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Site-specific gains and losses of heterochromatin accelerate the age-related neurodegeneration through the cascading destruction of KDM3B-centered epigenomic network

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ABSTRACT

Epigenetic alterations explained by the “loss of heterochromatin” model have been proposed as a universal mechanism of aging, but the region-specific changes of heterochromatin during aging are unclear. Here, we examine age-dependent transcriptomic profiling of mouse retinal neurons to identify epigenetic regulators involved in heterochromatin loss. RNA sequencing analysis revealed gradual down-regulation of Kdm3b during retinal aging. Disruption of Kdm3b (Kdm3b+/−) in 12-month-old mouse retina decreased the number of cone photoreceptors and changed the morphology of cone ribbon synapses. Integration of transcriptome profiling with epigenomic analysis demonstrated gain of heterochromatin feature in synapse assembly and vesicle transport genes via the accumulation of H3K9 mono- and di-methylation. However, the loss of heterochromatin in apoptotic genes exacerbated retinal neurodegeneration. We propose that this KDM3B-centered epigenomic network is crucial for maintaining cone photoreceptor homeostasis via the modulation of gene-set specific heterochromatin features during aging.
INTRODUCTION

Aging affects all organ systems, but age-related nervous system deficits are among the most prominent aging-related changes. Neuronal functions are gradually impaired during aging via aberrant neuronal network activity, increased oxidative stress, and disturbed energy homeostasis. The retina exhibits elaborate cellular patterns and circuitry and is one of the best-understood models of the vertebrate central nervous system; the differentiated retina, a functional aging model, is suitable for demonstrating the destruction between physical neuronal networks (synapses) and molecular regulatory mechanisms. Retinal aging leads to a reduction in visual acuity and visual field sensitivity, and an increased dark adaptation threshold, resulting in age-related diseases, such as age-related macular degeneration (AMD). Although these physiological changes are apparent, the molecular mechanisms underlying neurochemical changes and genome-wide epigenetic homeostasis during aging have not been identified.

Epigenetic regulation directly contributes to aging and age-related diseases, including changes in chromatin accessibility, accumulation of histone variants, aberrant histone modifications, and chromatin organization. The “loss of heterochromatin” is considered a common mechanism of aging across cell types and species. Age-related destabilization of tightly compacted chromatin leads to aberrant gene expression and cellular dysfunction. A typical mark of constitutive heterochromatin is the trimethylation of histone H3 on lysine 9 (H3K9me3), while H3K27me3 is usually enriched in facultative heterochromatin. Aging-associated losses of repressive histone mark occur at H3K9me3 and H3K27me3. For example, histone methyl transferase SUV39H1 expression levels in hematopoietic stem cells decrease during aging, resulting in a global reduction in H3K9me3 level and perturbed heterochromatin function. In addition, EZH2 is downregulated in stressed and senescent human lung cells, leading H3K27me3 loss. However, the region-specific changes in heterochromatin of the functionally active genes in differentiated cells have not been elucidated.

The epigenomic network is controlled by chromatin modification and the binding of transcription factors, resulting in changes in gene expression. The resulting variability in the pattern of epigenetic
information within individual cells in a population during aging leads to transcriptional drifts and genomic instability. Histone-modifying enzymes are regulatory factors for gene expression; among them, histone lysine methyl transferases and demethylases (KDMs) establish functional cell types through dynamic writing and erasing methylation marks on cell-type specific gene elements during development and aging\textsuperscript{7,8}. The lysine-specific demethylase 3 (KDM3) family includes KDM3A, KDM3B, and KDM3C, which are specific demethylases for mono-, and di-methylation at H3K9 and act as transcriptional activators\textsuperscript{9}. The KDM3 family is crucial for cell survival and proliferation in rapidly dividing cells, such as prostate cancer, leukemia, and hepatocellular carcinoma cells\textsuperscript{9-11}. Nevertheless, whether and how KDM3 contributes to non-mitotic differentiated cells in development, aging, and disease development is still poorly understood.

The functional physiology of differentiated cells is manifested through genetically encoded molecular interactions and complex networks. Multi-dimensional epigenomic networks can shed light on gene regulatory networks (GRNs) in \textit{in vivo} systems, resulting in the visualization of hidden biomolecular interactions. GRNs reveal cell-type specific development, differentiation, and cellular functions that control the transcriptional expression of signal molecules and transcription factors, and regulatory interactions\textsuperscript{12,13}. The network structure of integrated genomes and epigenomes contributes to better understanding of spatial and temporal regulation of gene expression during development and aging\textsuperscript{14}. It is also possible to measure the network strength of gene regulation-epigenetic modification via the perpetual imbalance of homeostasis during aging.

Herein, we aimed to understand these epigenomic networks using genetic approaches, integrated with physiological assays and multiple genome-wide analyses using fully differentiated retina as an aging model system. We found that haploinsufficiency of \textit{Kdm3b} is associated with a decreased number of cone photoreceptors via increased apoptosis and induced alterations of cone ribbon synapses due to the site-specific alteration of heterochromatin features. Furthermore, KDM3B is required for the maintenance of the integrated GRN that confers normal homeotic regulation in the mouse retina during aging.
RESULTS

Characterization of heterochromatin organization and transcriptomic profile involved in retinal aging. The retina is composed of six neuronal cell types, including rod and cone photoreceptors (Fig. 1a). To investigate the organization pattern of chromatin during retinal aging, we observed the distribution of heterochromatin in the nuclei of photoreceptors using DAPI staining. Heterochromatin was detected at the nuclear periphery and within chromocenters in cone photoreceptors of 2-month-old mice, but less condensed heterochromatin was observed in the center of the nucleus in 18-month-old mice (Fig. 1b). However, the distribution of heterochromatin did not change in the rods of both 2- and 18-month-old mice.

The number and mean area of chromocenters in cone nuclei decreased in the 18-month-old mice compared to that in the 2-month-old mice (Fig. 1c and Extended Data Fig. 1a). Several studies have shown that repressive histone modifications, such as H3K9me2/3, are hallmarks of pericentromeric heterochromatin that play a pivotal role in heterochromatin formation\(^{15,16}\). We also observed the distribution of silencing histone modification (H3K9me1/2/3) and active modification (H3K9ac and H3K4me3) in cones and rods during aging. H3K9me2/3 was abundant in the heterochromatin at the nuclear periphery in 2-month-old mice with conventional nuclear architecture, whereas H3K9me2/3 showed unusual distribution in both euchromatin and heterochromatin (but not in chromocenters) in 18-month-old mice (Extended Data Fig. 1b). We did not observe any differences in the distributions of H3K9me1 and active modification between 2-month-old mice and 18-month-old mice (Extended Data Fig. 1b). Furthermore, we confirmed the global histone modification of whole retina using immunoblotting (Extended Data Fig. 1c). H3K9me2 level was slightly decreased in 18-month-old mice, whereas H3K9me3 level was increased in whole retina of 18-month-old mice. These data indicate that genome-wide organization of chromatin is altered during retina aging.

To investigate whether epigenetic regulators are involved in “loss of heterochromatin features” during neuronal aging, we performed gene expression profiling using RNA-sequencing analysis of 1-, 6-,
12-, and 18-month-old mice retina. Strong agreement was observed between the results of each biological replicate from the different time points (Extended Data Fig. 2a). Given the criteria of FPKM > 30.0 and coefficient of variation between each biological replicate within 20%, 1,615 differentially expressed transcripts (DETs) were obtained by pairwise comparisons of the samples (Fig. 1d, Extended Data Fig. 2b and Supplementary Table 1). We performed a K-means clustering analysis of DETs with \( k = 10 \) (Fig. 1e, f and Extended Data Fig. 2c). Ten main clusters of expression patterns were discerned. We focused on cluster 8 and 9, which represented transcripts that are gradually downregulated during aging and included genes that are important in chromatin remodeling, such as \( \text{Kdm3b, Kdm5b, Kmt2e, and Park7} \) (Fig. 1e, Extended Data Fig. 2d and Supplementary Table 2). Interestingly, histone H3K9me1/2 demethylase \( \text{Kdm3b} \) is correlated with the abnormalities of human eye, including nystagmus, low vision, and learning of cerebellum-dependent optokinetic response (OKR)\(^{17} \). We confirmed that KDM3B mRNA and protein levels were approximately 25% decreased in 18-month-old mice compared to that in 2-month-old mice (Fig. 1g, h). Furthermore, KDM3B was highly expressed in heterochromatin and chromocenters in 2-month-old mice, whereas KDM3B level decreased in heterochromatin and chromocenters in 18-month-old mice (Extended Data Fig. 3a). Collectively, these results suggest that KDM3B may be crucial for the regulation of heterochromatin feature in mice retina during aging.

Abnormal eye phenotypes in \( \text{Kdm3b}^{+/+} \) mice. To determine the cellular and molecular functions of KDM3B in the retina, we generated \( \text{Kdm3b} \) functional knock-out mice via the gene trap technique (Extended Data Fig. 3b, c, and Supplementary note). We confirmed that the KDM3B mRNA and protein levels were reduced by approximately 50% to 80% in \( \text{Kdm3b}^{+/+} \) mouse retinas (Extended Data Fig. 3d, e, respectively). Additionally, publicly available mRNA-sequencing data (GSE 74660) of mouse retinal tissue showed that \( \text{Kdm3b} \) expression continued to increase up to stage P28 in whole retina and cones (Extended Data Fig. 3f). We found that KDM3B is highly enriched in the nucleus of cones, using immunohistochemistry (IHC), suggesting that it plays a role in the development or maintenance of cellular
functions in cone photoreceptor cells (Extended Data Fig. 3g).

To demonstrate the functional role of Kdm3b in the eye, we observed the morphology and the structure of the enucleated eyeball and retinal tissue. We clearly detected smaller eyes and abnormal corneal phenotypes in 12-month-old Kdm3b+/− mice compared to those in 1-month-old mice (Extended Data Fig. 3h). To investigate the structural and morphological differences in the retina, we measured the thickness of the entire retinas of 1-, 6-, and 12-month-old Kdm3b+/+ and Kdm3b+/− mice. The retinal outer nuclear layer (ONL) of the Kdm3b+/− mice was thinner than that of the Kdm3b+/+ mice (Fig. 2a, and Supplementary Fig. 1a,b). The mean ONL thickness of Kdm3b+/− mice was significantly reduced in 12-month-old mice (1,600 µm inferior: 97 ± 10 µm versus 57 ± 2.95 µm, 1,600 µm superior: 84.6 ± 9.35 µm versus 54.7 ± 2 µm) (Fig. 2b). However, there was no difference between the mean ONL thickness of 1-month-old Kdm3b+/+ and Kdm3b+/− mice (Fig. 2a, b). Consistent with these results, we observed that the number of nuclei in the ONL was markedly reduced in Kdm3b+/− mouse retinas among both 6-month-old (266 ± 3.06 versus 239 ± 2.34 nuclei/100 µm², P = 1.93E-5) and 12-month-old mice (239 ± 3.38 versus 205.1 ± 3.27 nuclei/100 µm², P = 9.0E-6) (Fig. 2c). We also observed that the mean thickness of the inner nuclear layer (INL) and the number of nuclei in the INL and ganglion cell layer (GCL) were decreased in 1- and 6-month-old mice (Extended Data Fig. 4a, b). The decrease in ONL thickness in the Kdm3b+/− retina could be because of reduced number of photoreceptor cell nuclei, including those of the rods and cones. To determine whether the rod and cone populations decreased in the ONL of the Kdm3b+/− retina, we performed IHC with cone-cell specific (anti-CAR; cone arrestin) and rod-cell specific (anti-RHO; rhodopsin) antibodies. We found that Kdm3b haploinsufficiency induced different morphologies and reduced the number of cones (14.6 ± 0.41 versus 9.8 ± 0.26 cones/100 µm² P = 4.93E-34) in 12-month-old mice, but it was not associated with differences in the rods (Fig. 2d, and Extended Data Fig. 4c).

Vertebrate rod and cone photoreceptors include outer segments (OS) and cell bodies that contribute to phototransduction, are required for cell viability, and a synaptic terminal able to signal to second-order neurons. To determine the progression of cone loss in aged Kdm3b+/− retinas, we examined the density of
cones and the morphological changes of cone OS by immunostaining analysis of whole mouse retinas.

Consistent with that of retina sections, the mean number of cones was significantly decreased in 12-month-old $Kdm3b^{+/+}$ mouse retinas, but the mean diameter of the cone-cell bodies was not significantly different between $Kdm3b^{+/+}$ and $Kdm3b^{++}$ mice (Fig. 2e, and Supplementary Fig. 1c,d). The mean length of the inner segment of cones was significantly decreased in 12-month-old $Kdm3b^{+/+}$ mouse retinas (Fig. 2f). However, the mean circumference of the cone OS was similar between $Kdm3b^{+/+}$ and $Kdm3b^{++}$ mouse retinas (Fig. 2f). Even though the overall mean cone lengths were similar, the mean lengths of the basal side and cone-cell body in the $Kdm3b^{+/+}$ mouse retinas were longer than those of the $Kdm3b^{++}$ mouse retinas (Extended Data Fig. 4d). Interestingly, we observed that the mean presynaptic area of cones (pedicle) was smaller in the 12-month-old $Kdm3b^{+/+}$ mouse retinas (Fig. 2g, and Supplementary Fig. 1e). These results suggest that $Kdm3b$ haploinsufficiency induced morphological changes and loss of cone photoreceptors in the aged mouse retina.

**Transcriptomic profiling of $Kdm3b^{++}$ mouse retinas.** After determining the morphological changes in the $Kdm3b^{++}$ mouse retinas, we analyzed differential transcript expression in $Kdm3b^{++}$ retina. We performed transcriptome profiling of 12-month-old $Kdm3b^{+/+}$ and $Kdm3b^{++}$ mouse retinas by RNA-sequencing analysis. Principal component analysis (PCA) plot showed that $Kdm3b^{++}$ retinas accounted for the largest variance, and biological replicates showed great reproducibility (Fig. 3a). Volcano plots present the statistical significance of differential transcript expressions with respective fold-changes ($P < 0.05$, absolute log$_2$ fold-change (log$_2$ FC) > 0.5) compared to the expression observed in the control group (Fig. 3b). Application of DEseq with conservative access to the RNA-sequencing data gained from the $Kdm3b^{++}$ retinal samples confirmed 3,978 differentially expressed transcripts (Fig. 3c and Supplementary Table 3). The significant upregulation or downregulation of approximately 20% genes was revealed in the $Kdm3b^{++}$ retina (19,485 annotated transcripts, Extended Data Fig. 5a). Gene ontology (GO) analysis was performed to reveal the biological connection among the upregulated (1,814 genes) and downregulated (1,852 genes)
DEGs in Kdm3b+/− mouse retinas (Fig. 3d and Supplementary Table 4). Clustering of the upregulated DEGs in Kdm3b+/− retinas enabled their classification into several categories that were associated with mRNA splicing and DNA damage stimuli, and downregulated DEGs were involved in synapse assembly and vesicle transport (Fig. 3d). Gene set enrichment analysis (GSEA), which shows the biological pathways and processes of the DEGs, also revealed that genes associated with cellular responses to DNA damage stimuli and apoptotic processes were upregulated, while the repressed genes in Kdm3b+/− mouse retinas were involved in synapse assembly and vesicle-mediated transport (Fig. 3e). The selected genes in these groups were upregulated or downregulated in Kdm3b+/− mouse retinas, indicating that KDM3B has a crucial role in cell death and synaptic transmission in the mouse retina (Fig. 3f, g). Since KDM3B haploinsufficiency induced the expression of gene sets for mRNA splicing, we also investigated alternative splicing events in RNA-sequencing data. The identified splice-site variants were categorized as 3,038 events for skipped exon (SE), 387 events for 5′-splice site (5′-SS), 642 events for 3′-splice site (3′-SS), 342 events for mutually exclusive exon (ME), and 906 events for retained intron (RI) (Extended Data Fig. 5b). These events showed the distribution of protein-coding genes (Extended Data Fig. 5c). RI is retained by the glycolytic enzyme, Aldolase A (Aldoa). The Aldoa transcript in Kdm3b+/+ mice did not include any introns between exon 3 and exon 5; however, the introns were not spliced in the Kdm3b+/− mouse retinas, suggesting a novel effect of KDM3B in transcript maturation (Extended Data Fig. 5d). These results showed that KDM3B ablation selectively regulated the expression of gene sets involved in neuronal apoptosis and synaptic transmission.

**Induction of apoptotic signals in aged Kdm3b+/− mouse retinas.** Previous studies suggested that apoptosis occurs in pathologic photoreceptor cell death in several mouse models of retinal degeneration. Since the number of cone photoreceptors was reduced in Kdm3b+/− aged mice, we investigated whether retinal cells in the ONL were damaged due to the death of photoreceptors. We detected the presence of Müller glial cells, which are involved in the clearance of damaged cells and retinal regeneration, using anti-GFAP antibodies. The mean Müller cell counts were significantly increased in 6- and 12-month-old Kdm3b+/− retinas.
compared to that in age-matched Kdm3b+/+ retinas (Fig. 4a, and Extended Data Fig. 6a). To further confirm whether retinal cell damage induces photoreceptor cell death through apoptosis, we stained 1- and 12-month-old Kdm3b+/+ and Kdm3b+/− retinas with the executive apoptotic marker, cytochrome c. The intensity of cytochrome c was significantly increased in 12-month-old Kdm3b+/− retinas compared to that in 1-month-old Kdm3b+/− retinas. However, this was not observed in 12- and 1-month-old Kdm3b+/+ retinas (Fig. 4b). Interestingly, we observed an increased release of cytochrome c in the cones of 12-month-old Kdm3b+/− retinas (Fig. 4c, and Supplementary Fig. 2a). Furthermore, we used TUNEL staining to determine whether degenerating cone photoreceptors underwent apoptosis through gDNA fragmentation. TUNEL-positive apoptotic cones also markedly increased in 12-month-old Kdm3b+/− retinas (Fig. 4d, Extended Data Fig. 6b, and Supplementary Fig. 2b). Cytochrome c and TUNEL-positive signals were densely detected in the outermost ONL, where most of the cone photoreceptors are located in the retina (Fig. 4c, d). To explore the potential molecular mechanism that mediated KDM3B-induced apoptosis, we investigated the levels of apoptosis-associated proteins using a mouse apoptosis antibody array. Several apoptosis-associated proteins were upregulated or downregulated in 8-month-old Kdm3b+/− retina (Fig. 4e, f, and Extended Data Fig. 6c, d). Compared to that in the Kdm3b+/+ retina, the expression of pro-apoptotic molecules, such as Bad, increased by 20% in 8-month-old Kdm3b+/− retina (Fig. 4e). Therefore, Kdm3b haploinsufficiency upregulated apoptotic signals in cone photoreceptors.

Synaptic functional morphology of cone photoreceptors in Kdm3b+/− mouse retinas. Photoreceptor ribbon synapses are specialized synaptic structures in the outer plexiform layer, where visual signals are transmitted from photoreceptors to bipolar and horizontal cells. Since functional KDM3B is required for the expression of synaptic and vesicle transport gene sets, we examined whether Kdm3b haploinsufficiency affected changes in the functional morphology of cone ribbon synapses. The mean area of cone synaptic terminals and the number of ribbon synapses was significantly reduced in 12-month-old Kdm3b+/− retinas (Fig. 5a, Supplementary Fig. 3, and Supplementary video). Furthermore, we performed transmission
electron microscopy (TEM) to elucidate the fine structure of photoreceptor synaptic regions. *Kdm3b* deficiency did not affect the length of ribbon synapses of both rods and cones (Extended Data Fig. 7a). The number and diameter of synaptic vesicles in the presynaptic regions of rod spherules and cone pedicles showed no differences between 12-month-old *Kdm3b*+/+ and *Kdm3b*+/- retinas (Fig. 5b and Extended Data Fig. 7b). However, the mean number of docked vesicles of cone ribbon synapses decreased in 12-month-old *Kdm3b*+/- retinas (3.8 ± 0.35 versus 2.9 ± 0.27, *P* = 0.0285) (Fig. 5c). These findings show that KDM3B is required for the synaptic components and the visual acuity associated with cone photoreceptors.

**Electrophysiological synaptic impairment of cone photoreceptors in *Kdm3b*+/- mouse retinas.** To investigate the physiological role of KDM3B *in vivo*, we compared electroretinograms (ERGs) of 3-, 6- and 12-month-old *Kdm3b*+/+ and *Kdm3b*+/- mice under dark-adapted (scotopic) and light-adapted (photopic) conditions. The amplitude of the scotopic ERG a-wave and b-wave reflects phototransduction in the OS of rods and rod bipolar cells and that of photopic ERGs, reflects both phototransduction in the OS of cones and cone bipolar cell activity. Under scotopic conditions, the amplitude of a-waves and b-waves, as well as rod-mediated outer retinal activity were comparable between 3-, 6-, and 12-month-old *Kdm3b*+/+ and *Kdm3b*+/- mice, indicating that rod function was not significantly affected (Fig. 5d-f, and Extended Data Fig. 7c-e). Under photopic conditions, the a-wave amplitude was not significantly different at both low and high stimuli in 3-, 6-, and 12-month-old mouse retinas (Fig. 5g, h and Extended Data Fig. 7f-h). However, photopic b-waves were significantly reduced in 6-month-old *Kdm3b*+/- mice compared to those in *Kdm3b*+/+ mice at higher stimulus luminance (194 ± 43.8 versus 129.6 ± 41.9 μV at 1.2 log cd sec/m², *P* = 0.0079), which may be attributable to the impaired synaptic transmission between cones and bipolar cells (Fig. 5i and Extended Data Fig. 7i). These results suggest that synapse transmission from cone photoreceptors to cone bipolar cells was impaired in 6-month-old *Kdm3b*+/- mice.

**Site-specific regulation of H3K9 methylation patterns by KDM3B in mouse retinas.** Because KDM3B
is required for the physiological and functional roles of cones, we performed ChIP-seq analysis to determine whether KDM3B was directly involved in gene expression via histone demethylase activity. Over 32 million reads were obtained for KDM3B ChIP-seq; these were aligned with the mouse reference genome (GRCm 38.99), and peak calling was performed (Supplementary Table 5). The majority of KDM3B peaks were globally found within 100 Kb from the transcription start site (TSS) (Fig. 6a). The distribution of KDM3B-binding peaks signified that the majority of KDM3B was localized in intergenic regions (50%) and introns (42%) (Fig. 6b). To further test the functional importance of KDM3B in gene expression regulation, we performed the GO analysis of the KDM3B-binding peaks. Interestingly, the KDM3B ChIP-seq signals were highly enriched for genes involved in nervous system development, such as synapse assembly and organization, neuron generation, and differentiation (Fig. 6c and Supplementary Table 6). Previous studies have determined that KDM3B has demethylase activity for H3K9me1 and H3K9me2 but not for H3K9me3. To determine whether KDM3B associated with H3K9me1, H3K9me2, and H3K9me3 marks the same target genes, we performed H3K9me1, H3K9me2, and H3K9me3 ChIP-seq using 2-month-old Kdm3b+/+ and Kdm3b+/− mouse retinas. Similar to the results of KDM3B enrichment, the majority of H3K9me1, H3K9me2, and H3K9me3 peaks were globally found within 100 Kb from the TSS, and most H3K9me1, H3K9me2, and H3K9me3 peaks were localized in intergenic regions and introns (Extended Data Fig. 8a, b). Consistent with the results of previous reports, KDM3B haploinsufficiency resulted in a dramatic increase in H3K9me1 and H3K9me2 levels, while the global patterns of H3K9me3 levels were not significantly different between Kdm3b+/+ and Kdm3b+/− mouse retinas (Fig. 6d). Next, all H3K9me1, H3K9me2 and H3K9me3 peaks were categorized into three clusters: present in both Kdm3b+/− and Kdm3b+/− mice (cluster 1), present only in Kdm3b+/− mice (cluster 2), and present only in Kdm3b+/− mice (cluster 3). Centralization of ChIP-seq peaks indicated that H3K9me2 levels in cluster 2 increased only in Kdm3b+/− retinas, indicating that H3K9me2 levels are regulated in a KDM3B activity-dependent manner. Also, the enrichment of H3K9me2 in cluster 3 proceeded as a demethylase-independent function of KDM3B (Fig 6e). The scatter-density plot of the H3K9me2 level at the TSS between Kdm3b+/+ and
Kdm3b+/− mouse retinas revealed a markedly negative correlation (Fig. 6f). Furthermore, using GO analysis, we found that the H3K9me2 enriched target genes in Kdm3b+/− retinas (cluster 2), indicating that repressed genes are involved in synaptic signaling, synapse organization, and chemical synaptic transmission (Fig. 6g). On the other hand, the H3K9me2 peaks in Kdm3b+/+ (cluster 3) were enriched for negative regulation of apoptosis and signal transduction (Fig. 6g). We obtained similar results for the global pattern of H3K9me1 peaks and biological processes by GO analysis (Fig. 6h-j). Together, these ChIP-seq analyses demonstrated that KDM3B is associated with H3K9me1 and H3K9me2, suggesting that KDM3B exclusively regulates gene sets between neuronal homeostatic genes (synaptic transmission, vesicle transport) and cell-death related genes.

KDM3B upregulates the expression of synaptic genes through the erasure of H3K9me1 and H3K9me2 signatures. The balance between histone methylation and demethylation is highly correlated with transcriptional regulation. We determined the molecular mechanism by which KDM3B reduced the stimulation of synapse assembly genes in Kdm3b+/− mice by integrating ChIP-seq and RNA-sequencing analysis. Approximately 31% (569 genes) and 43% (803 genes) of upregulated or downregulated DEGs, respectively, overlapped with all KDM3B ChIP-seq peaks (Fig. 7a, b). Consistent with RNA-sequencing analysis, GO analysis showed that genes bound by KDM3B and upregulated genes were involved in DNA damage stimuli and transcription regulation, while downregulated genes in Kdm3b+/− mice were exclusively enriched for neuronal development, synapse assembly, and vesicle-mediated transport (Fig. 7c-f and Supplementary Table 7). We next focused on the 803 downregulated genes in Kdm3b+/− mice to determine whether gene expression was modulated by the histone demethylation activity of KDM3B. H3K9me1 and H3K9me2 levels were significantly increased in association with downregulated genes in Kdm3b+/− mice, revealing the deposition of histone methylation due to the ablation of KDM3B enzymatic functions (Fig. 7g). Additionally, H3K9me3 level was slightly increased in some sets of downregulated genes (Fig. 7g). KDM3B was enriched in the synapse assembly genes, including Nrxn2 and Rims2, which were
downregulated in Kdm3b<sup>−/−</sup> mice (Fig. 7h). Based on ChIP-seq data, we selected ten target genes, such as synapse assembly genes and apoptosis-induced genes for validation by ChIP-qPCR. KDM3B was enriched in the target gene promoters (within ~2 Kb from TSS) representing the normal range of ChIP-seq peaks for H3K9me1 and H3K9me2 in Kdm3b<sup>+/+</sup>, and the signal was significantly elevated in Kdm3b<sup>−/−</sup> mouse retinas (Fig. 7i, and Extended Data Fig. 9, 10). These results suggest that KDM3B modulated the transcriptional dynamics of synaptic and apoptotic genes by balancing the H3K9 methylation status.

KDM3B associates with AP-1 transcription factors. Since KDM3B does not directly bind to DNA, KDM3B might be associated with DNA through binding to specific transcription factors. In leukemogenesis, KDM3B interacted with CBP, forming an activator complex during lmo2 transcription activation<sup>9</sup>. To identify potential new partners of KDM3B, we analyzed de novo motif discovery using the MEME-ChIP algorithm. We identified a list of 10 enriched motifs (Supplementary Table 8). Two highly enriched de novo motifs showed significant similarity to a known ZNF384 (motif enrichment E-value = 3.45e-05) and transcription factor of AP-1 family (motif enrichment E-value = 1.16E-04) (JASPAR database) (Extended Data Fig. 11a). Furthermore, we analyzed the positional distribution of ZNF384 and c-Jun enriched motifs using the Centrimo algorithm. Both the ZNF384 and c-Jun motifs were centrally enriched with respect to the KDM3B-ChIP peaks (Fig. 8a). De novo motif analysis of the KDM3B ChIP-seq regions yielded potential binding regions for transcription factors that might co-occupy KDM3B-regulated genomic sites and potentially modulate its target genes.

Since the AP-1 family regulates the various cellular processes, including cell proliferation, differentiation, and apoptosis<sup>23</sup>, it is reasonable to speculate that KDM3B modulates neuronal homeostatic genes and cell death-related genes by interacting with AP-1 transcription factors. To test this hypothesis, we first confirmed that AP-1 family (c-Fos, c-JUN, and JUNB) mRNA and protein levels were stably expressed up to 12 months in mouse retina (Extended Data Fig. 11b, c). Transcriptome profiling showed that c-Fos and JUNB mRNA levels increased in Kdm3b<sup>−/−</sup> mice retina (Extended Data Fig. 11d). In addition,
public mRNA-sequencing data (GSE 74660) from mice retina tissue showed that the expression of c-Fos, c-JUN and JUNB genes was higher in cone than in rod photoreceptors (Extended Data Fig. 11e). Next, we analyzed whether AP-1 transcription factors were involved in KDM3B recruitment in vivo. Finally, co-immunoprecipitation experiments showed that KDM3B was associated with c-Fos and JUNB in mice retina and human lung cancer cells (Fig. 8b and Extended Data Fig. 11f). Additionally, ChIP-qPCR with c-Fos and JUNB antibodies confirmed that both transcription factors were associated with KDM3B target genes (Fig. 8c, and Extended Data Fig. 11g). These results suggest that KDM3B, c-Fos, and JUNB can physically interact with and could regulate a set of KDM3B target genes.

**Kdm3b haploinsufficiency accelerated the destruction of epigenomic network robustness.** To determine the difference between Kdm3b+/+ and Kdm3b+/- networks, we constructed KDM3B-centered epigenomic networks using integrated RNA-sequencing and ChIP-Seq analysis. The Kdm3b+/+ network consisted of 1,452 genes with 2,906 links, while the Kdm3b+/- network consisted of 1,376 genes with 2,384 links (Fig. 8d). The clustering coefficient (Kdm3b+/+ = 0.18 versus Kdm3b+/- = 0.16) and the diameter of the nodes were similar between the Kdm3b+/+ and Kdm3b+/- networks, indicating a lack of a significant difference between the global properties of the networks. To strictly measure the structural differences, the network dissimilarity concept (D(G, G')) was introduced to the expanded network for four different ages of Kdm3b+/+ mouse retinas (1.5, 3, 6, and 12 months) compared to those of Kdm3b+/- mouse retinas. The D-value among the four different ages of Kdm3b+/+ mice showed relatively small dissimilarities, from 0.001 to 0.012 (Fig. 8e). In contrast, there was a significant difference in the mean D-value between Kdm3b+/+ and Kdm3b+/- networks (0.032 ± 0.001 at 12 months) (Fig. 8e). This indicated that the network dissimilarity was clear between the 12-month-old Kdm3b+/+ and Kdm3b+/- mouse retinas.

Network robustness is a central question in systems biology and medicine, helping us understand how subtle changes or failures lead to the development of disease. Therefore, we measured the network...
robustness between the Kdm3b+/+ and Kdm3b+/- genotypes by random link removal. We simulated random link removal with a fraction $f$ and measured the fraction of the largest cluster size $P(f)/P(0)$ as an order parameter (Fig. 8f). Since all epigenomic networks of KDM3B are based on centralized KDM3B networks and finite-sized networks, we can predict that the critical threshold $f_c$ goes to 1\cite{12}. Therefore, we focused on the difference of $P(f)/P(0)$ for various values of $f$. The order parameters of all four Kdm3b+/+ network stages showed more robustness for link failure than those of Kdm3b+/- (Fig. 8f). These results imply that the Kdm3b haploinsufficiency causes epigenomic network dissimilarity, resulting in the acceleration of the destruction of network robustness.
Aging is a complex multifactorial biological process shared by all Metazoa. Organismal aging holds significance for human health because it increases susceptibility to many diseases, including cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases. Environmental factors are the major effectors that increase or decrease life expectancy. Environmental conditions may affect the cellular and molecular epigenomic modifications including “loss of heterochromatin” to regulate gene expression and cell fate, which result in the aggravation or alleviation of the aging process.

Retinal aging is often associated with a decrease in visual acuity, ocular accommodation, and dark adaptation. Additionally, this aging phenotype is aggravated by several diseases, such as AMD. Recently, many researchers have investigated epigenetic changes, including DNA methylation and histone modification in retinal homeostasis. During the development of the vertebrate retina, multipotent progenitors differentiate into various neuronal subtypes as a result of gene expression regulation. For retinal development, the appropriate regulation of histone methylation is important for cell proliferation and differentiation. Histone H3K9 methyl transferase G9a (Ehmt2), mainly present in retinal progenitor cells, plays an essential role in proper retinal development by repressing the expression of progenitor genes in terminal differentiation. Kdm6b, which encodes H3K27 demethylase (JMJD3), is required for the survival of rod bipolar cells with the regulation of Bhlhb4 expression. Histone demethylase Kdm5b, known as histone H3K4 demethylase, functions as a regulatory factor for rod-specific genes in the retina.

Our study demonstrated that KDM3B is a putative regulator of apoptotic signaling and synaptic transmission, playing a balancing role in the retinal aging process. Moreover, instead of global heterochromatin loss, we found that KDM3B selectively modulates the heterochromatin features in a gene-function dependent manner, i.e., differently for apoptotic or neuro-functional genes. The synapses were also altered in cellular morphology and structure by downregulation of Kdm3b expression, indicating that Kdm3b haploinsufficiency disturbs retinal homeostasis. Screening the expression of apoptosis-related proteins in Kdm3b<sup>-/-</sup> mice retinas revealed the upregulation of pro-apoptotic protein Bad and the
downregulation of anti-apoptotic protein Catalase (Fig. 4e). Bad is a member of the Bcl-2 family, which was
identified as an intersection point between promotion and inhibition of apoptosis. Catalase protects against
oxidative stress-induced toxicity. Finally, dysregulated retinal homeostasis induces damage to cone
photoreceptors and initiates cell death in Kdm3b−/− mouse retinas.

AMD is characterized by reduced retinal pigment epithelium (RPE) function and photoreceptor
loss in the macular area. Genetic variant studies of AMD can help uncover disease mechanisms and provide
entry points into its therapy. Despite genetic studies investigating AMD, there is a limited understanding of
the underlying physiological and molecular pathogenetic mechanisms and therapies of this complex disease.

AMD pathogenesis is triggered by genetic and environmental factors. In particular, epigenetic alteration
is an important factor for gene regulation without genetic variation. Of the environmental risk factors that
have been investigated, smoking may be associated with a two-fold increased risk of developing AMD.

Therefore, the integration between genetic and epigenomic studies may contribute to more effective
functional analyses of the mechanisms underlying AMD pathogenesis. Previous evidence supports the role
of aberrant epigenetic modifications with significant increase in the mRNA expression of HDAC1, HDAC3,
HDAC6, DNMT1, and DNMT3a in the RPE cells of mice with excessive iron levels, which are, thus, at a
higher risk of developing AMD. A recent study showed KDM3B SNPs caused eye abnormalities (31%,
5/16 cases), such as nystagmus and strabismus in four individuals, as well as refraction anomalies and low
vision in three individuals. We generated heterozygous Kdm3b mice and focused on the function of
KDM3B in the mouse retina via physiological, genetic, and multi-dimensional epigenomic analysis. We
found that Kdm3b mRNA expression is similar between rods and cones during development, but the protein
level is highly enriched only in cones (Extended Data Fig. 3). Thus, KDM3B has cell type specificity and
different functional roles at the protein level. ONL thickness and cone photoreceptor populations can be
reduced in Kdm3b−/− mouse retina by regulating apoptotic genes (Fig. 2 and 4). Furthermore, KDM3B plays
an important role in synapse assembly and vesicle transport in cone ribbon synapses through multi-
dimensional epigenomic network analysis (Fig. 8d-f). We suggest that the cone-cell type specificity and
KDM3B functional network can cause age-related diseases, such as AMD. Future studies will demonstrate whether KDM3B is associated with cone-cell type specificity in the human retina.

The duplex retina in vertebrates constitutes specialized light-sensitive rod and cone photoreceptors. Rods permit energy conservation and maximum sensitivity at the expense of spatial and temporal resolution, whereas cones enable non-quenching, rapid responses to photons with high acuity in daylight. Rods and cones coordinate synaptic connections with bipolar and horizontal cells for visual transmission. Previous studies have shown the important roles of epigenetic regulation in retinal development and homeostatic maintenance. For instance, the histone H3K4 methyl transferase MLL1, is essential for retinal structure, functional synapse formation, horizontal cell differentiation, and maintenance. Ablation BMI1, a component of the PRC1 histone H3K27 methyl transferase complex, leads to increased retinal cell death in bipolar and cone cells. Despite these observations, the exact role of epigenetic regulation in photoreceptor development and degeneration is largely unknown. We demonstrated the functional morphology and physiology of cone synapses in the Kdm3b+/− retina. Reduced ERG waves in Kdm3b+/− mice imply defective phototransduction in cones. A higher decrease in B-waves than in A-waves in photopic ERGs suggests abnormalities in visual transmission in cones. The number of cone ribbon synapses and docked vesicles in cone pedicles were reduced in Kdm3b+/− mice (Fig. 5). Additionally, Kdm3b+/− mouse retinas showed that the synapse assembly genes including Rims2, Nrnx2, CtBP2, Nedd4, and vesicle-transport genes, including Tmed10, Vamp4 were downregulated by increasing H3K9 methylation (Fig. 7). This suggests that the presynaptic terminal abnormalities in cones proceed from epigenetic modification complications followed by incorrect assembly of ribbon synapses.

Rising evidence suggested that epigenetic modulators often exhibit dual function, both activator and repressor, in gene transcriptional regulation, development, and cancer progression. However, how these dual functions are coordinated in specific cellular contexts remains poorly understood. Therefore, it is interesting that KDM3B, an active histone demethylase, unexpectedly activates apoptotic genes in aged Kdm3b+/− mouse retinas. Since KDM3B lacks DNA binding ability, it may require a transcription factor for
gene-set specific regulation. Consistent with this hypothesis, our RNA-sequencing analysis supported an increasing expression pattern of AP-1 family transcription factors during retinal aging. In addition, motif analysis of KDM3B ChIP-seq data uncovered a list of transcription factors that were associated with specific target genes. In particular, KDM3B peaks carried a motif predicted by AP-1 family transcription factors such as c-Fos and JUNB. We demonstrated that KDM3B co-occupies a subset of KDM3B target genes via physical interaction; however, it remains to be determined if this interaction is direct or indirect (Fig. 8). The immediate early gene c-Fos is expressed in various cell types by numerous stimuli and conditions. C-Fos is well known to dimerize with c-JUN family proteins, which are a major component of the AP-1 transcriptional complex. Previous reports indicate that c-Fos is implicated in the induction of cell death in several types of neuronal cells. c-Fos is required for both regeneration of retina ganglion cells and apoptotic cell death\(^{43,44}\). Consistent with the results from previous studies, we observed that c-Fos and JUNB are associated with KDM3B at the regulatory regions of apoptosis-induced genes and synapse assembly genes in mice retina. This finding further supports the assertion that c-Fos and JUNB might fine-tune the transcription of KDM3B target genes acting as on-off switches during aging. Although detailed mechanisms are yet to be elucidated, our data shed some light on novel aging regulatory mechanisms.

GRNs affect all biological phenomena associated with maturation (development), rapid destruction (disease), and slow destruction (aging) (Fig. 8g). In terms of network topology, conventional GRNs cannot differentiate between subtle changes, such as environmental adverse effects and aging. We suggest that the integration of multi-dimensional epigenomic networks and GRNs will help improve the understanding of biological phenomena by allowing for the measurement of network strength during aging. We focused on an epigenetic regulator (KDM3B)-centered network during retinal aging. Even though many studies have investigated epigenetic regulation in cancers or stem cells, the precise mechanism of epigenomic modification in completely differentiated and aging cells is largely unknown. KDM3B is an H3K9me1 and H3K9me2 demethylase that belongs to the KDM3 family and acts as a transcriptional activator\(^9,45\). KDM3B accelerates leukemogenesis by regulating Lmo2 expression via the reduction of
H3K9me2 methylation\textsuperscript{22}. Additionally, the demethylation activity of KDM3A/B activates Wnt target genes, which increase the survival of human colorectal cancer stem cells\textsuperscript{22}. Previous reports implicate H3K9 methylation remodeling in aging. Histone methyl transferase SUV39H1 decreases in line with age, resulting in a reduction of global H3K9me level and disturbed heterochromatin function\textsuperscript{46}. Another report showed that the disruption of Kdm4A causes shortening of the lifespan of male \textit{Drosophila}, indicating that KDM4A is involved in longevity\textsuperscript{47}. We established \textit{Kdm3b\textsuperscript{+/-}} mice and demonstrated the functional roles of KDM3B via morphological and physiological changes, as well as genome-wide transcriptional and epigenomic analysis during retinal aging. Importantly, our data suggest that site-specific changes of H3K9 methylation in \textit{Kdm3b\textsuperscript{+/-}} mice accelerate the destruction of GRN leading to “loss of heterochromatin features” affecting cellular lifespan depending on organism. Furthermore, we constructed the KDM3B-centered GRN using integrated RNA-Seq and ChIP-Seq analysis, which showed that the strength of the network between a transcriptional regulator and its target genes weakened in \textit{Kdm3b\textsuperscript{+/-}} mice. Although the network structural properties are not significantly different between \textit{Kdm3b\textsuperscript{+/-}} and \textit{Kdm3b\textsuperscript{+/-}} mice (Fig. 8d), we found interior topological differences by measuring network dissimilarity and robustness (Fig. 8f). Eventually, \textit{Kdm3b} haploinsufficiency diminished the structural gene network via heterochromatin misregulation and induced cascading destruction of network integrity; these delicate alterations emerged as post-transcriptional and translational regulations. For example, disrupted alternative splicing of the \textit{CPSF1} and \textit{CNOT3} genes leads to the attenuation of biological processes in retinitis pigmentosa (RP)\textsuperscript{48}. We identified the retained intron events on the \textit{Aldoa} gene in \textit{Kdm3b\textsuperscript{+/-}} mouse retinas (Extended Data Fig. 5d). ALDOA is a glycolytic enzyme that catalyzes the reversible conversion of fructose-1, 6-bisphosphate to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate\textsuperscript{49}. This result suggests that \textit{Kdm3b} haploinsufficiency changes the normal transcriptome profile, which is a potential cause of retinal disease via energy metabolism regulation during aging.

In summary, our findings provide new insight into how site-specific epigenetic regulation contributes to the maintenance of retinal homeostasis during aging. We demonstrated the presence of an
epigenomic network in the retina, which might illuminate the selective roles of KDM3B in synaptic transmission and apoptotic signaling pathways during retinal aging. We integrated epigenomic network analysis with physiological and morphological analyses of the Kdm3b\(^{+/−}\) mouse retina. Ablation of Kdm3b results in presynaptic cone abnormalities by inducing apoptotic signals. Kdm3b haploinsufficiency downregulates a subset of synapse assembly genes, with H3K9 methylation-associated silencing. Overall, these findings suggest that integrating genomic and epigenomic data in network studies can help to predict whether subtle changes in network strength increase susceptibility to age-related neuronal disease.
METHODS

Animals. *Kdm3b*<sup>+/−</sup> mice were developed by a gene trapping system using pGT0lf plasmid inserted between exons 12 and 13 of the *Kdm3b* gene. *Kdm3b*<sup>+/−</sup> genotype was confirmed by PCR using genotyping primers: common forward, 5’-GGA ACC AGA CCC TGG GAG CTA G-3’; WT reverse, 5’-CAC CCA CGA CCT GGC TTA CAC C-3’; and KO reverse 5’-CAC CCA CGA CCT GGC TTA CAC C-3’. Mice were maintained in a 12-h light-dark cycle (lights were turned off at 20:00) with free access to water and mouse feed. The mice were weaned at the age of 3 weeks and housed 1-6 per cage. Mice were approved by the Institutional Review Board of Chung-Ang University (updated IRB number: 2019-00059 and 2020-00061).

Immunohistochemistry (IHC). *Kdm3b*<sup>+/−</sup> mice retinas were excised quickly by removing the lenses on a cold plate submerged in PBS. After fixation of retinal tissues with 4% paraformaldehyde (#15710; Electron Microscopy Sciences, Hatfield, PA, USA), they were incubated successively in 10%, 20%, 30% sucrose-PBS for 1, 3, and 12 h, each. The fixed retina was embedded with 7% agarose gel or OCT compound (4583, SAKURA, CA, USA), and the retinas were sectioned with vibratome (7000SMZ, Campden Instruments, England) and cryotome (CM1850, Leica, Germany). After permeabilization in PBS with 0.1% Triton X-100 for 10 min, the sections were incubated for 1 h in blocking solution, which included 5% normal goat serum in PBS with 0.1% Triton X-100. Primary antibodies were incubated overnight at 4°C. Secondary antibodies were incubated for 1 h at 23-25°C in the dark after being washed with PBS. The list of antibodies is presented in Supplementary Table 9. The nucleus was counterstained with 5 μg/ml 40, 60-diamidino-2-phenylindole (DAPI) for 3 min in the dark. Fluorescence images were generated with Zeiss Axio Observer Z1 LSM 700 confocal microscope and ZEN program (ZEN lite 2011) (Carl Zeiss, Oberkochen, Germany).

Immunoprecipitation and immunoblotting. The retina tissues were lysed in a buffer containing 1% Triton X-100, 150 mM NaCl, 50 mM Tris-HCl, pH 7.5, 0.1% sodium dodecyl sulfate (SDS), 1% Nonidet P-40,
and 1 mM PMSF. The cell suspensions of retina tissue were homogenized on ice and centrifuged at 15,000
g at 4°C for 10 min. For immunoprecipitation assays, the supernatants were precleaned with 20 μl of protein A/G
magnetic agarose beads (50% slurry) and then incubated at 4°C overnight with 40 μl of protein A/G magnetic agarose beads in the presence of appropriate antibodies. The beads were washed 3 times in PBS, resuspended in SDS sample buffer, and boiled for 10 min. The protein samples from the retina were electrophoresed on a 10% SDS-PAGE and transferred to a nitrocellulose membrane (Protran™; Whatman, Maidstone, UK). The membrane was blocked with 5% skim milk in TBS-T buffer (137 mM NaCl, 20 mM Tris–HCl, pH 7.6 and 0.1% Tween-20) and incubated with suitable diluted primary antibody overnight at 4°C. The list of antibodies is presented in Supplementary Table 9. The membranes were washed thrice for 10 min each with TBST and incubated with a 1:5,000 dilution of horseradish peroxidase-conjugated anti-mouse or anti-rabbit antibodies for 1 hr. Blots were washed with TBST three times and developed with the Western blotting luminol reagent (sc-2048, Santa Cruz), according to the manufacturer’s protocols.

**Total RNA isolation and RT-qPCR.** Total RNA was extracted using the TRIzol solution (15596018, Invitrogen, CA, USA) according to the manufacturer’s specifications. Contaminated genomic DNA was removed from 10 μg of total RNA by incubation with 20 units of Rnase-free Dnase I (New England Biolabs) and 4 units of RNase inhibitor (New England Biolabs) in DEPC-treated water. The reaction mix was incubated for 1 hr at 37°C and then for 10 min at 50°C. RNA samples were quantified spectrophotometrically at 260 nm and all RNA extracts had an OD$_{260}$:OD$_{280}$ between 1.8 and 2.0, demonstrating that RNA was extracted clearly. Oligo-dT (6110A, Takara) was applied as the primer in the first step of cDNA synthesis. Total RNA (1 μg) was combined with 1 μl of oligo dT and H$_2$O and then preheated at 70°C for 10 min to denature the secondary structures of RNA. The mix was then quickly cooled to 4°C, then 10 mM DTT, 2 μl of 10X reverse-transcriptase buffer, and 200 units of reverse transcriptase (18064022, Invitrogen, CA, USA) were added to make a total volume of 20 μl. The reverse-transcriptase mixture was incubated at 40°C for 60 min, subsequently the reaction was stopped by heating at 94°C for
20 s. The cDNA stock was stored at -20°C.

The specificity of each of the amplified products was confirmed by melting curve analysis. For real-time quantitative PCR, the iQ SYBR Green PCR Supermix (#1708880, Bio-Rad) and the CFX96 Real-time PCR detection system (Bio-Rad) were used to detect amplified cDNA samples according to the manufacturer’s instructions. The β-actin gene was used for normalization. The relative mRNA expression was calculated by the 2^(-ΔΔCt) method.

**ERG analysis.** Mice were given full field flash ERG to assess retinal function under scotopic and photopic conditions. Mice were adapted to dark overnight for scotopic measurements and anaesthetized with 2X avertin solution (200 µl per 20 g mouse) with tribromoethanol (T48402, SIGMA) and tert-amyl alcohol (240486, SIGMA). Their pupils were dilated with isopto atropine (Alcon, Republic of Korea), and a small drop of 1% hypromellose (Samil, Republic of Korea) was added to each eye before measurements. Ground and reference subdermal electrodes were placed subcutaneously near the hindquarter and between the eyes, respectively and the mice were placed on heated pads (37 ℃). Recording electrodes were placed on the cornea, and ERG was carried out under scotopic conditions for both eyes simultaneously, with increasing green light stimulus strengths. After the mice adapted to 1.6 log cd·sec/m² for 5 min, the photopic responses were recorded.

**Mouse apoptosis antibody array.** The expression of apoptosis-related proteins in mice retinas was analyzed using a Mouse Apoptosis Array Kit (R&D Systems, Minneapolis, MN, USA). All procedures were performed according to manufacturer’s instructions. Nitrocellulose-membrane sheets containing 21 apoptosis-related proteins were incubated with blocking buffer at room temperature for 1 h, then the mouse retina lysates were incubated overnight with the membranes at 4°C. After rinsing, the array was incubated with a cocktail of biotinylated detection antibodies at room temperature for 1 hr and probed with HRP-conjugated streptavidin for 30 min. A signal produced at each capture spot corresponding to the amount of
protein bound was detected. Relative protein levels were estimated by comparing the pixel densities of protein spots and were quantified using ImageJ.

Transmission electron microscopic (TEM) analyses. Mice retinas were enucleated and fixed in 4% paraformaldehyde overnight at 4°C. After three washes with 0.1 M sodium cacodylate buffer, pH 7.2, they were fixed in 1% osmium tetroxide/0.1 M cacodylate buffer. The retinas were dehydrated using an ethanol gradient up to 100%, and embedded in epoxy resin. Ultrathin sections were prepared using an ultracut diamond knife and stained with 2% uranyl acetate and 4% lead citrate. Specimens were visualized with a TEM (Tecnai F20 G2).

RNA sequencing and bioinformatic analysis. RNA-sequencing library was generated by TruSeq mRNA Library Prep Kit (Illumina, Inc., USA) according to the manufacturer’s instructions. Briefly, 100 ng total RNA from the retinas was isolated and an oligo-dT primer including an Illumina-compatible sequence at its 5’ end was hybridized to the RNA and reverse transcription was carried out. After the degradation of the RNA template, second strand synthesis was initiated by a random primer containing an Illumina-compatible linker sequence at its 5’ end. The double-stranded library was purified by employing AMPure magnetic beads (A63881, Beckman coulter, CA, USA) to get rid of all reaction components. The library was amplified to add the complete adapter sequences required for cluster generation. The finished library was purified from PCR components. High-throughput sequencing was carried out as paired-end 75-sequencing reads using NextSeq 500 (Illumina, Inc., USA). Alignment of mRNA-Sequencing reads was performed using STAR-2.5.4b (Supplementary Table 10). Indices were either produced from genome assembly sequence or the representative transcript sequences for aligning to the genome and transcriptome. The alignment file was applied for assembling transcripts, estimating their abundances, and detecting differential expression of genes. Differentially expressed transcripts were determined based on counts from unique and multiple alignments using Edge R within R version 3.6.3 (R development Core Team, 2011)
Chromatin immunoprecipitation (ChIP) sequencing. Retina tissue samples were cross-linked with 1% paraformaldehyde for 10 min at 23°C and glycine (final concentration: 125 mM) was added to quench the reaction. The samples were homogenized in SDS lysis buffer (1% SDS, 10 mM EDTA, and 50 mM Tris-HCl, pH 8.1), and chromatin was then sheared using a bioruptor sonicator (Diagenode) for 40 cycles (30s on / 30s off, repeated 2 times) at high power setting. After centrifugation for 10 min at 18,500 g, the samples were resuspended in ChIP dilution buffer (0.01% SDS, 1.2 mM EDTA, 1.1% Triton X-100, 167 mM NaCl, and 16.7 mM Tris-HCl, pH 8.1). The sonicated lysates were subjected to immunoprecipitation using the indicated antibodies (5 μg of antibodies for each immunoprecipitation (IP) reaction) overnight. Protein A/G magnetic beads (26162, Thermo scientific, USA) (30 μl) were added, and immunoprecipitations were continued for an additional 4 h, and washed with low-salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 150 mM NaCl, 20 mM Tris-HCl, pH 8.1), high-salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 500 mM NaCl, 20 mM Tris-HCl, pH 8.1), LiCl immune-wash buffer (0.25M LiCl, 1% NP40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris-HCl, pH 8.1), and Tris-EDTA buffer (1 mM EDTA, 10 mM Tris-HCl, pH 8.0). Following the final wash, the precipitates were eluted with 100 μl of IP elution buffer (1% SDS, 0.1 M NaHCO₃) twice and reverse cross-linked by incubating overnight at 65°C overnight. DNA/protein complexes were precipitated with 100% ethanol, air-dried, and dissolved in 20 μl of distilled water (W4502, Sigma). The list of primers used for validation of ChIP-seq is described in Supplementary Table 11.

ChIP-seq library preparation and bioinformatic analysis. ChIP-seq library was generated by TruSeq ChIP Library Preparation Kit (Set A, IP-202-1012, Set B, IP-202-1024, Illumina, CA, USA) according to the manufacturer’s instructions. Briefly, 5 μg of input and ChIP-enriched DNA were end-repaired, A-tailed, adapter ligated with TruSeq index adapters, and amplified. Paired-end sequencing of all ChIP libraries was
performed on the Illumina NextSeq 500 platform. For processing of KDM3B and histone modification
ChIP-seq in the retina, all ChIP reads in FASTQ format were aligned to the GRCm 38.99 mouse genome
using Bowtie2 (v2.3.4.1) and redundant reads were removed (Supplementary Table 12). Peak calling was
performed with EPIC2 (v0.0.41) with the default parameters: bin size = 200; fragment size = 150; Gap
allowed = 3, FDR cutoff < 0.05). After confirming the consistency between each replicate, we pooled
extended reads to generate a bigwig track for visualization with IGV (v2.8.7).

Motif discovery. Overrepresented motif analysis of sequences enriched under KDM3B ChIP-seq peaks
was performed using the de novo motif finder MEME-ChIP [http://meme.nbcr.net/meme/tools/meme-chip,
date last accessed, July 2015]. A 300-bp sequence surrounding each of the ChIP-Seq peak summits
(extending 150 bp on each side) was supplied to MEME-ChIP as input and analyzed with the default settings
for motif width and significance thresholds.

GO and GSEA analysis. DAVID (http://david.abcc.ncifcrf.gov) allowed significant DEGs and ChIP-seq
peaks in respective gene sets to be clustered into functional gene ontologies. Enriched gene ontology terms
were identified using Metascape, and scatter plots of ontology terms were made using REVIGO 51. GSEA
analysis was performed using (GSEA 4.1.0; http://www.broadinstitute.org/gsea/index.jsp) the MsigDB v7.1
mouse database.

Epigenomic network construction. KDM3B-centered network was constructed by integrating RNA-
sequencing and ChIP-Seq data analysis as follows: First, the nearest neighbor (NN) genes for KDM3B were
selected by filtering condition (ChIP-count > 10 per each gene). Next, 2nd NN genes of KDM3B were
identified using NetworkAnalyst 52. The selected 2nd NN gene was linked with the 1st NN gene. All networks
were considered by the 3rd NN. For all candidate genes of a network, genes from RNA-sequencing were
filtered using FPKM > 10. For measurement of network robustness, we compared the impact of random
link removal on the KDM3B-centered networks between $Kdm3b^{+/+}$ (1.5, 3, 6, and 12 months) and $Kdm3b^{+/-}$ (12 months) mice.

**Measurement of network structural dissimilarities.** Network dissimilarity measurement was done following the approach proposed by Shieber et al.\textsuperscript{24}. This method compared network structures based on quantifying differences among distance distributions, node dispersion metric, and $\alpha$-centrality.

\[
D(G, G') = w_1 \frac{I(\mu_G, \mu_{G'})}{\log 2} + w_2 \sqrt{NND(G) - NND(G')} + \frac{w_3}{2} \left( \frac{J(P_{ag}, P_{ag'})}{\log 2} + \frac{J(P_{ag^c}, P_{ag'^c})}{\log 2} \right)
\]

Here, $J$ represents Jensen-Shannon divergence. The first term represents dissimilarity in average node connectivity $\mu_G$ is the graphs averaged node-distance distribution. The second term shows dissimilarity in a node dispersion metric ($NND(G)$). And the last term denotes the difference for distribution of $\alpha$-centrality ($P_{ag}, P_{ag'^c}$) that measures the influence of a node in the given graph $G$ and its complement graph ($G^c$). We measured network dissimilarity following the algorithm\textsuperscript{24}, using $w_1 = 0.45$, $w_2 = 0.45$, $w_3 = 0.1$ values.

**Image analysis.** The number of nuclei in ONL was counted on the middle of the $Kdm3b^{+/+}$ and $Kdm3b^{+/-}$ mice retina. The thickness of ONL was measured using the ‘length measurement’ plugin of ImageJ software. The number of cone photoreceptors was counted in the middle of $Kdm3b^{+/-}$ mouse inferior retina. Size of cone photoreceptor synapse was measured by using the ‘area measurement’ plugin of ImageJ software. Intensities of GFAP and cytochrome c were measured using the ‘histogram’ plugin of ImageJ software. The 3D reconstruction image was generated using Oxford Bitplane Imaris (v9.3.1, Bitplane, CT, USA).

**Statistical analysis.** Student’s $t$-test was used to examine the significance of differences between data with GraphPad PRISM. The results are denoted as mean ± standard error of the mean (SEM), which are obtained
from two or three separated experiments. \( P \) values less than 0.05 were considered statistically significant.

Data availability. RNA-sequencing and ChIP-seq data that support the findings of this study have been deposited in NCBI GenBank with the primary accession code GSE160793. All other data are present in the article and its Supplementary files, or are available from the corresponding author upon reasonable request. Source data are provided with this paper. Recheck and GEO confirm.
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AUTHOR CONTRIBUTIONS

Overall scientific conceptualization, J-WK; Methodology and investigation, M-JA, J-YK, JP, JhK, D-HK, G-SS, H-ML, C-HK, MJK, and J-WK; Data analysis & scientific comments, M-JA, J-YK, JP, JhK, D-HK, JkK, SR, S-BS, and J-WK; Statistical & bioinformatics analysis, JP, JhK; Writing original draft, M-JA, J-YK, and J-WK; Funding acquisition, J-WK; Supervision and project administration, J-WK.
Fig. 1 Characterization of heterochromatin organization and transcriptomic profiling involved during retinal aging. a, Schematic representation of retinal structure. A; amacrine, B; bipolar, G; ganglion, H; horizontal cells. b, Representative immunofluorescence confocal images of mouse retinas stained using 4’,6-diamidino-2-phenylindole (DAPI). c, Quantification of the number of chromocenters in nuclei of cones. d, Hierarchically clustered heatmap of transcripts (a total of 1,615 annotated transcripts (pre-filtered by FPKM > 30) that were differentially expressed in 1-, 6-, 12-, and 18-month-old C57BL/6J mouse retinas). e, Cluster analysis was performed by the K-means method for the gene expression profiles. f, tSNE plot revealing 10 distinct clusters identified from 1-, 6-, 12-, and 18-month-old C57BL/6J mouse retinas. g, The expression level of Kdm3b transcripts in 1-, 6-, 12-, and 18-month-old C57BL/6J mouse retinas. h, Whole retinal lysates of 2- and 18-month-old C57BL/6J mouse were immunoblotted using anti-KDM3B antibody. β-actin was used for internal loading control. Signal intensity of KDM3B was quantified using ImageJ software.
**Figure 2**

The morphological changes in rods and cones in Kdm3b⁻/⁻ mouse retinas.  

**a.** Cross-sectioned retinas are stained with DAPI and H&E from 1-, 6-, and 12-month-old Kdm3b⁺⁺⁺ and Kdm3b⁻⁻⁻ mice. Scale bar: 20 μm.  

**b.** Measurements of ONL thickness were made along the vertical axes of the eyes in 1, 6, and 12-month-old Kdm3b⁺⁺⁺ and Kdm3b⁻⁻⁻ mice (n ≥ 6).  

**c.** Quantification of the number of nuclei in ONL in 100 μm² of retina.  

**d.** Immunostaining of cone arrestin (CAR) in 1- and 12-month-old Kdm3b⁺⁺⁺ and Kdm3b⁻⁻⁻ mouse retinas (left). Scale bar, 40 μm. The number of cones in 100 μm² of retina is quantified (right).  

**e.** Whole-mount retinas stained with CAR antibodies (red) in 12-month-old Kdm3b⁺⁺⁺ and Kdm3b⁻⁻⁻ mice. The number of cones and diameter of the cone cell body (μm) (e), and the length of cone segments (μm) (f) were measured.  

**f.** Cone pedicles in 12-month-old Kdm3b⁺⁺⁺ and Kdm3b⁻⁻⁻ mouse retinas (left). Scale bar: 10 μm. The diameter of cone pedicles (μm) and area of cone pedicles (pixel × 10⁴) were measured (right). Error bars show mean ± SEM. P values obtained by Student’s t-test. ***P < 0.001, *P < 0.05, n.s. not significant. OS; outer segment, IS; inner segment, ONL; outer nuclear layer, OPL; outer plexiform layer, INL; inner nuclear layer, IPL; inner plexiform layer, GCL; ganglion cell layer.
Fig. 3 Alteration of transcriptomic dynamics in Kdm3b<sup>−/−</sup> mouse retinas. a, PCA plot of RNA-sequencing from 12-month-old Kdm3b<sup>−/−</sup> and Kdm3b<sup>−/+</sup> mouse retinas. Small circles symbolize each sample, and larger circles indicate each group. b, Volcano plot showing changes of gene expression patterns in Kdm3b<sup>−/−</sup> and Kdm3b<sup>−/+</sup> mice. Expressed transcripts with strict filtering (log₂FC > 0.5, P value < 0.05) highlight only upregulated (red dots) and downregulated (green dots) transcripts. c, Hierarchical clustering of transcripts from volcano plot (a total of 3,978 transcripts) in Kdm3b<sup>−/−</sup> and Kdm3b<sup>−/+</sup> mice. A clear alteration in transcriptome landscape is obvious between Kdm3b<sup>−/−</sup> and Kdm3b<sup>−/+</sup> mouse retinas. d, Scatter plot of confidence scores for enriched gene ontologies associated with differentially expressed genes, with ontologies clustered by functional similarity in the semantic space (left). Gene ontology of biological process is identified by the DAVID showing upregulated and downregulated genes in Kdm3b<sup>−/−</sup> mouse retinas (right). e, GSEA scores for genes involved in cellular responses to DNA damage stimuli, apoptotic processes, synapse-assembly, and vesicle-mediated transport in synapses. f, Volcano plot described in (b) with three gene set categories: apoptosis-induced genes (red), synapse assembly genes (blue), and vesicle-mediated transport genes (cyan). g, Expression of genes in three categorized groups is represented in log<sub>2</sub> of fold-change (FPKM). When multiple transcripts are expressed, the expression level of the most highly expressed transcript is indicated. Error bars show mean ± SEM.
Fig. 4 Induction of apoptotic response of cones in Kdm3b<sup>+/−</sup> mice. a, Immunostaining of glial fibrillary acidic protein (GFAP) for the specific staining of Müller glial cells in 1- and 12-month-old Kdm3b<sup>+/−</sup> and Kdm3b<sup>−/−</sup> mice (left). Scale bar: 40 μm. GFAP-intensity of Müller glial cells was measured using ImageJ software in 1- and 12-month-old Kdm3b<sup>+/−</sup> and Kdm3b<sup>−/−</sup> retinas (right). b, Immunostaining of cytochrome c in 1- and 12-month-old Kdm3b<sup>+/−</sup> and Kdm3b<sup>−/−</sup> retinas (left). Scale bar: 40 μm. Cytochrome c intensity of the retinas in 1- and 12-month-old Kdm3b<sup>+/−</sup> and Kdm3b<sup>−/−</sup> mice was measured using ImageJ (right). c, Double immunostaining of cytochrome c (red) and CAR (white) in 12-month-old Kdm3b<sup>+/−</sup> and Kdm3b<sup>−/−</sup> retinas. Scale bar: 40 μm. High magnification images in the white box showing cytochrome c (red) expression in cones (white arrowhead). Scale bar: 20 μm. The signal intensity of cytochrome c-positive cones was measured. d, Apoptotic cells were detected by TUNEL assay (BrdU positive cells) in 12-month-old Kdm3b<sup>+/−</sup> and Kdm3b<sup>−/−</sup> retinas. Scale bar: 50 μm. High magnification images in the white box showing BrdU (red) staining in cone photoreceptors (white). Scale bar: 20 μm. The percentage of BrdU-positive cones. Error bars show mean ± SEM. P values obtained by Student's t-test. *** P < 0.001, * P < 0.05, n.s., not significant. e, f, Relative protein expression levels were estimated after quantifying the pixel densities in autoradiograms using ImageJ.
Figure 5

The alteration of synaptic morphology of cones in Kdm3b<sup>−/−</sup> mice. a, 3D reconstruction images showing ribbon synapses in the cone photoreceptors of 12-month-old Kdm3b<sup>+/+</sup> and Kdm3b<sup>−/−</sup> mice. Scale bar: 5 μm. Immunostaining with CibP2 (C-terminal binding protein) and CAR antibodies examined in 1 and 12-month-old Kdm3b<sup>+/+</sup> and Kdm3b<sup>−/−</sup> mice (left). The number of ribbon synapses is measured in a single cone pedicle (right). Scale bar: 2 μm. b, Transmission electron microscopy (TEM) images of the vesicles in rod spherules and cone pedicles of 12-month-old Kdm3b<sup>+/+</sup> and Kdm3b<sup>−/−</sup> mice (left). The number of vesicles in a single rod spherule and cone pedicle (0.16 μm<sup>2</sup>) (right). Scale bar: 0.2 μm. c, TEM images of docked vesicles (shaded with light cyan) near the rod and cone ribbon synapse (left). Scale bar: 0.2 μm. The number of docked vesicles near the rod and cone ribbon synapse in 12-month-old Kdm3b<sup>+/+</sup> and Kdm3b<sup>−/−</sup> mice (0.1 μm of each ribbon synapse) (right). Error bars show mean ± SEM. P values obtained by Student's t-test. * P < 0.05, n.s., not significant. d-i, Electoretinograms (ERGs) of 6-month-old Kdm3b<sup>+/+</sup> and Kdm3b<sup>−/−</sup> mice in 17 different stimulus flashes (from -1.7 to 3.1 log<sub>10</sub> cd/deg²/m²) presented in darkness (scotopic) and superimposed upon a steady rod-desensitizing adapting field (photopic). d, Representative scotopic ERGs elicited by two different stimuli (OFF, -0.1, and 1.6 log<sub>10</sub> cd/deg²/m²) from 6-month-old Kdm3b<sup>+/+</sup> and Kdm3b<sup>−/−</sup> mice. e, f. The scotopic amplitudes of a-waves (e) and b-waves (f) are shown as functions of the stimulus intensity. g, Representative photopic ERGs elicited by two different stimuli (OFF, -0.1, and 1.6 log<sub>10</sub> cd/deg²/m²) from 6-month-old Kdm3b<sup>+/+</sup> and Kdm3b<sup>−/−</sup> mice. h, i, The photopic amplitudes of a-waves (h) and b-waves (i) are shown as functions of the stimulus intensity. Graphs show the mean ± SEM, n = 7 per genotype.
Figure 6

The disruption of global H3K9 methylation in *Kdm3b*<sup>−/−</sup> mouse retinas. a, Bar graph shows the distribution of KDM3B peaks around transcriptional start sites. b, Pie chart of KDM3B enrichment distribution at genomic loci within the genome, including promoters, exons, introns, 5′-untranslated regions (5′-UTRs), 3′-UTRs, and intergenic regions (outside ±10 to +10 Kb of genes) in Kdm3b<sup>−/−</sup> mouse retinas. c, Scatter plot of confidence scores for enriched gene ontologies associated with KDM3B ChIP-seq signal, with ontologies clustered by functional similarity in the semantic space (left). Gene ontology of biological process is identified by the DAVID showing KDM3B-enriched peaks (right). d, Plots of H3K9me1, H3K9me2, and H3K9me3 ChIP-seq signal intensity relative to the center of KDM3B occupied sites (±5Kb) in Kdm3b<sup>+/+</sup> and Kdm3b<sup>−/−</sup> mouse retinas. e, Heatmap view of H3K9me2 (e) and H3K9me1 (h) ChIP-Seq-read intensity around H3K9me2 (e) and H3K9me1 (h) peak center (±3 Kb) detected in Kdm3b<sup>+/+</sup> and Kdm3b<sup>−/−</sup> mouse retinas. The plot shows the mean H3K9me2 and H3K9me1 binding intensities to the center of each peak. f, i, Scatter density plot of input-normalized H3K9me2 (f), and H3K9me1 (i) RPKM between Kdm3b<sup>+/+</sup> and Kdm3b<sup>−/−</sup> at H3K9me2 peak TSS regions (f), and at H3K9me1 peak TSS regions (i). g, j, Gene ontology of biological process is identified by the DAVID showing H3K9me2 (g) and H3K9me1 (j) enriched peaks in Kdm3b<sup>+/+</sup> and Kdm3b<sup>−/−</sup> mouse retinas.
Fig. 7 The global correlation between transcriptome and epigenome dynamics during mouse retinal aging. a, b, Venn diagram showing the overlap between the genes including KDM3B peaks and upregulated (a) or downregulated (b) KDM3B-target genes based on RNA-sequencing data. c, d, Scatter plot of confidence scores for enriched gene ontologies associated with upregulated genes (c) and downregulated genes (d) and their enrichments for KDM3B, with ontologies clustered by functional similarity in the semantic space. Gene ontology of biological process shows upregulated and downregulated genes integrated with RNA-sequencing and ChIP-seq analysis. e, f, Scatter plot showing changes in expression of upregulated (e) or downregulated genes (f) in Kdm3b<sup>+/−</sup> and Kdm3b<sup>+/+</sup> mouse retinas and their enrichments for KDM3B. g, Plots of H3K9me1, H3K9me2, and H3K9me3 ChIP-seq signal intensity relative to the center of downregulated KDM3B occupied sites (± 5Kb) in Kdm3b<sup>−/−</sup> and Kdm3b<sup>+/+</sup> mouse retinas. h, ChIP-seq tracks of KDM3B in Kdm3b<sup>−/−</sup> along the Nrnn2 and Rims2 locus. Selected genomic elements (shaded with light cyan) indicate KDM3B ChIP-seq peaks at Nrnn2 and Rims2 promoter regions. i, Recruitment and presence of KDM3B, H3K9me1, H3K9me2, and H3K9me3 at Nrnn2 and Rims2 promoter regions are validated by ChIP-qPCR. Results are presented as the mean ± SEM (error bars). P values obtained by Student’s t-test. *** P < 0.001, ** P < 0.01, n.s., not significant.
**Figure 8**

The measurement of structural difference and robustness of KDM3B-centered networks. 

**a.** The graph represents the distribution of occurrence probabilities of the consensus sequences enriched under KDM3B ChIP-Seq peaks with respect to ZNF384 and JUN motifs. 

**b.** Immunoblot showing co-immunoprecipitation of endogenous KDM3B with c-Fos and JUNB in mouse retinas. 

**c.** Recruitment and presence of c-Fos and JUNB at Nrxn2 and Rims2 promoter regions are validated by ChIP-qPCR. Error bars show mean ± SEM. P values obtained by Student’s t-test. *P < 0.05.**

**d.** The KDM3B-centered networks in Kdm3b^-/- and Kdm3b^+/- mouse retinas. The circles indicate individual nodes (i.e., regulator and target genes), and the line indicates the interaction between regulatory hubs and genes. The KDM3B-centered network is shown as a yellow circle in the center of networks. The differential expression nodes are represented by the blue circles (downregulated genes in Kdm3b^-/-) and red circles (upregulated genes in Kdm3b^+/-). 

**e.** The matrix of dissimilarity D’s measured from each pair of networks. 

**f.** The plot of fraction of nodes that belong to the largest component in the network for link removal fraction f. All quantities are averaged over 300 realizations for each network. The error bars are estimated from all realizations. 

**g.** Schematic representation of KDM3B-centered network during mouse retinal aging. The integrated KDM3B-centered network with gene expression (mRNA), chromatin status (H3K9me1, H3K9me2), protein was visualized in the cube. Subtle change of network strength in Kdm3b^-/- mouse retina leads to the breakdown of functional physiology in the cone photoreceptors.
Extended Data Fig. 1 Characterization of heterochromatin organization during retinal aging. a, The area of chromocenter (μm²) in cone and rod. b, Whole retinal lysates of 2- and 18-month-old Kdm3b+/- mice were immunoblotted using histone specific modification antibodies. Histone H3 antibody was used for internal loading control. c, Immunostaining of histone mark (red) and DAPI in 2- and 18-month-old Kdm3b+/- mouse retinas.
Extended Data Fig. 2 Transcriptomic profiling during retinal aging. **a**, PCA plot of RNA-seq from 1-, 6-, 12- and 18-months mouse retinas. **b**, Hierarchically clustered heatmap of transcripts (a total of 19,484 annotated transcripts were prefiltered by FPKM > 1 that were differentially expressed in 1-, 6-, 12- and 18-months mouse retinas). **c**, Plot of correlation within group for the number of k centers and fitting the data with generalized logistic function. Inset of (c): Plots of first and second-order to the fitted function. **d-h**, Hierarchically clustered heatmap of transcripts involved in DNA-modification, euchromatin, heterochromatin, histone-binding, and nucleosome-assembly.
Extended Data Fig. 3 Generation of Kdm3b−/− mice. a, Immunostaining of KDM3B (red) and DAPI in 2- and 18-month-old Kdm3b+/− mouse retinas. b, Gene trap vector (pGTO0Lx) was inserted into the intron region between exon 12 and exon 13. c, Genotyping of Kdm3b+/− and Kdm3b−/− mice was performed by conventional PCR analysis using isolated genomic DNA (gDNA) from mouse tails representing each genotype. The bands of 475 bp and 282 bp correspond to WT and mutant alleles, respectively. d, qRT-PCR analysis of Kdm3b mRNA expression (fold-change) in retina, brain, and liver of Kdm3b+/− and Kdm3b−/− mice. e, Whole retinal lysates of Kdm3b+/− and Kdm3b−/− mice were immunoblotted using anti-KDM3B antibody (left). β-actin was used for internal loading control. Signal intensity of KDM3B was quantified using ImageJ software (right). f, Expression level of Kdm3b transcripts in whole retina (WT) and s-cone-like cells (Nrl−/GFP;Nrl−/−) plotted for six developmental time points (P2, P4, P6, P10, P14, and P28) using public RNA-sequencing data (GSE 74660). g, Immunostaining of KDM3B (red) and DAPI in 2-month-old Kdm3b+/− mouse retinas. Scale bar: 20 μm. High magnification image in the white box showing KDM3B (red) staining in cones (middle panel). Scale bar: 10 μm. h, Eyeballs were enucleated from 12-month-old Kdm3b+/− and Kdm3b−/− mice. White arrows indicate the abnormal cornea phenotype. Scale bar: 2 mm. The sizes of eyeballs in 1- and 12-month-old Kdm3b+/− and Kdm3b−/− mice was measured. Error bars show mean ± SEM. P values obtained by Student’s t-test. ** P < 0.01, * P < 0.05.
Extended Data Fig. 4 The morphological changes of rod and cone photoreceptors in Kdm3b−/− mouse retinas. a, Thickness of INL was measured along the vertical meridians of the retinas of 1-, 6-, and 12-month-old Kdm3b−/− and Kdm3b+/− mice (n ≥ 6). b, The number of nuclei in INL was quantified in 100 μm² of retina (left) and linearized GCL in 100 μm of retina (right). c, Immunostaining of rhodopsin (Rho) in 1 and 12-month-old Kdm3b−/− and Kdm3b+/− mouse retinas. Scale bar: 40 μm. d, Measurements of the length of cone basal side (μm) and cell body (μm) by ImageJ software. Error bars show mean ± SEM. P values obtained by Student’s t-test. *** P < 0.001, n.s. not significant.
Extended Data Fig. 5 Alternative splicing in Kdm3b<sup>−/−</sup> mouse retinas. a, Hierarchically clustered heatmap of transcripts (a total of 19,485 annotated transcripts (pre-filtered by FPKM > 1) that were differentially expressed in Kdm3b<sup>+/+</sup> and Kdm3b<sup>−/−</sup> mouse retinas). b, Alternative splicing events were analyzed with Rmats (v4.1), which identified 5,315 genes having alternative splicing events (P value < 0.05). Types of alternative splicing events detected and frequencies in group-wise comparisons. c, Protein coding genes obtained from alternative splicing events indicated as the percentages of counted genes. d, Differential retained introns between Kdm3b<sup>+/+</sup> and Kdm3b<sup>−/−</sup> mouse retinas. The genomic area of the Aldoa gene is shown, which comprises eight exons and seven introns, and RNA-sequencing coverage plots of Kdm3b<sup>+/+</sup> and Kdm3b<sup>−/−</sup> in the area of exons two through five. Red box indicates the clear retained intron region in Kdm3b<sup>−/−</sup> mouse retinas.
Extended Data Fig. 6 Increase of apoptotic cell death in cones in Kdm3b<sup>−/−</sup> retinas. a, Immunostaining of GFAP in 6-month-old Kdm3b<sup>+/+</sup> and Kdm3b<sup>−/−</sup> mouse retinas (left). Scale bar: 20 μm. GFAP-intensity of Müller glial cells was measured by ImageJ in 6-month-old Kdm3b<sup>+/+</sup> and Kdm3b<sup>−/−</sup> mouse retinas (right). b, Apoptotic cells were detected by TUNEL assay (BrdU positive cells) in 12-month-old Kdm3b<sup>+/+</sup> and Kdm3b<sup>−/−</sup> mouse retinas. Scale bar: 50 μm. BrdU-positive cones in red dashed box. c, d, The entire apoptosis proteome profile array in 8-month-old Kdm3b<sup>+/+</sup> and Kdm3b<sup>−/−</sup> mouse retinas.
Extended Data Fig. 7  The alteration of synaptic morphology of cone photoreceptors in Kdm3b<sup>−/−</sup> mice retina.  

**a.** TEM images of ribbon synapse in rod spherules and cone pedicles of 12-month-old Kdm3b<sup>−/−</sup> and Kdm3b<sup>+/−</sup> mice. Scale bar: 0.2 μm. 

**b.** Measurements of the length of ribbon synapses (μm) (left) and diameter of vesicles (μm) (right) by ImageJ software. Error bars show mean ± SEM. P values obtained by Student’s t-test. ** P < 0.01, n.s, not significant. 

**c-e.** Representative scotopic ERGs elicited from 3- (c), 6- (d), and 12- (e) month-old Kdm3b<sup>−/−</sup> and Kdm3b<sup>+/−</sup> mice.  

**f-h.** Representative photopic ERGs elicited from 3- (f), 6- (g), and 12- (h) month-old Kdm3b<sup>−/−</sup> and Kdm3b<sup>+/−</sup> mice.  

**i.** Quantification of ERG responses at 1.2 log<sub>10</sub> cd/sec/m² light intensity from 3-, 6-, and 12-month-old Kdm3b<sup>−/−</sup> and Kdm3b<sup>+/−</sup> mice. Graphs show the mean ± SEM, and data are representative of n > 3 per each genotype. P values obtained by Student’s t-test. ** P < 0.01, n.s, not significant.
Extended Data Fig. 8 KDM3B is required for global H3K9 demethylation in mouse retinas. a, b, Bar graph shows the distribution of H3K9me1, H3K9me2, and H3K9me3 peaks around transcriptional start sites (left). Pie chart of H3K9me1, H3K9me2, and H3K9me3 enrichment distribution at genomic loci (right) in Kdm3b^−/− (a) and Kdm3b^−/- (b) mouse retinas.
Extended Data Fig. 9 KDM3B regulates synapse assembly genes via erasing H3K9me1 and H3K9me2 marks. a, b, ChIP-seq tracks of KDM3B in Kdm3b<sup>-/-</sup> and H3K9me1, H3K9me2, and H3K9me3 peaks in Kdm3b<sup>-/-</sup> and Kdm3b<sup>-/-</sup> mouse retinas along the Nr2f2 (a) and Rims2 (b) locus. Selected genomic elements (shaded with light cyan) indicate KDM3B ChIP-seq peaks at Nr2f2 and Rims2 promoter regions. c, Recruitment and presence of KDM3B, H3K9me1, H3K9me2, and H3K9me3 at indicated target gene promoters were validated by ChIP-qPCR. Error bars show mean ± SEM. P values obtained by Student’s t-test. *** P < 0.001, ** P < 0.01, n.s., not significant.
Extended Data Fig. 10 KDM3B regulates synapse assembly genes via erasing H3K9me1 and H3K9me2 marks. a, ChIP-seq tracks of KDM3B in Kdm3b<sup>−/−</sup> and H3K9me1, H3K9me2, and H3K9me3 peaks in Kdm3b<sup>−/−</sup> and Kdm3b<sup>−/−</sup> mouse retinas along the Dapk1 locus. Selected genomic element (shaded with light cyan) indicates KDM3B ChIP-seq peaks at Dapk1 promoter region. b, Recruitment and presence of KDM3B, H3K9me1, H3K9me2, and H3K9me3 at apoptotic gene promoters were validated by ChIP-qPCR. Error bars show mean ± SEM. *P values obtained by Student’s t-test. ***P < 0.001, **P < 0.01, n.s. not significant.
Extended Data Fig. 11 The AP-1 transcription factors are associated with KDM3B in mice retinas. a, The sequence logos representation of the 2 motifs identified using MEME-ChIP. De novo motif analysis was performed using sequences within a ±150 bp window of KDM3B ChIP-Seq peaks. The sequence logo size indicates nucleotide frequency and the P-values represent the significance of motif enrichments compared to that of the genomic background. b, Whole retinal lysates of 2-, 6-, and 12-month-old mice were immunoblotted using anti-c-Fos and JUNB antibodies. β-actin was used for internal loading control. c, The expression level of Kdm3b, c-Fos, JUN, and JUNB transcripts in whole retina plotted for four developmental time points (1.5-, 3-, 6-, and 12-month-old). d, The expression level of Kdm3b, c-Fos, JUN, and JUNB transcripts in Kdm3b+/+ and Kdm3b−/− mice retinas. e, The expression level of c-Fos, JUN, and JUNB transcripts in rod cell and s-cone-like cells (Nrlp-GFP; Nrl−/−) plotted for three developmental time points (P10, P14, and P28) using public RNA-sequencing data (GSE 74660). f, Immunoblot showing co-immunoprecipitation of endogenous KDM3B with c-Fos and JUNB in human lung cancer cells. g, Recruitment and presence of c-Fos and JUNB at indicated gene promoters were validated by ChiP-qPCR. Error bars show mean ± SEM. P values obtained by Student's t-test. *** P < 0.001, ** P < 0.01, n.s, not significant.
Supplementary Fig. 1 The morphological changes of rods and cones in Kdm3b<sup>+/+</sup> and Kdm3b<sup>-/-</sup> mouse retina. a, Cross-sectioned retinas were stained with DAPI from 1-, 6-, and 12-month-old Kdm3b<sup>+/+</sup> and Kdm3b<sup>-/-</sup> mice. Scale bar: 500 μm. b, Representative images of hematoxylin and eosin (H&E) staining for retinal thickness and total number of nuclei in ONL, INL, and GCL in the retinas of 1-, 6-, and 12-month-old Kdm3b<sup>+/+</sup> and Kdm3b<sup>-/-</sup> mice. c,d, Immunostaining with CAR antibodies (red) of whole mount retina and DAPI (blue) in 12-month-old Kdm3b<sup>+/+</sup> and Kdm3b<sup>-/-</sup> mice. Scale bar: 20 μm e, The magnification of the cone pedicles in 1- and 12-months-old Kdm3b<sup>+/+</sup> and Kdm3b<sup>-/-</sup> mice. Scale bar: 10 μm.
Supplementary Fig. 2 Induced apoptotic response of cone photoreceptors in $Kdm3b^{+/+}$ retinas. 

**a.** Immunostaining of cytochrome c (red) and CAR (green) in 12-month-old $Kdm3b^{+/+}$ and $Kdm3b^{+/-}$ retinas. Scale bar: 20 μm. Cytochrome c-intensity is measured in red dashed box. 

**b.** Apoptotic cells were detected by TUNEL assay (BrdU positive cells) in 12-month-old $Kdm3b^{+/+}$ and $Kdm3b^{+/-}$ mouse retinas. Scale bar: 50 μm. BrdU-positive cones in red dashed box are quantified.
Supplementary Fig. 3 The alteration of synaptic morphology of cone photoreceptors in Kdm3b<sup>+/−</sup> mice retina. a, Immunostaining of Cone arrestin (CAR) and CtBP2 in 12-month-old Kdm3b<sup>+/−</sup> and Kdm3b<sup>+/−</sup> mouse retina sections. Scale bar: 2 μm. b, The number of ribbon synapses in cone photoreceptor is quantified in 1- and 12-month-old Kdm3b<sup>+/−</sup> and Kdm3b<sup>+/−</sup> mouse retinas (n = 30) Scale bar: 20 μm. c, 3D reconstruction images showing ribbon synapses in cone photoreceptors of 12-month-old Kdm3b<sup>+/−</sup> and Kdm3b<sup>+/−</sup> mouse retinas. Scale bar: 5 μm
Supplementary Files

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