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 (“m⁶A erasers”) and methyltransferases (“m⁶A writers”). Since the fate of m⁶A-modified transcripts are decoded by the m⁶A binding proteins (“m⁶A 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 1B*). Conversely, the expression of YTHDF2 in other layers and cells of the retina is much lower or absent (*Figure 1A; Figure 1—figure supplement 1B-D*). Another two m⁶A readers YTHDF1 and YTHDF3 show similar expression patterns (*Figure 1—figure supplement 1E,F*). 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 1G,H*). 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 sh*Ythdf2* (*Figure 1C,D; Figure 1—figure supplement 1I,J*). In contrast, the dendrite branching of RGCs with YTHDF1 KD using sh*Ythdf1* was not significantly different from control shRNA (*Figure 1—figure supplement 1K*), while YTHDF3 KD using sh*Ythdf3* caused a slight (statistically significant in several Sholl radii) decrease of RGC dendrite branching compared with control shRNA (*Figure 1—figure supplement 1L*). 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, horizontal cells, Müller glia, or astrocytes were not affected in *Ythdf2* cKO retina (*Figure 2—figure supplement 1B-K; Figure 2—figure supplement 2A-D*), suggesting that YTHDF2 is not involved in the generation or development of these cells. This is in line with the low or no 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 sublaminal 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 sh*Ythdf2*-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-E, the mRNA levels of *Kalrn7*, *Kalrn9*, *Kalrn12*, *Strn* and *Ubr4* were dramatically increased after KD of YTHDF2, KO of *Ythdf2*, or KD of 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 1F*). 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 1G*), 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 sh*Kalrn12* and sh*Ubr4*, 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 and show better RGC survival compared with control AAV (*Figure 8E-G*).

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. We have validated this effect on several RGC subtypes. Since there are dozens of RGC subtypes, it is technically challenging but still interesting to test whether this effect is universal to all subtypes or there is specificity for RGC subtypes.

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

Animals and generation of the *Ythdf2* cKO mice

Ythdf2^{fl/fl} 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'-GCAGCTCTGACTATTCTAAAACCTCC-3'; the second *Ythdf2-loxP* site, 5'-CTCATAACATCCATAGCCACAGG-3' and 5'-CCAAGAGATAGCTTTCCTAATG-3'. *Six3-cre* site, 5'-CCTTCCTCCCTCTCTATGTG-3' and 5'-GAACGAACCTGGTCGAAATC-3'. *Rosa26-eYFP* wild type site, 5'-CTGGCTTCTGAGGACCG-3' and 5'-CAGGACAACGCCACACA-3'; the mutant site, 5'-AGGGCGAGGAGCTGTTCA-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

400 Lentiviral knockdown plasmids encoding shRNA (shCtrl: 5'-GCATCAAGGTGAACTTCAAGA-3'; sh*Ythdf2*:
 401 5'-GGACGTTCCCAATAGCCAACT-3'; sh*Ythdf1*: 5'- GGACATTGGTACTTGGGATAA-3'; sh*Ythdf3*: 5'-
 402 GGATTTGGCAATGATACTTTG-3'; sh*Mettl14#6*: 5'-GCTGGACCTGGGATGATATTA-3'; sh*Mettl14#7*: 5'-
 403 CCCAGCTTGACTTTGCTTA-3') were generated from pLKO.1-TRC and lentivirus preparation process
 404 was described previously (Yu et al. 2018). All siRNA were chosen from previous studies and the
 405 target sequences of siRNA are as following: siCtrl (RNAi negative control): 5'-
 406 UUCUCCGAACGUGUCACGUTT-3' (Yu et al. 2018); si*Kalrn7*: 5'- AGUACAAUCCUGGCAUGUTT-3' (Xie
 407 et al. 2007); si*Kalrn9*: 5'-ACUGGACUGGACUUCUAUUTT-3' (Yan et al. 2015); si*Kalrn12*: 5'-
 408 CGAUGAGCUGAUGGAAGAATT-3' (Yan et al. 2015); si*Strn*: 5'-GGUGAAGAUCGAGAUACAATT-3'
 409 (Breitman et al. 2008); si*Ubr4*: 5'-AAUGAUGAGCAGUCAUCUATT-3' (Shim et al. 2008). AAV
 410 knockdown plasmids encoding shRNA (shCtrl: 5'-TTCTCCGAACGTGTCACGTAA-3'; sh*Kalrn12*: 5'-
 411 TGATGAGCTGATGGAAGAA-3'; sh*Ubr4*: 5'-AATGATGAGCAGTCATCTC-3') were generated using
 412 pHBAAV-U6-MCS-CMV-EGFP and packaged in serotype-9 by Hanbio (1.5×10¹² genomic copies per ml).
 413 AAV overexpression plasmids of *Hspa12a* (NM_175199.3; PCR primer for mouse *Hspa12a*: 5'-
 414 ATGGCGGACAAGGAAGCTGG-3' and 5'-GTAATTTAAGAAGTCGATCCCC-3') and *Islr2* (NM_001161541.1;
 415 PCR Primer for mouse *Islr2*: 5'-ATGGGGCCCTTTGGAGC-3' and 5'-GCCCCGTGTCTGCCTGTAG-3') were
 416 generated from pHBAAV-CMV-MCS-3flag-T2A-ZsGreen and packaged serotype-9 by Hanbio (1.2×10¹²
 417 genomic copies per ml).

418 GeneSilencer® Transfection Reagent (Genlantis) was used in siRNA transfection following the
 419 manufacturer's protocols. Culture medium was changed after 1 day of lentiviral shRNA infection or
 420 siRNA transfection. For lentiviral shRNA assay, puromycin (Thermo or Sigma, 1 µg/ml) was added
 421 after 2 days of infection. Immunofluorescence, RNA or protein preparation was performed after
 422 shRNA or siRNA worked for 3 days. For AAV intravitreal injection, P0-P1 mouse pups were
 423 anesthetized in ice and then eyes were pierced at the edge of corneal by 30G × 1/2 needle (BD,
 424 305106) under stereomicroscope. Then 1 µl AAV was intravitreally injected with 10 µl Syringe
 425 (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 × 1/2 needle successively. 2 µl AAV was intravitreally injected with 10 µL 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 PrimeScript™ RT Master Mix (TaKaRa). Synthesized cDNA was used for qPCR by 2× ChamQ™ Universal SYBR qPCR Master Mix (Vazyme) on StepOnePlus™ 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'-CTATTGGGCCTCTTCACCCC-3'; mouse *Ubr4*: 5'- TGAGTGAGGACAAGGGCAAC-3' and 5'-GGGTTGGATCGAACGAAGGT-3'; mouse *Ythdf2*: 5'- GAGCAGAGACCAAAGGTCAAG-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

For tissue sections, mouse embryonic eyes were fixed with 4% PFA (Sigma) in 0.1 M Phosphate Buffer (PB) for 30-45 min at room temperature (RT); eyes of mouse pups (< P10) were pre-fixed briefly and

452 then eyecups were dissected and fixed for 45 min-1 hr at RT; for P20-30 or adult mice, eyecups were
453 dissected after myocardial perfusion with 0.9% NaCl, followed by fixation for 1 hr. After PBS (3 × 5
454 min) washing, tissues were dehydrated with 30% sucrose in 0.1 M PB overnight at 4°C, then
455 embedded with O.C.T. (SAKURA) and cryosectioned at 12 µm (20 µm for Thy1-GFP section analysis)
456 with Leica CM1950 Cryostat. Tissue sections were permeabilized and blocked with 10%
457 ChemiBLOCKER (Millipore) and 0.5% Triton x-100 (Sigma) in PBS (PBST) for 1 hr at RT and incubated
458 in PBST overnight at 4°C with following primary antibodies: chicken anti-GFP (1:1000, Abcam
459 ab13970), chicken anti-MAP2 (1:10000, Abcam ab5392), goat anti-VACht (1:1000, Millipore ABN100),
460 guinea pig anti-RBPMS (1:1000, PhosphoSolutions 1832-RBPMS), mouse anti-AP2α (1:1000, DSHB
461 3B5), mouse anti-Bassoon (1:2500, Enzo Life Sciences ADI-VAM-PS003), mouse anti-Brn3a (1:300,
462 Millipore MAB1585), mouse anti-Calbindin-D-28K (1:200, Sigma C9848), mouse anti-PKCα (1:500,
463 Santa Cruz sc-8393), rabbit anti-Strn (Striatin) (1:500, BD Biosciences 610838), rabbit anti-CART
464 (1:2000, Phoenix Pharmaceuticals H-003-62), rabbit anti-m⁶A (1:200, Synaptic Systems 202003),
465 rabbit anti-melanopsin (1:1000, Thermo PA1-780), rabbit anti-PKCα (1:1000, Cell Signaling CST-2056),
466 rabbit anti-PSD95 (1:1000, Abcam ab18258), rabbit anti-Recoverin (1:1000, Millipore AB5585), rabbit
467 anti-YTHDF2 (1:1000, Proteintech 24744-1-AP), rabbit anti-YTHDF1 (1:1000, Proteintech 17479-1-AP),
468 rabbit anti-YTHDF3 (1:1000, Abcam ab103328), rabbit anti-Ubr4 (1:300, Abcam ab86738), sheep anti-
469 Chx10 (1:1000, Exalpha X1179P), chicken anti-GFAP (1:500, Millipore AB5541), goat anti-Lhx2 (1:200,
470 Santa Cruz Biotechnology sc-19344), rabbit anti-Lhx2 (1:500, Abcam ab184337). After three times of
471 PBS washing, sections were incubated in PBST for 1 hr at RT with secondary antibodies: Alexa 488
472 donkey anti-chicken (1:500, Jackson 703-545-155), Alexa 488 donkey anti guinea pig (1:500, Jackson
473 706-545-148), Alexa 488 donkey anti-mouse (1:500, Thermo A21202), Alexa 488 donkey anti-rabbit
474 (1:500, Thermo A21206), Alexa 555 donkey anti-goat (1:1000, Thermo A21432), Alexa 555 donkey
475 anti-mouse (1:1000, Thermo A31570), Alexa 555 donkey anti-rabbit (1:1000, Thermo A31572), Alexa
476 555 donkey anti-sheep (1:1000, Thermo A21436), Alexa 555 goat anti-chicken (1:1000, Thermo

477 A21437), or Alexa 647 donkey anti-mouse (1:200, Thermo A31571) and then mounted with the
478 VECTASHIELD Antifade Mounting Medium with DAPI (Vector Laboratory).

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

482 For wholemount immunostaining of retina, eyes were dissected after myocardial perfusion with
483 0.9% NaCl. Then retinas were separated from sclera and fixed with 4% PFA in 0.1M PB for 1 hr at RT.
484 Then retinas were blocked with 5% normal goat serum (Novus), 0.4% Triton x-100 in PBS overnight at
485 4°C. Primary antibodies such as chicken anti-GFP (1:1000, Abcam ab13970), mouse anti-Brn3a (1:300,
486 Millipore MAB1585), mouse anti-SMI-32 (1:200, BioLegend 801701), or rabbit anti-Melanopsin
487 (1:1000, Thermo PA1-780), rabbit anti-CART (1:2000, Phoenix Pharmaceuticals H-003-62) were
488 diluted in 5% normal goat serum, 0.4% Triton x-100 in PBS and incubated overnight at 4°C. Then
489 retinas were incubated with Alexa 488 donkey anti-chicken (1:500, Jackson 703-545-155), Alexa 488
490 donkey anti-mouse (1:500, Thermo A21202), Alexa 555 donkey anti-mouse (1:1000, Thermo A-31570)
491 and Alexa 555 donkey anti-rabbit (1:1000, Thermo A31572) second antibodies in 5% normal goat
492 serum (Novus), 0.4% Triton x-100 in PBS and finally mounted with the VECTASHIELD Antifade
493 Mounting Medium with DAPI.

494 All images were captured on Nikon A1R confocal microscope or Zeiss LSM 800 confocal
495 microscope with identical settings for each group in the same experiment. A region of interest (ROI),
496 length or thickness in immunofluorescence experiments were obtained with ImageJ. The number of
497 neurons in specific area was counted blindly and manually. To quantify RGC dendrite lamination in
498 IPL with Thy1-GFP, z-stack and maximum projection were performed during the analysis. GFP
499 intensity values across IPL depth were measured by ImageJ/Analyze/Plot Profile function (*Liu et al.*
500 **2018**). To quantify the numbers of Bassoon⁺/PSD-95⁺ excitatory synapses in IPL, the colocalization
501 puncta was measured by ImageJ/Analyze/Puncta Analyzer as described previously (*Ippolito and*
502 *Eroglu 2010*).

Sholl analysis

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

Optomotor response assay.

Ythdf2 cKO and control mice aged about 6 weeks were dark-adapted overnight before experiment and used in the optomotor response assay following the previously reported protocols (*Douglas et al. 2005; Sergeeva et al. 2018*). Using the Matlab program, 0.2 cycle/degree (15 sec per direction of 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 0.55 c/deg (30 sec per direction of rotation) were used in the following recordings. Mouse behaviors were analyzed in real time during the experiment and re-checked with video recordings. Finally, data for each mouse were determined by the minimal spatial frequency between left and right optomotor response.

CTB Labelling of Optic Nerve

To label RGC axon terminals in mouse brain, RGC axons were anterogradely labeled by CTB (Cholera Toxin Subunit B) conjugated with Alexa Fluor™ 555 (Invitrogen, C34776) through intravitreal injection 48 hr before sacrifice. After PFA perfusion, the brains were fixed with 4% PFA in 0.1 M PB overnight, 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 retinal 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. RIP experimental steps, RNA sample preparation and sequencing, and sequence data analysis followed the procedures reported previously (*Yu et al. 2021*).

MS analysis

E15.5 retinal neurons were cultured and infected with lenti viral sh*Ythdf2* or shCtrl. Sample collection and lysis, protein and peptide preparation were performed following procedures reported previously (*Yu et al. 2021*). 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 MeRIPTM 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 μ l/g) based on body weight and the Compound Tropicamide Eye Drops were used to scatter pupil. The anterior chamber was penetrated using the 32G $\times$ 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 preformed 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.).

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
- Furuta Y, Lagutin O, Hogan BL, Oliver GC. 2000. Retina- and ventral forebrain-specific Cre recombinase activity in transgenic mice. *Genesis* **26**:130-132. PMID: 10686607
- Hattar S, Liao HW, Takao M, Berson DM, Yau KW. 2002. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science* **295**:1065-1070. DOI: <https://doi.org/10.1126/science.1069609>, PMID: 11834834

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

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

coincides with reduced cortical thickness. *Nat Commun* **5**:4858. DOI: <https://doi.org/10.1038/ncomms5858>, 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

Figure legends, supplementary files, source data

Figure 1. Knockdown of YTHDF2 leads to a robust increase of RGC dendrite branching.

(A) Representative confocal images showing high expression of YTHDF2 in RGCs (marked by RBPMS) in P0 retina. Note that all RGCs marked by the pan-RGC marker RBPMS express YTHDF2 while all YTHDF2-expressing cells are RBPMS⁺ RGCs. GCL, ganglion cell layer. Scale bars: 20 μ m.

(B) Western blotting (WB) confirming efficient knockdown (KD) of YTHDF2 in cultured RGCs using sh*Ythdf2*. Data of WB quantification are mean $\pm$ SEM and are represented as dot plots: *** p = 0.00012 (n = 3 replicates); by unpaired Student's t test.

(C) Examination of RGC dendrite development after YTHDF2 KD. As shown, significantly increased branching of dendrites marked by MAP2 immunofluorescence was observed in cultured RGCs marked by Brn3a. Dendrite traces were drawn for the corresponding RGCs. Scale bar: 10 μ m.

(D) Quantification of dendrite branching **(C)** using Sholl analysis. As shown, numbers of interactions are significantly greater in sh*Ythdf2* groups (n = 68 RGCs) than shCtrl groups (n = 72 RGCs) in Sholl radii between 10-30 μ m. Data are mean $\pm$ SEM. **** p = 4.32E-05 (10 μ m), *** p = 0.00038 (15 μ m), **** p = 2.85E-05 (20 μ m), *** p = 0.00084 (25 μ m), * p = 0.020 (30 μ m), by unpaired Student's t test.

Figure 1—figure supplement 1. RGC have high level of m⁶A modification and strong expression of YTHDFs.

(A-F) Representative confocal images showing high levels of m⁶A modification **(A)**, strong expressions of YTHDF2 **(B)**, YTHDF1 **(E)**, and YTHDF3 **(F)** in RGCs (marked by Brn3a) in P0 retina, and no expression of YTHDF2 in Müller glia (marked by Lhx2, **C**) and astrocytes (marked by GFAP, **D**) in P20 retina. Scale bars: 20 μ m.

(G, H) Western blotting (WB) confirming efficient knockdown (KD) of YTHDF1 and YTHDF3 in cultured RGCs using sh*Ythdf1* and sh*Ythdf3*, respectively.

872 (I, J) Quantification of total length (I) and length of maximum branch (J) of RGC dendrites after
 873 YTHDF2 KD. Data are represented as box and whisker plots: $n = 36$ RGCs for shCtrl, $n = 32$ RGCs for
 874 sh*Ythdf2*; *** $p = 0.00062$ for I; $p = 0.22$ for J; ns, not significant; by unpaired Student's t test.
 875 (K, L) Quantification of dendrite branching using Sholl analysis after YTHDF1 KD (K) and YTHDF3 KD
 876 (L). Data are mean $\pm$ SEM. In K, $n = 29$ RGCs for shCtrl, $n = 32$ RGCs for sh*Ythdf1*, all not significant; in
 877 L, $n = 32$ RGCs for shCtrl, $n = 27$ RGCs for sh*Ythdf3*, ** $p = 0.0028$ (20 μm), ** $p = 0.0028$ (30 μm), ** p
 878 $= 0.0052$ (35 μm); by unpaired Student's t test.

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

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

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

887 (C, D) RGC neurogenesis not affected in the *Ythdf2* cKO retina. Wholemount immunostaining using a
 888 Brn3a antibody was carried out in P20 retina (C). Numbers of Brn3a⁺ RGC per 10000 μm^2 of retina
 889 were quantified and showed no difference between the *Ythdf2* cKO and their littermate controls (D).
 890 $n = 12$ confocal fields for each genotype. Data are represented as box and whisker plots: ns, not
 891 significant ($p = 0.79$); by unpaired Student's t test. Scale bar: 25 μm .

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

896 (F) Quantification of RGC dendrite branching (E) using Sholl analysis. Data are mean $\pm$ SEM. Numbers
 897 of interactions are significantly greater in *Six3-cre*^{+/+};*Ythdf2*^{fl/fl} groups ($n = 68$ RGCs) than *Ythdf2*^{fl/fl}

groups ($n = 42$ RGCs) in Sholl radii between 10-30 μm : *** $p = 0.00030$ (10 μm), **** $p = 1.19\text{E-}05$ (15 μm), *** $p = 0.00018$ (20 μm), * $p = 0.021$ (25 μm), ** $p = 0.0022$ (30 μm), by unpaired Student's t test.

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

(H) Quantification of CART⁺ RGC dendrite branching (G) using Sholl analysis. Data are mean $\pm$ SEM. Numbers of interactions are significantly greater in *Six3-cre*^{+/+}, *Ythdf2*^{fl/fl} groups ($n = 77$ RGCs) than *Ythdf2*^{fl/fl} groups ($n = 90$ RGCs) in Sholl radii between 10-30 μm : **** $p = 3.17\text{E-}05$ (10 μm), **** $p = 6.50\text{E-}11$ (15 μm), **** $p = 5.14\text{E-}12$ (20 μm), **** $p = 5.00\text{E-}07$ (25 μm), *** $p = 0.00020$ (30 μm), by unpaired Student's t test.

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

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

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

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

(F, G) Bipolar cells not changed in the *Ythdf2* cKO retina. PKC α and CHX10 IF were used to label bipolar cells in P15 retina **(F)**. Numbers of PKC α ⁺/CHX10⁺ bipolar cells per 100 μ m of layer width in retina vertical sections were quantified and showed no difference between the *Ythdf2* cKO ($n = 18$ sections) and littermate controls ($n = 18$ sections) **(G)**.

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

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

All quantification data are represented as box and whisker plots: ns, not significant; $p = 0.41$ for **C**, $p = 0.16$ for **E**, $p = 0.82$ for **G**, $p = 0.89$ for **I**, $p = 0.19$ for **K**; by unpaired Student's t test. Scale bars: 20 μ m.

Figure 2—figure supplement 2. *Ythdf2* cKO does not change numbers of Müller glia or astrocytes.

(A, B) Müller glia numbers not changed in the *Ythdf2* cKO retina. Lhx2 IF was used to label Müller glia in P20 retina **(A)**. Numbers of Lhx2⁺ Müller glia per 100 μ m of layer width in retina vertical sections were quantified and showed no difference between the *Ythdf2* cKO ($n = 10$ sections) and littermate controls ($n = 9$ sections) **(B)**. Data are represented as box and whisker plots: ns, not significant; $p = 0.69$; by unpaired Student's t test.

(C, D) Astrocytes not affected in the *Ythdf2* cKO retina. GFAP IF was used to label astrocytes in P20 retina (arrowheads in **C**). Numbers of GFAP⁺ astrocytes per 100 μ m of layer width in retina vertical sections were quantified and showed no difference between the *Ythdf2* cKO ($n = 14$ sections) and

littermate controls ($n = 13$ sections) (**D**). Data are represented as box and whisker plots: ns, not significant; $p = 0.23$; by unpaired Student's t test.

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 μm .

(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 μm : ** $p = 0.0041$ (50 μm), ** $p = 0.0059$ (60 μm), *** $p = 0.00036$ (70 μm), ** $p = 0.0058$ (80 μm), ** $p = 0.0018$ (90 μm), ** $p = 0.0064$ (100 μm), ** $p = 0.0045$ (110 μm), * $p = 0.040$ (120 μm), 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 μm .

(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 μm : ** $p = 0.0083$ (20 μm), * $p = 0.018$ (40 μm), *** $p = 0.00068$ (50 μm), *** $p = 0.00027$ (60 μm), * $p = 0.048$ (70 μm), ** $p = 0.0048$ (80 μm), ** $p = 0.0023$ (100 μm), 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 μm .

(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 μm : $**p = 0.0044$ (40 μm), $**p = 0.0035$ (50 μm), $***p = 0.00021$ (60 μm), $****p = 2.63\text{E-}05$ (70 μm), $****p = 2.38\text{E-}06$ (80 μm), $****p = 1.68\text{E-}06$ (90 μm), $****p = 6.76\text{E-}06$ (100 μm), $****p = 5.72\text{E-}05$ (110 μm), $**p = 0.0011$ (120 μm), $**p = 0.0032$ (130 μm), $*p = 0.047$ (140 μm), by unpaired Student's t test.

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; $****p = 1.39\text{E-}06$ for C; $*p = 7.89\text{E-}08$ 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 μm .

(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%), $****p =$

999 3.48E-05 (45%), **** $p = 4.76E-05$ (40%), **** $p = 6.85E-05$ (35%), ** $p = 0.0034$ (30%), by unpaired
1000 Student's t test. Arrows indicate peaks of VACHT signals.

1001 (H) Cross-sections of the IPL showing dendritic sublaminal patterning of melanopsin⁺ ipRGCs in P20
1002 control and *Ythdf2* cKO retina. Scale bar: 20 μ m.

1003 (I) Quantification and distribution of melanopsin IF intensities from melanopsin⁺ ipRGC dendrites
1004 through the depth of IPL shown in (H). Melanopsin IF intensities are increased in the *Ythdf2* cKO
1005 retina compared with their littermate controls, but the general patterning is similar between the two
1006 genotypes. Data are mean $\pm$ SEM ($n = 11$ neurons for control, $n = 8$ neurons for *Ythdf2* cKO): * $p =$
1007 0.049 (95%), * $p = 0.010$ (90%), * $p = 0.039$ (75%), * $p = 0.022$ (70%), * $p = 0.019$ (65%), * $p = 0.018$
1008 (60%), * $p = 0.016$ (55%), * $p = 0.013$ (50%), ** $p = 0.0095$ (45%), ** $p = 0.0044$ (40%), ** $p = 0.0053$
1009 (35%), ** $p = 0.0091$ (30%), * $p = 0.014$ (25%), ** $p = 0.0074$ (20%), ** $p = 0.0071$ (15%), ** $p = 0.0053$
1010 (10%), ** $p = 0.0025$ (5%), ** $p = 0.0029$ (0%), by unpaired Student's t test.

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1012

1013 **Figure 4. IPL of the *Ythdf2* cKO retina is thicker and has more synapses.**

1014 (A) Cross-sections of P6 *Six3-cre*^{+/+}, *Ythdf2*^{fl/fl} retina showing increased IPL thickness by MAP2 staining
1015 compared with littermate control. ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner
1016 nuclear layer; IPL, inner plexiform layer; GCL, granule cell layer. Scale bar: 20 μ m.

1017 (B, C) Quantification showing increased IPL thickness and MAP2 IF intensity per area in IPL of the
1018 *Ythdf2* cKO retina (A). Quantification data are represented as box and whisker plots: *** $p = 1.28E-07$
1019 for B ($n = 12$ sections for each genotype), by unpaired Student's t test; ** $p = 0.0045$ for C ($n = 12$
1020 sections for each genotype), by paired Student's t test.

1021 (D, E) Representative confocal images showing the excitatory synapses labeled by co-localization of
1022 Bassoon (presynaptic) and PSD-95 (postsynaptic) in the IPL of P30 retina (D). There are significantly
1023 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 μm .

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

(A-D) Six3-Cre-mediated $Ythdf2$ cKO showing normal animal development and body weight (male in A, female in C). Quantification data of body weight (B, D) are represented as box and whisker plots: $p = 0.41$ in B (male, $n = 24$ for control, $n = 18$ for cKO); $p = 0.08$ in D (female, $n = 23$ for control, $n = 25$ for cKO); ns, not significant; by unpaired Student's t test.

(E) The setup of optomotor response assay is illustrated by schematic drawing.

(F, G) Optomotor response assay demonstrating improved visual acuity in the $Ythdf2$ cKO mice. Quantification data are mean $\pm$ SEM: $*p = 0.048$ in F (male, $n = 24$ control, $n = 16$ cKO); $*p = 0.015$ in G (female, $n = 21$ control, $n = 25$ cKO); by unpaired Student's t test.

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

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

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

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

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

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 $\pm$ 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.

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

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

(C) Increased dendrite branching of cultured RGCs prepared from E14.5 *Ythdf2* cKO (Y2 cKO) retina was rescued by KD of target mRNAs using siRNAs. Data are mean $\pm$ SEM. Ctrl, *Ythdf2*^{fl/fl}; Y2 cKO, *Six3*-

1101 *cre^{+/-}, Ythdf2^{fl/fl}*. In **C1**, “Ctrl, siCtrl” (*n* = 35 neurons) vs “Y2 cKO, siCtrl” (*n* = 52 neurons), **p* = 0.038 (15
1102 μm), **p* = 0.045 (20 μm), ***p* = 0.0036 (30 μm); “Y2 cKO, siKalrn7” (*n* = 55 neurons) vs “Y2 cKO,
1103 siCtrl”, **p* = 0.020 (15 μm), **p* = 0.025 (20 μm), **p* = 0.031 (25 μm); “Y2 cKO, siKalrn9” (*n* = 66
1104 neurons) vs “Y2 cKO, siCtrl”, **p* = 0.020 (10 μm), **p* = 0.013 (15 μm), **p* = 0.031 (20 μm), **p* = 0.017
1105 (25 μm), **p* = 0.031 (30 μm), **p* = 0.031 (45 μm); “Y2 cKO, siKalrn12” (*n* = 80 neurons) vs “Y2 cKO,
1106 siCtrl”, **p* = 0.015 (10 μm), ***p* = 0.0018 (15 μm), **p* = 0.015 (20 μm), **p* = 0.027 (40 μm). In **C2**, “Ctrl,
1107 siCtrl” (*n* = 50 neurons) vs “Y2 cKO, siCtrl” (*n* = 47 neurons), ***p* = 0.0031 (10 μm), ***p* = 0.0013 (15
1108 μm), **p* = 0.029 (20 μm), ***p* = 0.0015 (30 μm), **p* = 0.014 (35 μm); “Y2 cKO, siStrn” (*n* = 45 neurons)
1109 vs “Y2 cKO, siCtrl”, ****p* = 0.00016 (10 μm), ***p* = 0.0043 (15 μm), **p* = 0.010 (20 μm), **p* = 0.018 (30
1110 μm); “Y2 cKO, siUbr4” (*n* = 57 neurons) vs “Y2 cKO, siCtrl”, ****p* = 0.00084 (10 μm), *****p* = 4.89E-05
1111 (15 μm), ***p* = 0.0058 (20 μm), ***p* = 0.0045 (30 μm). All by unpaired Student’s *t* test.

1112 **(D)** Increased dendrite branching of RGC subtypes in *Ythdf2* cKO (Y2 cKO) retina was rescued by KD of
1113 target mRNAs through intravitreal injection of AAV shRNAs in vivo. Data are mean ± SEM. Ctrl,
1114 *Ythdf2^{fl/fl}*; Y2 cKO, *Six3-cre^{+/-}, Ythdf2^{fl/fl}*. In **D1** (CART⁺/eGFP⁺ ooDSGCs), “Ctrl, shCtrl” (*n* = 10 neurons)
1115 vs “Y2 cKO, shCtrl” (*n* = 6 neurons), **p* = 0.010 (10 μm), ****p* = 0.00049 (20 μm), ***p* = 0.0021 (30
1116 μm), ***p* = 0.0047 (40 μm), **p* = 0.028 (50 μm), **p* = 0.011 (60 μm), **p* = 0.030 (90 μm), **p* = 0.042
1117 (110 μm); “Y2 cKO, shKalrn12” (*n* = 8 neurons) vs “Y2 cKO, shCtrl”, **p* = 0.012 (20 μm), **p* = 0.014 (30
1118 μm); “Y2 cKO, shUbr4” (*n* = 6 neurons) vs “Y2 cKO, shCtrl”, **p* = 0.011 (10 μm), ***p* = 0.0084 (20 μm),
1119 **p* = 0.029 (30 μm). In **D2** (SMI-32⁺ αRGCs), “Ctrl, shCtrl” (*n* = 14 neurons) vs “Y2 cKO, shCtrl” (*n* = 14
1120 neurons), **p* = 0.032 (40 μm), ***p* = 0.0019 (50 μm), ***p* = 0.0014 (60 μm), **p* = 0.015 (70 μm), **p* =
1121 0.044 (90 μm); “Y2 cKO, shKalrn12” (*n* = 26 neurons) vs “Y2 cKO, shCtrl”, ***p* = 0.0023 (20 μm), ****p*
1122 = 0.00076 (30 μm), ****p* = 0.00030 (40 μm), ****p* = 0.00020 (50 μm), **p* = 0.015 (60 μm); “Y2 cKO,
1123 shUbr4” (*n* = 15 neurons) vs “Y2 cKO, shCtrl”, **p* = 0.042 (30 μm), **p* = 0.024 (40 μm), **p* = 0.018 (50
1124 μm). All by unpaired Student’s *t* test.

1127

1128 **Figure 7—figure supplement 1. YTHDF2 target mRNAs were characterized and validated.**

1129 (A) Upregulation of target mRNAs-encoding proteins Strn and Ubr4 in *Ythdf2* cKO retina in vivo.

1130 Enrichment and higher levels of these proteins were detected in the IPL of P6 *Ythdf2* cKO retina

1131 compared with control by IF. Scale bar: 20 μ m.

1132 (B) Upregulation of target mRNA levels after YTHDF2 KD. RT-qPCR confirmed upregulation of the

1133 candidate target mRNAs after KD of YTHDF2 in cultured RGCs using sh*Ythdf2*. Data are mean $\pm$ SEM

1134 and are represented as dot plots ($n = 3$ replicates): *** $p = 0.00084$ for *Kalrn7*; ** $p = 0.0039$ for

1135 *Kalrn9*; ** $p = 0.0026$ for *Kalrn12*; **** $p = 1.11E-06$ for *Strn*; *** $p = 0.00011$ for *Ubr4*; by unpaired

1136 Student's t test.

1137 (C) Upregulation of target mRNA levels in the *Ythdf2* cKO retina were confirmed by RT-qPCR. Data are

1138 mean $\pm$ SEM and are represented as dot plots ($n = 3$ replicates): * $p = 0.030$ for *Kalrn7*; ** $p = 0.0064$

1139 for *Kalrn9*; *** $p = 0.00049$ for *Kalrn12*; * $p = 0.041$ for *Strn*; ** $p = 0.0027$ for *Ubr4*; by unpaired

1140 Student's t test.

1141 (D) Confirmation of METTL14 KD in cultured RGCs using sh*Mettl14* by RT-qPCR. Data are mean $\pm$ SEM

1142 and are represented as dot plots ($n = 3$ replicates): *** $p = 0.00012$ (sh*Mettl14#6* vs shCtrl); ** $p =$

1143 0.0079 (sh*Mettl14#7* vs shCtrl); by unpaired Student's t test.

1144 (E) Upregulation of target mRNA levels after METTL14 KD. RT-qPCR confirmed upregulation of the

1145 candidate target mRNAs after KD of METTL14 in cultured RGCs using sh*Mettl14*. Data are mean $\pm$

1146 SEM and are represented as dot plots ($n = 3$ replicates): for *Kalrn7*, ** $p = 0.0034$ (sh*Mettl14#6* vs

1147 shCtrl), * $p = 0.016$ (sh*Mettl14#7* vs shCtrl); for *Kalrn9*, ** $p = 0.0010$ (sh*Mettl14#6* vs shCtrl), ** $p =$

1148 0.0067 (sh*Mettl14#7* vs shCtrl); for *Kalrn12*, ** $p = 0.0079$ (sh*Mettl14#6* vs shCtrl), * $p = 0.026$

1149 (sh*Mettl14#7* vs shCtrl); for *Strn*, **** $p = 5.45E-06$ (sh*Mettl14#6* vs shCtrl), * $p = 0.025$ (sh*Mettl14#7*

1150 vs shCtrl); for *Ubr4*, ** $p = 0.0029$ (sh*Mettl14#6* vs shCtrl), ** $p = 0.0066$ (sh*Mettl14#7* vs shCtrl); by

1151 unpaired Student's t test.

(F) Confirmation of KD by siRNAs against target mRNAs. Data are mean $\pm$ SEM and are represented as dot plots ($n = 3$ replicates): $**p = 0.0020$ for *Kalrn7*; $**p = 0.0025$ for *Kalrn9*; $***p = 0.00020$ for *Kalrn12*; $**p = 0.0021$ for *Strn*; $**p = 0.0033$ for *Ubr4*; by unpaired Student's t test.

(G) KD of target mRNAs all together using a siRNA cocktail causing further decrease of dendrite branching of cultured RGCs compared with single siRNA against each target mRNA. Data are mean $\pm$ SEM: $n = 32$ RGCs for siCtrl, $n = 33$ RGCs for si*Kalrn7*, $n = 32$ RGCs for si*Kalrn9*, $n = 35$ RGCs for si*Kalrn12*, $n = 35$ RGCs for si*Strn*, $n = 36$ RGCs for si*Ubr4*, $n = 36$ RGCs for siCocktail. si*Kalrn7* vs siCtrl: $*p = 0.031$ (10 μ m), $*p = 0.046$ (15 μ m); si*Kalrn9* vs siCtrl: $**p = 0.0011$ (10 μ m), $**p = 0.0090$ (15 μ m); si*Kalrn12* vs siCtrl: $**p = 0.0061$ (10 μ m), $**p = 0.0086$ (15 μ m); si*Strn* vs siCtrl: $***p = 0.00056$ (10 μ m), $*p = 0.025$ (15 μ m); si*Ubr4* vs siCtrl: $**p = 0.0018$ (10 μ m), $*p = 0.026$ (15 μ m), $*p = 0.048$ (20 μ m), $*p = 0.011$ (25 μ m); siCocktail vs siCtrl: $****p = 3.44E-06$ (10 μ m), $****p = 4.07E-06$ (15 μ m), $***p = 0.00077$ (20 μ m), $**p = 0.0010$ (25 μ m), $**p = 0.0049$ (30 μ m), $**p = 0.0094$ (35 μ m). si*Kalrn7* vs siCocktail: $**p = 0.0092$ (10 μ m), $**p = 0.0040$ (15 μ m), $**p = 0.0028$ (20 μ m), $**p = 0.0034$ (25 μ m); si*Kalrn9* vs siCocktail: $**p = 0.0042$ (15 μ m), $*p = 0.034$ (20 μ m); si*Kalrn12* vs siCocktail: $*p = 0.029$ (10 μ m), $*p = 0.019$ (15 μ m), $**p = 0.0014$ (20 μ m), $**p = 0.0091$ (25 μ m), $**p = 0.0063$ (30 μ m); si*Strn* vs siCocktail: $*p = 0.043$ (10 μ m), $**p = 0.0051$ (15 μ m), $**p = 0.0045$ (20 μ m), $****p = 3.79E-06$ (25 μ m), $***p = 0.00022$ (30 μ m); si*Ubr4* vs siCocktail: $*p = 0.049$ (10 μ m), $*p = 0.011$ (15 μ m). All by unpaired Student's t test.

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

1178 significantly greater in *Six3-cre*^{+/+}, *Ythdf2*^{fl/fl} retina than *Ythdf2*^{fl/fl} control retina in both RGC subtypes
 1179 after AOH: for melanopsin⁺ ipRGCs in **A**, *Ythdf2*^{fl/fl}/AOH (*n* = 51 RGCs) vs cKO/AOH (*n* = 64 RGCs), ****p*
 1180 = 0.00015 (10 μm), ***p* = 0.0017 (20 μm), **p* = 0.034 (30 μm), ****p* = 0.00035 (40 μm), *****p* =
 1181 3.02E-05 (50 μm), *****p* = 2.63E-05 (60 μm), ***p* = 0.0029 (70 μm), ***p* = 0.0028 (80 μm), ****p* =
 1182 0.00035 (90 μm), ***p* = 0.0032 (100 μm), ***p* = 0.0014 (110 μm), ***p* = 0.0043 (120 μm), ***p* =
 1183 0.0014 (130 μm), **p* = 0.023 (140 μm), **p* = 0.013 (150 μm); for SMI-32⁺ αRGCs in **B**, *Ythdf2*^{fl/fl}/AOH
 1184 (*n* = 21 neurons) vs cKO/AOH (*n* = 15 neurons), ***p* = 0.0052 (40 μm), ***p* = 0.0057 (50 μm); all by
 1185 unpaired Student's *t* test.
 1186 **(C, D)** *Ythdf2* cKO retina showing less severe RGC loss after AOH. AOH was performed using adult
 1187 mice and retinas were collected after AOH for wholemount immunostaining using a Brn3a antibody
 1188 **(C)**. Numbers of Brn3a⁺ RGCs per 10000 μm² of retina were quantified for different genotypes and
 1189 conditions (confocal fields for analysis: *n* = 117 for *Ythdf2*^{fl/fl}/Ctrl; *n* = 98 for *Ythdf2*^{fl/fl}/AOH; *n* = 110
 1190 for cKO/Ctrl; *n* = 104 for cKO/AOH). Data are represented as box and whisker plots **(D)**: ns, not
 1191 significant (*p* = 0.16; *Ythdf2*^{fl/fl}/Ctrl vs cKO/Ctrl); ***p* = 0.0077 (*Ythdf2*^{fl/fl}/AOH vs cKO/AOH); by
 1192 unpaired Student's *t* test. Scale bar: 25 μm.
 1193 **(E, F)** Overexpression (OE) of YTHDF2 targets *Hspa12a* and *Islr2* protecting retina from RGC dendrite
 1194 degeneration in the AOH model. Wild type (WT) mice were intravitreally injected with AAV
 1195 overexpressing *Hspa12a* or *Islr2* and then operated with AOH. Wholemount immunostaining of
 1196 CART/ZsGreen and SMI-32/ZsGreen was carried out to visualize the dendrite arbors of corresponding
 1197 RGC subtype, respectively. Dendrite traces were drawn as previously shown and quantification of
 1198 dendrite branching was done using Sholl analysis. Data are mean ± SEM. Numbers of interactions are
 1199 significantly greater in retina with OE of *Hspa12a* or *Islr2* than control retina in both RGC subtypes
 1200 after AOH. For CART⁺ ooDSGCs in **E**: OE-Ctrl/AOH (*n* = 11 RGCs) vs OE-Hspa12a/AOH (*n* = 11 RGCs), **p*
 1201 = 0.014 (20 μm), ***p* = 0.0025 (30 μm), **p* = 0.018 (40 μm); OE-Ctrl/AOH vs OE-*Islr2*/AOH (*n* = 6
 1202 RGCs), **p* = 0.024 (10 μm), ****p* = 0.00031 (20 μm), ***p* = 0.0038 (30 μm), **p* = 0.013 (40 μm). For
 1203 SMI-32⁺ αRGCs in **F**: OE-Ctrl/AOH (*n* = 49 neurons) vs OE-Hspa12a/AOH (*n* = 46 neurons), ***p* =

1204 0.0023 (30 μm), *** p = 0.00080 (40 μm), ** p = 0.0059 (50 μm), ** p = 0.0051 (60 μm), ** p = 0.0036
1205 (70 μm), *** p = 0.00070 (80 μm), ** p = 0.0015 (90 μm), * p = 0.016 (100 μm), * p = 0.011 (110 μm);
1206 OE-Ctrl/AOH vs OE-IsIr2/AOH (n = 13 RGCs), * p = 0.010 (30 μm), ** p = 0.0093 (40 μm), ** p = 0.0019
1207 (50 μm), *** p = 0.00085 (60 μm), *** p = 0.00067 (70 μm), **** p = 4.25E-05 (80 μm), **** p = 2.54E-
1208 05 (90 μm), *** p = 0.00020 (100 μm), ** p = 0.0016 (110 μm). All by unpaired Student's t test.

1209 **(G)** Overexpression (OE) of YTHDF2 targets *Hspa12a* and *IsIr2* alleviating RGC loss in the AOH model.
1210 Wild type (WT) mice were intravitreally injected with AAV overexpressing *Hspa12a* or *IsIr2* and then
1211 operated with AOH. Wholemout immunostaining of Brn3a was performed to label RGCs. Numbers
1212 of Brn3a⁺ RGCs per 10000 μm^2 of retina were quantified for different conditions (confocal fields for
1213 analysis: n = 19 for OE-Ctrl; n = 26 for OE-Hspa12a; n = 24 for OE-IsIr2). Data are represented as box
1214 and whisker plots: * p = 0.034 (OE-Hspa12a vs OE-Ctrl; * p = 0.029 (OE-IsIr2 vs OE-Ctrl); by unpaired
1215 Student's t test.

1216

1217

1218 **Figure 8—figure supplement 1. *Hspa12a* and *IsIr2* are two target mRNAs of YTHDF2 in adult retina.**

1219 **(A, B)** The curves from *Figure 3D,F* and *Figure 8A,B* were plotted together for easy comparison. The
1220 error bars and the asterisks were removed from these graphs for easy reading and these information
1221 can still be seen in *Figure 3D,F* and *Figure 8A,B*.

1222 **(C)** Upregulation of YTHDF2 target mRNA *Hspa12a* and *IsIr2* in adult *Ythdf2* cKO retina compared with
1223 control by RT-qPCR. Data are mean $\pm$ SEM and are represented as dot plots (n = 3 replicates): ** p =
1224 0.0035 for *Hspa12a*; *** p = 0.00012 for *IsIr2*; by unpaired Student's t test.

1225 **(D)** Verification of m⁶A modification of *Hspa12a* and *IsIr2* mRNAs by anti-m⁶A pulldown followed by
1226 RT-qPCR. ND, not detected. Data are mean $\pm$ SEM and are represented as dot plots (n = 3 replicates):
1227 **** p = 7.07E-06 for *IsIr2*; **** 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.

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

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

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

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

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

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

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

1254

1255 **Figure 1-figure supplement 1-source data 1. Source data for Figure 1-figure supplement 1E,F.**

1256 (A) WB of anti YTHDF1 after KD of YTHDF1.

1257 (B) WB of anti β -actin after KD of YTHDF1.

1258 (C) WB of anti YTHDF3 after KD of YTHDF3.

1259 (D) WB of anti β -actin after KD of YTHDF3.

1260

1261 **Figure 1-figure supplement 1-source data 2. Source data for Figure 1-figure supplement 1E.**

1262 Original file of the full raw unedited blot of anti YTHDF1 after KD of YTHDF1.

1263

1264 **Figure 1-figure supplement 1-source data 3. Source data for Figure 1-figure supplement 1E.**

1265 Original file of the full raw unedited blot of anti β -actin after KD of YTHDF1.

1266

1267 **Figure 1-figure supplement 1-source data 4. Source data for Figure 1-figure supplement 1F.**

1268 Original file of the full raw unedited blot of anti YTHDF3 after KD of YTHDF3.

1269

1270 **Figure 1-figure supplement 1-source data 5. Source data for Figure 1-figure supplement 1F.**

1271 Original file of the full raw unedited blot of anti β -actin after KD of YTHDF3.

Key resources table				
Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
strain, strain background (mouse)	Mouse: Ythdf2 ^{fl/fl}	(Yu et al. 2021)	N/A	
strain, strain background (mouse)	Mouse: Tg(Six3-cre)69Frt ^y /GcoJ	Jackson Laboratory	Cat#: JAX_019755 RRID: IMSR_JAX:019755	
strain, strain background (mouse)	Mouse: B6.Cg-Tg(Thy1-EGFP)OJrs/GfngJ	Jackson Laboratory	Cat#: JAX_007919 RRID: IMSR_JAX:007919	
strain, strain background (mouse)	Mouse: B6.129X1-Gt(Rosa)26Sor ^{tm1(EYFP)C} ^{os} /J	Jackson Laboratory	Cat#: JAX_006148 RRID: IMSR_JAX:006148	
antibody	anti-GFP (Chicken polyclonal)	Abcam	Cat#: ab13970, RRID: AB_300798	IF (1:1000)
antibody	anti-MAP2 (Chicken polyclonal)	Abcam	Cat#: ab5392, RRID: AB_2138153	IF (1:10000)
antibody	anti-RBPMS (Guinea pig polyclonal)	PhosphoSolutions	Cat#: 1832-RBPMS, RRID: AB_2492226	IF (1:1000)
antibody	anti-VACHT (Goat polyclonal)	Millipore	Cat#: ABN100, RRID: AB_2630394	IF (1:1000)

antibody	anti- β Actin (Mouse monoclonal)	Abcam	Cat#: ab6276, RRID: AB_2223210	WB (1:30000)
antibody	anti- β Actin (Mouse monoclonal)	ABclonal	Cat#: AC004, RRID: AB_2737399	WB (1:30000)
antibody	anti-AP2 α (Mouse monoclonal)	DSHB	Cat#: 3B5, RRID: AB_2313947	IF (1:1000)
antibody	anti-Bassoon (Mouse monoclonal)	Enzo Life Sciences	Cat#: ADI-VAM-PS003, RRID: AB_10618753	IF (1:2500)
antibody	anti-Brn3a (Mouse monoclonal)	Millipore	Cat#: MAB1585, RRID: AB_94166	IF (1:300)
antibody	anti-Calbindin-D-28K (Mouse monoclonal)	Sigma-Aldrich	Cat#: C9848, RRID: AB_476894	IF (1:200)
antibody	anti-PKC α (Mouse monoclonal)	Santa Cruz Biotechnology	Cat#: sc-8393, RRID: AB_628142	IF (1:500)
antibody	anti-SMI-32 (Mouse monoclonal)	BioLegend	Cat#: 801701, RRID: AB_2564642	IF (1:200)
antibody	anti-Strn (Striatin) (Mouse monoclonal_	BD Biosciences	Cat#: 610838, RRID: AB_398157	IF (1:500)
antibody	anti-CART (Rabbit polyclonal)	Phoenix Pharmaceuticals	Cat#: H-003-62, RRID: AB_2313614	IF (1:2000)
antibody	anti m ⁶ A (Rabbit polyclonal)	Synaptic Systems	Cat# 202003, RRID: AB_2279214	IF (1:200)
antibody	anti-melanopsin (Rabbit polyclonal)	Thermo Fisher Scientific	Cat#: PA1-780, RRID: AB_2267547	IF (1:1000)
antibody	anti-PKC α (Rabbit	Cell Signaling	Cat#: CST-2056	IF (1:1000)

	polyclonal)			
antibody	anti-PSD-95 (Mouse monoclonal)	Abcam	Cat#: ab2723, RRID: AB_303248	IF (1:500)
antibody	anti-Recoverin (Rabbit polyclonal)	Millipore	Cat#: AB5585, RRID: AB_2253622	IF (1:1000)
antibody	anti-YTHDF2 (Rabbit polyclonal)	Proteintech	Cat#: 24744-1-AP, RRID: AB_2687435	IF (1:1000)
antibody	anti-YTHDF1 (Rabbit polyclonal)	Proteintech	Cat#: 17479-1-AP, RRID: AB_2217473	IF (1:1000)
antibody	anti-YTHDF3 (Rabbit polyclonal)	Abcam	Cat#: ab103328, RRID: AB_10710895	IF (1:1000)
antibody	anti-Ubr4 (Rabbit polyclonal)	Abcam	Cat#: ab86738, RRID: AB_1952666	IF (1:300)
antibody	anti-Chx10 (Sheep polyclonal)	Exalpha	Cat#: X1179P	IF (1:1000)
antibody	anti-GFAP (Chicken polyclonal)	Millipore	Cat#: AB5541, RRID: AB_177521	IF (1:500)
antibody	anti-Lhx2 (Goat polyclonal)	Santa Cruz Biotechnology	Cat#: sc-19344, RRID: AB_2135660	IF (1:200)
antibody	anti-Lhx2 (Rabbit monoclonal)	Abcam	Cat#: ab184337	IF (1:500)
antibody	anti-chicken IgY (Alexa 488 donkey)	Jackson ImmunoResearch	Cat#: 703-545-155, RRID: AB_2340375	IF (1:500)
antibody	anti-G. pig IgG (Alexa 488 donkey)	Jackson ImmunoResearch	Cat#: 706-545-148, RRID: AB_2340472	IF (1:500)

antibody	anti-mouse IgG (Alexa 488 donkey)	Thermo Fisher Scientific	Cat#: A-21202, RRID: AB_141607	IF (1:500)
antibody	anti-rabbit IgG (Alexa 488 donkey)	Thermo Fisher Scientific	Cat#: A-21206, RRID: AB_141708	IF (1:500)
antibody	anti-goat IgG (Alexa 555 donkey)	Thermo Fisher Scientific	Cat#: A-21432, RRID: AB_2535853	IF (1:1000)
antibody	anti-mouse IgG (Alexa 555 donkey)	Thermo Fisher Scientific	Cat#: A-31570, RRID: AB_2536180	IF (1:1000)
antibody	anti-rabbit IgG (Alexa 555 donkey)	Thermo Fisher Scientific	Cat#: A-31572, RRID: AB_162543	IF (1:1000)
antibody	anti-sheep IgG (Alexa 555 donkey)	Thermo Fisher Scientific	Cat# A-21436, RRID: AB_2535857	IF (1:1000)
antibody	anti-chicken IgY (Alexa 555 goat)	Thermo Fisher Scientific	Cat#: A-21437, RRID: AB_2535858	IF (1:1000)
antibody	anti-mouse IgG (Alexa 647 donkey)	Thermo Fisher Scientific	Cat#: A-31571, RRID: AB_162542	IF (1:200)
antibody	anti-mouse IgG (HRP donkey)	Abcam	Cat#: ab97030, RRID: AB_10680919	WB (1:2500)
antibody	anti-rabbit IgG (HRP donkey)	Abcam	Cat#: ab16284, RRID: AB_955387	WB (1:2500)
antibody	anti-mouse IgG (HRP VHH)	AlpaLife	Cat#: KTSM1321	WB (1:5000)
antibody	anti-rabbit IgG (HRP VHH)	AlpaLife	Cat#: KTSM1322	WB (1:5000)
recombinant	Plasmid: pLKO.1-TRC	Addgene	Addgene plasmid #10878,	

DNA reagent			RRID: Addgene_10878	
sequence-based reagent	shRNA targeting sequence of negative control	This paper	N/A	GCATCAAGGTGAACTTCAAGA
sequence-based reagent	shRNA targeting sequence of mouse <i>Ythdf2</i>	(Yu et al. 2018)	N/A	GGACGTTCCCAATAGCCAACT
sequence-based reagent	shRNA targeting sequence of mouse <i>Ythdf1</i>	This paper	N/A	GGACATTGGTACTTGGGATAA
sequence-based reagent	shRNA targeting sequence of mouse <i>Ythdf3</i>	This paper	N/A	GGATTTGGCAATGATACTTTG
sequence-based reagent	shRNA targeting sequence of mouse <i>Mettl14#6</i>	This paper	N/A	GCTGGACCTGGGATGATATTA
sequence-based reagent	shRNA targeting sequence of mouse <i>Mettl14#7</i>	This paper	N/A	CCCAGCTTGTA CTTTGCTTTA
sequence-based reagent	shRNA targeting sequence of negative control (AAV)	This paper	N/A	TTCTCCGAACGTGTCACGTAA
sequence-based reagent	shRNA targeting sequence of mouse <i>Kalrn12</i>	This paper	N/A	TGATGAGCTGATGGAAGAA
sequence-based reagent	shRNA targeting sequence of mouse <i>Ubr4</i>	This paper	N/A	AATGATGAGCAGTCATCTC
sequence-based reagent	siRNA targeting sequence of negative control	(Yu et al. 2018)	N/A	UUCUCCGAACGUGUCACGUTT
sequence-based reagent	siRNA targeting sequence of mouse <i>Kalrn7</i>	(Xie et al. 2007)	N/A	AGUACAAUCCUGGCCAUGUTT

sequence-based reagent	siRNA targeting sequence of mouse <i>Kalrn9</i>	(Yan et al. 2015)	N/A	ACUGGACUGGACUUCUAUUTT
sequence-based reagent	siRNA targeting sequence of mouse <i>Kalrn12</i>	(Yan et al. 2015)	N/A	CGAUGAGCUGAUGGAAGAATT
sequence-based reagent	siRNA targeting sequence of mouse <i>Strn</i>	(Breitman et al. 2008)	N/A	GGUGAAGAUCGAGAUACAATT
sequence-based reagent	siRNA targeting sequence of mouse <i>Ubr4</i>	(Shim et al. 2008)	N/A	AAUGAUGAGCAGUCAUCUATT
sequence-based reagent	qPCR primers of mouse <i>18s</i>	(Wang et al. 2018)	N/A	Fwd: GCTTAATTTGACTCAACACGGGA Rev: AGCTATCAATCTGTCAATCCTGTC
sequence-based reagent	qPCR primers of mouse <i>Gapdh</i>	(Mains et al. 2011)	N/A	Fwd: TTGTCAGCAATGCATCCTGCACCA CC Rev: CTGAGTGGCAGTGATGGCATGG AC
sequence-based reagent	qPCR primers of mouse <i>Ythdf2</i>	This paper	N/A	Fwd: GAGCAGAGACCAAAAGGTCAAG Rev: CTGTGGGCTCAAGTAAGTTC
sequence-based reagent	qPCR primers of mouse <i>Kalrn7</i>	(Mains et al. 2011)	N/A	Fwd: GATACCATATCCATTGCCTCCAGG ACC Rev: CCAGGCTGCGCGCTAAACGTAAG
sequence-based reagent	qPCR primers of mouse <i>Kalrn9</i>	(Mains et al. 2011)	N/A	Fwd: GCCCCTCGCCAAAGCCACAGC Rev: CCAGTGAGTCCCGTGGTGGGC

sequence-based reagent	qPCR primers of mouse <i>Kalrn12</i>	(Mains et al. 2011)	N/A	Fwd: CAGCAGCCACGTGCCTGCAGC Rev: TCTTGACATTGGGAATGGGCCGCAC
sequence-based reagent	qPCR primers of mouse <i>Strn</i>	This paper	N/A	Fwd: TGAAGCCTGGAATGTGGACC Rev: CTATTGGGCCTCTTCACCCC
sequence-based reagent	qPCR primers of mouse <i>Ubr4</i>	This paper	N/A	Fwd: TGAGTGAGGACAAGGGCAAC Rev: GGGTTGGATCGAACGAAGGT
sequence-based reagent	qPCR Primer for mouse <i>Hspa12a</i>	This paper	N/A	Fwd: GGGTTTGACAGGCTAAGGA Rev: TCTGATGGACGGTCAGGTCT
sequence-based reagent	qPCR Primer for mouse <i>Islr2</i>	This paper	N/A	Fwd: GAAGCTCCCTTAGACTGTCACC Rev: CCCCATCGTGACTCCTGCTG
sequence-based reagent	PCR Primer for mouse <i>Hspa12a</i> CDS	This paper	N/A	Fwd: ATGGCGGACAAGGAAGCTGG Rev: GTAATTTAAGAAGTCGATCCCC
sequence-based reagent	PCR Primer for mouse <i>Islr2</i> CDS	This paper	N/A	Fwd: ATGGGGCCCTTTGGAGC Rev: GCCCGCTGTCTGCCTGTAG
sequence-based reagent	Mouse genotyping primers for <i>Ythdf2</i> loxp site 1	This paper	N/A	GCTTGTAGTTATGTTGTGTACCAC and GCAGCTCTGACTATTCTAAACCTCC
sequence-based reagent	Mouse genotyping primers for <i>Ythdf2</i> loxp site 2	This paper	N/A	CTCATAACATCCATAGCCACAGG and CCAAGAGATAGCTTTCCTAATG
sequence-based	Mouse genotyping	Chunqiao	N/A	CCTTCCTCCCTCTCTATGTG and

reagent	primers for <i>Six3-cre</i>	Liu's lab		GAACGAACCTGGTCGAAATC
sequence-based reagent	Mouse genotyping primers for <i>Thy1-GFP</i>	The Jackson Laboratory website	N/A	CGGTGGTGCAGATGAACTT and ACAGACACACACCCAGGACA
sequence-based reagent	Mouse genotyping primers for Rosa-YFP mutant site	The Jackson Laboratory website	N/A	AGGGCGAGGAGCTGTTCA and TGAAGTCGATGCCCTTCAG
sequence-based reagent	Mouse genotyping primers for Rosa-YFP wildtype site	The Jackson Laboratory website	N/A	CTGGCTTCTGAGGACCG and CAGGACAACGCCACACA
peptide, recombinant protein	Insulin	Sigma	Cat#: I6634	
peptide, recombinant protein	Recombinant Human/Murine/Rat BDNF	PeproTech	Cat#: 450-02	
peptide, recombinant protein	Recombinant Human NT-3	PeproTech	Cat#: 450-03	
peptide, recombinant protein	Recombinant Murine EGF	PeproTech	Cat#: 315-09	
peptide, recombinant protein	Recombinant Human FGF-basic	PeproTech	Cat#: 100-18B	
commercial assay or kit	Pierce™ BCA Protein Assay Kit	Thermo Fisher Scientific	Cat#: 23227	
commercial assay or kit	GeneSilencer® Transfection Reagent	Genlantis	Cat#: T500750	
commercial assay or kit	Magna MeRIP™ m ⁶ A Kit	Millipore	Cat#: 17-10499	
commercial assay or kit	EZ-Magna RIP™ RNA-Binding Protein Immunoprecipitation	Millipore	Cat#: 17-701	

	Kit			
chemical compound, drug	cpt-cAMP, 8-(4-Chlorophenylthio) Adenosine 3':5'-CY	Sigma	Cat#: C3912	
chemical compound, drug	N-acetyl-L-cysteine (NAC)	Sigma	Cat#: A8199	
chemical compound, drug	Forskolin	Sigma	Cat#: F6886	
chemical compound, drug	Puromycin	Thermo Fisher Scientific	Cat#: A11138-03	
chemical compound, drug	Puromycin	Sigma	Cat#: P8833	
chemical compound, drug	Paraformaldehyde	Vetec	Cat#: V900894-100G	
chemical compound, drug	Triton x-100	Sigma	Cat#: V900502	
software, algorithm	GraphPad Prism 7.0	GraphPad	https://www.graphpad.com , RRID: SCR_002798	
software, algorithm	STAR v2.5	(Dobin et al. 2013)	https://github.com/alexdobin/STAR/	
software, algorithm	HTSeq	(Anders et al. 2015)	https://pypi.org/project/HTSeq/	
software, algorithm	ImageJ (Fiji)	(Schindelin et al. 2012)	http://fiji.sc , RRID:SCR_002285	
software,	Matlab	Matlab	https://www2.ma	

algorithm			thworks.cn	
other	TRIzol Reagent	Life	Cat#: 15596018	
other	PrimeScript™ RT Master Mix	Takara	Cat#: RR036B	
other	2× ChamQ™ Universal SYBR qPCR Master Mix	Vazyme	Cat#: Q711-02	
other	DMEM, high glucose	Gibco	Cat#: 11965-092	
other	Dulbecco's Modified Eagle's Medium, 10×, low glucose	Sigma	Cat#: D2429	
other	DMEM, high glucose	Hyclone	Cat#: SH30022.01	
other	Fetal Bovine Serum (FBS)	Gibco	Cat#: 10270-106	
other	Dulbecco's Phosphate-Buffered Saline, 1× without calcium and magnesium (DPBS)	Corning	Cat#: 21-031-CVR	
other	poly-D-lysine, Cultrex	Trevigen	Cat#: 3439-100-01	
other	Laminin (mouse), Culrex	Trevigen	Cat#: 3400-010-01	
other	DMEM/F-12, GlutaMAX™	Gibco	Cat#: 10565-018	
other	Neurobasal™ Medium, minus phenol red	Gibco	Cat#: 12348-017	
other	Penicillin-Streptomycin	Life	Cat#: 15140-122	
other	B27 serum-free supplement, 50×	Life	Cat#: 17504044	
other	N-2 Supplement, 100×	Gibco	Cat#: 17502-048	
other	O.C.T. Compound and	SAKURA	Cat#: 4583	

	Cryomolds, Tissue-Tek			
other	ChemiBLOCKER	Millipore	Cat#: 2170	
other	CTB (Cholera Toxin Subunit B) conjugated by Alexa Fluor™ 555	Invitrogen	Cat#: C34776	
other	VECTASHIELD Antifade Mounting Medium with DAPI	Vector Laboratory	Cat#: H-1200	
other	Mounting Medium, antifading (with DAPI)	Solarbio	Cat#: S2110	
other	Normal Goat Serum	Novus	Cat#: NBP2-23475	

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A *Ythdf2*^{fl/fl} *Six3-cre*^{+/-},
Ythdf2^{fl/fl}

C *Ythdf2*^{fl/fl} *Six3-cre*^{+/-},
Ythdf2^{fl/fl}

E left eye activated

right eye activated

Spatial frequency

All graphs:

Ythdf2^{fl/fl}

Six3-cre^{+/-}, *Ythdf2*^{fl/fl}

A **B** **C** **D**

All graphs: ■ *Ythdf2*^{fl/fl} ■ *Six3-cre*^{+/-}, *Ythdf2*^{fl/fl}