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2 **Banp regulates DNA damage response and chromosome**
 3 **segregation during the cell cycle in zebrafish retina**

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 14 tp53

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18

19 Abstract

20 Btg3-associated nuclear protein (Banp) was originally identified as a nuclear matrix-
 21 associated protein and it functions as a tumor suppressor. At molecular level, Banp
 22 regulates transcription of metabolic genes via a CGCG-containing motif called the Banp
 23 motif. However, its physiological roles in embryonic development are unknown. Here
 24 we report that Banp is indispensable for DNA damage response and chromosome
 25 segregation during mitosis. Zebrafish *banp* mutants show mitotic arrest and apoptosis in
 26 developing retina. We found that DNA replication stress and tp53-dependent DNA
 27 damage responses were activated to induce apoptosis in *banp* mutants, suggesting that
 28 Banp is required for integrity of DNA replication and DNA damage repair. Furthermore,
 29 in *banp* mutants, chromosome segregation was not smoothly processed from
 30 prometaphase to anaphase, leading to a prolonged M-phase. Our RNA- and ATAC-
 31 sequencing identified 31 candidates for direct Banp target genes that carry the Banp
 32 motif. Interestingly, two chromosome segregation regulators, *cenpt* and *ncapg*, are
 33 included in this list. Thus, Banp directly regulates transcription of *cenpt* and *ncapg* to
 34 promote chromosome segregation during mitosis. Our findings provide the first *in vivo*
 35 evidence that Banp is required for cell-cycle progression and cell survival by regulating
 36 DNA damage responses and chromosome segregation during mitosis.

37

38 (197 words)

39 **Introduction**

40 The vertebrate retina is composed of six major classes of neurons and one type of glia,
 41 namely retinal ganglion cells (RGCs), three types of interneurons (amacrine cells,
 42 bipolar cells, and horizontal cells), two types of photoreceptors (rods and cones), and
 43 Müller glia. These retinal cells are assembled to form the neural circuit responsible for
 44 phototransduction and visual processing (Dowling, 2012). During development, retinal
 45 progenitor cells are multipotent and give rise to all seven retinal cell types in response to
 46 extrinsic and intrinsic cues (Boije et al., 2014). In addition, retinal neurogenesis is
 47 spatially and temporally regulated among vertebrates. In zebrafish, neurogenesis starts
 48 in the ventro-nasal retina at 25 h post-fertilization (hpf) and then spreads into the entire
 49 neural retina by 72 hpf (Hu and Easter, 1999; Masai et al., 2000). In general, early-born
 50 post-mitotic cells differentiate into RGCs and cone photoreceptors, followed by
 51 amacrine cells, bipolar cells, and horizontal cells, and late-born cells differentiate into
 52 rod photoreceptors and Müller glia, indicating stage-dependent retinal histogenesis.

53 Another important characteristic of retinal development is that retinal
 54 neurogenesis is linked to cell-cycle regulation. In zebrafish, cell-cycle length of retinal
 55 progenitor cells is around 32–49 h during 6 to 25 hpf, but shortened to 8–10 h once
 56 retinal neurogenesis starts at 25 hpf (Li et al., 2000), suggesting that activation of cell-
 57 cycle progression is associated with neurogenesis. It is generally accepted that active
 58 cell proliferation may increase DNA replication errors, which induces DNA replication
 59 stress and subsequent DNA damage (Berti et al., 2020; Blackford and Jackson, 2017;
 60 Waterman et al., 2020). Thus, DNA damage response is essential to ensure genomic
 61 integrity (Murga et al., 2009). Otherwise, retinal progenitor cells undergo cell-cycle
 62 arrest or apoptosis. Consistently, in zebrafish, retinal apoptosis occurs in the absence of
 63 DNA damage response regulators such as DNA damage-binding protein 1 (DDB1) (Hu
 64 et al., 2015), Fanconi anemia group D2 (Fancd2) (Liu et al., 2003), and Ku80 (Xrcc80)
 65 (Bladen et al., 2005). We also showed that retinal apoptosis occurs in zebrafish *prim1*
 66 mutants, in which DNA replication stress is chronically elevated. In this mutant, a
 67 central regulator of the DNA replication checkpoint, Ataxia telangiectasia-mutated
 68 (ATM) and Rad3-related (ATR), is activated, which subsequently activates DNA
 69 damage response regulators, ATM and Chk2, leading to tp53-mediated apoptosis. Thus,
 70 DNA replication checkpoint and DNA damage response function as safeguards to

71 ensure integrity of retinal cell proliferation and neurogenesis (Yamaguchi et al., 2008).
72 However, it remains to be seen how the DNA damage response pathway is integrated
73 into retinal cell proliferation and neurogenesis.

74 BTG3-associated nuclear protein (Banp) (also known as Scaffold/matrix
75 attachment region binding protein 1 (SMAR1)) was identified as a nuclear protein that
76 binds to the matrix-associated region (MAR) of the β -locus of murine T cell receptors
77 (Biro et al., 2000; Chattopadhyay et al., 2000). MARs are often found near gene
78 promoters (Scheuermann and Garrard, 1999) and constitute a periodic loop domain,
79 through which chromosomal DNA is topologically organized inside the nucleus
80 (Blasquez et al., 1989; Hart and Laemmli, 1998). Very recently, it was reported that
81 Banp binds to the nucleotide sequence TCTCGCGAGA, called the “Banp motif”, which
82 is enriched near the transcription initiation site of CpG island promoters, and regulates
83 gene transcription in a DNA methylation-dependent manner. Banp promotes
84 transcription of essential metabolic genes in pluripotent stem and terminal differentiated
85 neuronal cells in humans and mice (Grand et al., 2021). Furthermore, Banp functions as
86 a tumor suppressor (Kaul et al., 2003; Kaul-Ghanekar et al., 2005). In humans, the *banp*
87 gene is located on chromosome 16q24, the genomic region of which often suffers from
88 loss-of-heterozygosity in various tumors (Tsuda et al., 1994; Wang et al., 1999). Banp
89 interacts with tumor suppressor tp53 to modulate its functions (Kaul et al., 2003;
90 Pavithra et al., 2009; Sinha et al., 2012; Sinha et al., 2010). Overexpression of Banp
91 suppresses cancer growth (Bhagat et al., 2018; Liu et al., 2014; Taye et al., 2018).
92 However, these cellular functions of Banp were proposed mostly from *in vitro* studies,
93 and genetic deletion of *banp* was reportedly unsuccessful (Grand et al., 2021), whereas
94 *banp* knockout mice show embryonic lethality (Chemmannur et al., 2015), which makes
95 *in vivo* analysis difficult. Thus, physiological functions of Banp are still unknown.

96 In this study, we identified zebrafish *banp* mutants, which show mitotic arrest
97 and apoptosis in the developing retina. DNA replication checkpoint and tp53-dependent
98 DNA damage response are activated to induce apoptosis in *banp* mutant retinas. Thus,
99 Banp is required for integrity of DNA replication and DNA damage repair. Furthermore,
100 live imaging revealed that chromosome segregation fails to proceed smoothly during
101 mitosis in *banp* mutants, resulting in a prolonged M-phase. Our RNA-sequencing and
102 ATAC-sequencing enabled us to identify 31 candidates for direct Banp target genes that

103 carry the Banp motif near their promoters. Interestingly, two chromosome segregation
 104 regulators, *cenpt* and *ncapg*, are included in this list. Thus, Banp directly regulates the
 105 transcription of *cenpt* and *ncapg* to promote chromosome segregation during mitosis.
 106 Taken together, these data suggest that Banp is required for cell-cycle progression and
 107 cell survival in zebrafish retinas by regulating DNA damage response and chromosome
 108 segregation during mitosis.
 109

110 **Results**

111 **Zebrafish *rw337* mutants show mitotic cell accumulation and cell death in the** 112 **retina after 2.5 dpf**

113 We performed large-scale mutagenesis using zebrafish and identified a zebrafish mutant,
114 *rw337*. *rw337* mutants have smaller eyes and a smaller optic tectum at 4 days post-
115 fertilization (dpf) (Fig. 1A). Around 6–7 dpf, *rw337* mutants die, indicating a lethal
116 mutation. Next, we examined retinal phenotypes (Fig. 1B). At 1.5 dpf, the retina
117 consists of retinal progenitor cells and early differentiating neurons. There was no
118 difference in retinal morphology between *rw337* mutants and wild-type siblings.
119 However, at 2 dpf, mitotic-like round cells had accumulated in the apical region of the
120 retina in *rw337* mutants, indicating mitotic arrest. At 2.5 dpf, in wild-type retinas, three
121 nuclear layers, the RGC layer, the inner nuclear layer (INL) and the outer nuclear layer
122 (ONL), and two plexiform layers, the inner plexiform layer (IPL) and the outer
123 plexiform layer (OPL), had formed. In *rw337* mutants, only the RGC layer was
124 observed; however, many pyknotic nuclei were observed in the outer retinal region. At
125 3 dpf, the RGC layer, IPL and INL appeared in *rw337* mutants. However, pyknotic
126 nuclei were densely packed in the outer region of *rw337* mutant retinas. At 4 dpf, most
127 dead cell debris had decreased in *rw337* mutants. The RGC layer, IPL, and INL were
128 formed; however, no ONL was observed, despite very small, patchy photoreceptor-like
129 cells. At 3 and 4 dpf, the ciliary marginal zone (CMZ), where retinal stem and
130 progenitor cells are located, was observed in *rw337* mutants. Thus, RGCs differentiate,
131 but mitotic arrest and cell death occur after 2.5 dpf in *rw337* mutants.

132 Next, we examined neuronal differentiation in *rw337* mutants at 4 dpf. The
133 transgene *Tg[ath5:GFP]* drives GFP expression in RGCs (Fig. 1-figure supplement 1A)
134 (Masai et al., 2000). Pax6 is strongly expressed in amacrine cells and weakly in RGCs
135 (Fig. 1-figure supplement 1C) (Macdonald and Wilson, 1997). Prox1 is expressed in
136 bipolar cells and horizontal cells (Fig. 1-figure supplement 1E) (Jusuf and Harris, 2009).
137 zpr1 antibody labels double cone photoreceptors (Fig. 1-figure supplement 1G) (Larison
138 and Bremiller, 1990). Anti-Glutamate synthetase (GS) antibody labels Müller cells (Fig. 1-figure supplement 1I)
139 (Peterson et al., 2001). We observed that all these markers are expressed in *rw337*

mutants (Fig. 1-figure supplement 1B, 1D, 1F, 1H, 1J). However, the numbers of strong Pax6⁺ cells and GS⁺ cells were decreased in *rw337* mutants (Fig. 1-figure supplement 1D, 1J), suggesting that amacrine cells and Müller cells were reduced. Prox1⁺ cells were misplaced and less numerous in *rw337* mutants, suggesting that horizontal and bipolar cells are reduced (Fig. 1-figure supplement 1F). *zpr1*⁺ cells were not detected, except for a small patch of *zpr1*⁺ cells in the ventral retina, where photoreceptor differentiation initially occurs, in *rw337* mutants (Fig. 1-figure supplement 1H), suggesting that most cone photoreceptors fail to differentiate. Anti-PCNA antibody labels retinal stem and progenitor cells in the CMZ (Fig. 1-figure supplement 1K), which was not affected in *rw337* mutants (Fig. 1-figure supplement 1L). Phalloidin labeling shows IPL and OPL in wild-type retinas (Fig. 1-figure supplement 1M). *rw337* mutants showed IPL, but not OPL (Fig. 1-figure supplement 1N), which is consistent with *zpr1* labeling (Fig. 1-figure supplement 1H). Thus, retinal stem and progenitor cells are maintained. RGCs do differentiate; however, amacrine cells, horizontal cells, bipolar cells, and Müller cells are decreased in number and photoreceptors fail to differentiate in *rw337* mutants.

158

***rw337* mutant gene encodes Banp**

We mapped the *rw337* mutation to zebrafish chromosome 25 (Fig. 1C). Two polymorphic markers, 070702B and zC93F2D, restricted the genomic region encompassing *rw337* mutation, in which only four candidate genes: *BTG3-associated nuclear protein (banp)*; *programmed cell death 2-like (pdcd2l)*; *carboxylesterase 2b (ces2b)*; *protein disulfide isomerase family A, member 3 (pdia3)*, were annotated. We cloned all these cDNAs from mRNA prepared from *rw337* homozygous mutants, sequenced them, and found that a part of the intron sequence between exons 4 and 5 (intron 4/5) failed to be spliced out of the *banp* cDNA (Fig. 1D). A nucleotide T at the exon-intron junction of exon 4 is converted to G, which results in an alteration of the donor splice site of exon 4 from GT to GG. This mutation leads to the abnormal insertion of a 52-bp intron sequence after exon 4, resulting in a premature stop codon in *banp* cDNA (Fig. 1D). The *banp* gene encodes a protein containing a coiled-coil domain, a BEN domain and a DNA-binding domain, which includes a MAR-binding domain (Fig. 1E). Overall, zebrafish Banp protein shows high similarity

174 to those of humans and mice, especially in the BEN domain (Fig. 1-figure supplement
175 2). This mutation is predicted to generate nonfunctional, truncated Banp protein in
176 *rw337* mutants (Fig. 1E). There was no mutation in the other three genes in *rw337*
177 mutants, suggesting that the *rw337* mutant gene encodes Banp.

178 To confirm that the loss of Banp causes retinal phenotypes in *rw337* mutants, we
179 carried out three sets of experiments. First, we examined another nonsense mutation
180 allele, *banp*^{sa12976}, which has a premature stop codon in exon 4 (Fig. 1-figure
181 supplement 3A). Homozygous *banp*^{sa12976} mutant embryos and trans-heterozygous
182 *banp*^{rw337}; *banp*^{sa12976} mutants phenocopied *rw337* mutants (Fig. 1-figure supplement
183 3B). Second, we inhibited Banp using ATG-morpholino antisense oligos against Banp
184 (*banp* MO). We confirmed MO specificity, because *banp* MO inhibited translation of
185 mRNA encoding C-terminal EGFP-tagged wild-type Banp (*banp*(wt)-EGFP), whereas
186 the control, 5-mismatch *banp* MO (*banp* 5misMO) did not (Fig. 1-figure supplement 4A
187 and 4B). *banp* morphants phenocopied *rw337* mutants (Fig. 1F). Third, we
188 overexpressed wild-type Banp in *banp*^{rw337} mutants. We injected mRNA encoding
189 Banp(wt)-EGFP into wild-type embryos at the one-cell stage. In the developing retinas,
190 Banp(wt)-EGFP protein was localized in the nuclei during interphase, whereas it spread
191 into the whole cell region during mitosis (Fig. 1-figure supplement 4C and 4D),
192 confirming nuclear localization of zebrafish Banp. We expressed Banp(wt)-EGFP, N-
193 terminal EGFP tagged wild-type (EGFP-Banp(wt)) and the *rw337* mutant form of Banp
194 (EGFP-Banp(*rw337*)) in wild-type retinas. Only EGFP-Banp(*rw337*) was not stably
195 maintained (Fig. 1-figure supplement 4E), suggesting that the mutant form of Banp
196 protein is degraded, probably by nonsense mutation decay. Finally, overexpression of
197 Banp(wt)-EGFP rescued retinal phenotypes in *rw337* mutants at 2 dpf, but
198 overexpression of EGFP-Banp(*rw337*) did not (Fig. 1G). Taken together, retinal
199 phenotypes of *rw337* mutants are caused by the loss of Banp.

200

201 ***banp* mRNA is prominently expressed in the developing CNS of zebrafish**

202 We examined *banp* mRNA expression by *in-situ* hybridization (Fig. 1-figure
203 supplement 5A). *banp* mRNA shows ubiquitous expression from the one-cell stage to
204 the 21-somite stage, suggesting maternal and zygotic expression. mRNA was restricted
205 to the brain, including the retina at 30 and 48 hpf. Frontal sections show that mRNA is

expressed in neural retina at 24 hpf, but restricted in the CMZ at 48 hpf, suggesting that retinal progenitor cells express *banp* mRNA (Fig. 1-figure supplement 5B). In addition to the brain and retinas, *banp* mRNA expression was observed in fin and notochord at 48 hpf and in neuromasts of the lateral line after 72 hpf. *banp* mRNA expression was not detected in *banp^{rw337}* homozygous mutants at 3 dpf (Fig. 1-figure supplement 5C), suggesting nonsense mutation-mediated decay. Thus, *banp* mRNA is prominently expressed in the developing brain in zebrafish.

***banp* mutants show cell cycle arrest followed by cell death of retinal progenitor cells**

Although retinal progenitor cells produce RGCs in *banp^{rw337}* mutant retinas, the number of other neurons, especially photoreceptors, was decreased at 4 dpf (Fig. 1-figure supplement 1). Indeed, mitotic-like round cells and pyknotic nuclei were accumulated in the outer region of *banp^{rw337}* mutant retinas from 2 to 3 dpf (Fig. 1B), suggesting that mitotic arrest and cell death eliminate retinal neurons. Accordingly, we performed TdT-mediated dUTP Nick End Labeling (TUNEL), which visualizes apoptosis. TUNEL+ cells were rarely observed in wild-type retinas throughout the stages from 29 to 101 hpf, as well as in *banp^{rw337}* mutant retinas at 24 hpf (Fig. 2A). However, in *banp^{rw337}* mutants, TUNEL+ cells appeared in the neural retina at 53 hpf, and became more localized to the outer retina at 77 hpf (Fig. 2A) and to the interface between the central retina and the CMZ at 77 and 101 hpf (Fig. 2A). After 53 hpf, the number of TUNEL+ cells was significantly higher in *banp^{rw337}* mutants than in wild-type siblings (Fig. 2C). Thus, retinal cells undergo apoptosis in *banp^{rw337}* mutants. Next, we examined mitosis by labeling with anti-phosphorylated (Ser-10) Histone H3 (pH3) antibody, which labels mitotic cells in pro-metaphase (Prigent and Dimitrov, 2003) (Fig. 2B). The number of pH3+ cells did not differ between wild-type and *banp^{rw337}* mutant retinas at 24 hpf. However, pH3+ cells were markedly increased and located at the apical surface of the neural retina in *banp^{rw337}* mutant retinas at 53 hpf (Fig. 2B), suggesting mitotic arrest. At 77 and 101 hpf, pH3+ cells disappeared from the central retina, but still were accumulated in the CMZ in *banp^{rw337}* mutants (Fig. 2B). The number of pH3+ cells in *banp^{rw337}* mutant retinas increased transiently at 53 hpf, and then decreased, but was

237 still significantly higher than that of wild-type sibling retinas after 77 hpf because of
238 accumulation of pH3+ cells at the CMZ (Fig. 2D).

239 To clarify how mitotic arrest and apoptosis are induced during retinal cell
240 proliferation and neurogenesis in *banp*^{rw337} mutants, we examined BrdU incorporation
241 and ath5:EGFP expression at 48 hpf (Fig. 2-figure supplement 1A and 1B). The
242 percentage of BrdU+ area to total retinal area did not differ significantly between wild-
243 type siblings and *banp*^{rw337} mutants (Fig. 2-figure supplement 1C), whereas the
244 percentage of ath5:EGFP+ area to the total retinal area was slightly, but significantly
245 lower in *banp*^{rw337} mutants than in wild-type siblings (Fig. 2-figure supplement 1C),
246 suggesting that retinal neurogenesis is slightly reduced in *banp*^{rw337} mutants at 48 hpf,
247 probably due to mitotic arrest or apoptosis.

248 Next, we examined the spatial position of mitotic arrest and apoptosis at 48 hpf
249 by labeling with anti-pH3 and anti-activated caspase 3 antibodies (Fig. 2-figure
250 supplement 1D and 1E). Both caspase 3+ and pH3+ cells were increased in *banp*^{rw337}
251 mutants (Fig. 2-figure supplement 1F). Furthermore, caspase 3+ cells were mostly
252 located in the intermediate zone of the neural retina along the apico-basal axis, whereas
253 pH3+ cells were localized at the apical surface of the neural retina (Fig. 2-figure
254 supplement 1G), suggesting that apoptosis occurs mostly in retinal progenitor cells
255 undergoing G1 or S phase, or differentiating neurons. Next, we examined whether
256 apoptosis occurs in early differentiating retinal neurons, using double labeling with anti-
257 activated caspase 3 antibody and ath5:EGFP (Fig. 2-figure supplement 1H). The
258 percentage of ath5:EGFP- and caspase 3-double-positive cells to the total number of
259 caspase 3+ cells was 9.44±13.1% in *banp* mutants at 48 hpf and higher than that of
260 wild-type siblings (0.00±0.00%), although the difference was not significant (Fig. 2-
261 figure supplement 1I). Next, we examined whether apoptosis occurs in mature retinal
262 neurons. HuC/D is expressed in maturing neurons (Park et al., 2000). At 48 hpf, anti-
263 HuC/D antibody labeled RGCs in zebrafish (Fig. 2-figure supplement 1J). The fraction
264 of caspase 3- and HuC/D-double-positive cells in the total caspase 3+ cells was almost
265 zero in both wild-type and *banp*^{rw337} mutants (Fig. 2-figure supplement 1K), suggesting
266 that apoptosis does not occur in maturing retinal neurons. Taken together, in *banp*^{rw337}
267 mutants, retinal progenitor cells undergo mitotic arrest at 48 hpf. On the other hand,
268 apoptosis is induced in retinal progenitor cells at G1 or S-phase or in newly

differentiating retinal neurons, although apoptosis in newly differentiating retinal neurons is minor (Fig. 2-figure supplement 1L). Since mitotic arrest was transiently observed at 53 hpf (Fig. 2B), but apoptosis continues in the central retina of *banp*^{rw337} mutants after 53 hpf (Fig. 2A), it is likely that apoptosis occurs following mitotic arrest (Fig. 2E and 2F).

tp53 pathway mediates retinal apoptosis in *banp* mutants

To understand molecular mechanisms of mitotic arrest and apoptosis in *banp*^{rw337} mutants, we identified differentially expressed mRNAs by RNA-seq analysis. We prepared mRNA from 48-hpf wild-type siblings and *banp*^{rw337} mutants. Illumina sequencing generated more than 50 million paired-end reads from cDNA libraries. 27,771 protein-coding genes were detected. Among them, 258 genes were upregulated, and 81 genes were downregulated in *banp*^{rw337} mutants (Fig. 3A). The top 15 upregulated genes were enriched regulators of tp53 dependent-DNA damage response (Fig. 3B). Quantitative RT-PCR revealed that four tp53 target genes: *p21(cdkn1a)*, *puma (bbc3)*, *ccng1*, and *mdm2* are significantly upregulated in *banp*^{rw337} mutants (Fig. 3C). Western blotting showed that full length tp53 (FL tp53) protein is stabilized and more than 11 fold higher in *banp*^{rw337} mutants than in wild type (Fig. 3D and 3E). We previously showed that the ATR/ATM-Chk2-tp53 DNA damage pathway induces retinal apoptosis in zebrafish (Yamaguchi et al., 2008). Indeed, mRNA expression of ATR and ATM was upregulated in *banp*^{rw337} mutants (Fig. 3F). So, we examined whether retinal apoptosis depends on tp53 in *banp*^{rw337} mutants. Injection of tp53 antisense morpholino (tp53 MO) completely inhibited retinal apoptosis in *banp*^{rw337} mutants at 48 hpf (Fig. 3G and 3H). However, retinal apoptosis reappeared in *banp*^{rw337} mutants injected with tp53 MO at 72 hpf (Fig. 3G), although there was no statistical difference in apoptotic cell number between wild-type and *banp*^{rw337} mutant retinas at 72 hpf (Fig. 3H). Thus, retinal apoptosis in *banp*^{rw337} mutants depends on tp53, although it is possible that some tp53-independent mechanism induces retinal apoptosis after 48 hpf.

Transcription of $\Delta 113$ -tp53 is preferentially elevated in *banp* mutants

RNA-seq analysis showed upregulation of tp53 mRNA (Fig. 3B); however, surprisingly, quantitative RT-PCR showed that the mRNA level of FL tp53 is not significantly different between *banp*^{rw337} mutants and wild-type siblings at 48 hpf (Fig. 4A). There are several isoforms of tp53 protein (Jorruiz et al., 2020; Jorruiz and Bourdon, 2016). Importantly, in zebrafish, the isoform Δ 113tp53 is generated from the alternative transcription start site in intron 4. Δ 113tp53 preferentially activates the transcription of target genes related to cell-cycle arrest to promote DNA repair and cell survival, whereas FL tp53 preferentially activates transcription of target apoptotic genes to eliminate severely DNA-damaged cells (Gong et al., 2015; Gong et al., 2020). Differential expression of tp53 isoforms is thought to regulate the balance between cell survival and apoptosis in response to DNA damage. Indeed, quantitative RT-PCR confirmed that only Δ 113tp53 was upregulated in *banp*^{rw337} mutants (Fig. 4A). ATAC-seq revealed that a genomic region in intron 4 of tp53 is specifically open in *banp*^{rw337} mutants but not in wild-type siblings (Fig. 4B, ATAC-seq). Consistently, transcription of the Δ 113tp53 mRNA was specifically activated in *banp*^{rw337} mutants, but not in wild-type siblings (Fig. 4B, RNA-seq). Thus, the internal promoter at intron 4 (Chen et al., 2009; Chen et al., 2005) is specifically activated in *banp*^{rw337} mutants (Fig. 4B). These data raise the possibility that the lack of Banp increases DNA replication stress and promotes an accumulation of DNA damage, resulting in activation of ATR and ATM, which stabilize FL tp53, such that FL tp53 triggers Δ 113tp53 transcription in *banp*^{rw337} mutants (Fig. 4G).

321

322 DNA double-strand breaks accumulate in *banp* mutant retinas

To examine whether DNA damage is increased due to loss of Banp functions, we labeled 48-hpf retinas with anti- γ -H2AX antibody, which marks DNA double-strand breaks (Rogakou et al., 1998; Shroff et al., 2004). The number of γ -H2AX+ cells increased in both *banp*^{rw337} mutant (Fig. 4C, 4F) and *banp* morphant retinas (Fig. 4-figure supplement 1A, 1B), confirming an accumulation of DNA damage in the absence of Banp. Next, to determine how DNA damage is induced in *banp*^{rw337} mutants, we examined the fraction of S-phase cells, mitotic cells, and post-mitotic neurons in γ -H2AX+ retinal cells in *banp*^{rw337} mutants at 48 hpf. First, wild-type and *banp*^{rw337} mutant retinas carrying the transgene *Tg[ath5:EGFP]* were labeled with anti-BrdU and

anti- γ -H2AX antibodies (Fig. 4-figure supplement 1C). The fraction of γ -H2AX+ cells increased in *banp*^{rw337} mutant retinas, whereas the fraction was almost zero in wild-type sibling retinas (Fig. 4-figure supplement 1D). Next, we compared the fraction of BrdU+ cells between the total retinal cells and γ -H2AX+ retinal cells in *banp*^{rw337} mutants (Fig. 4-figure supplement 1E). The BrdU+ fraction was significantly higher in γ -H2AX+ cells than in the total retinal cells (Fig. 4-figure supplement 1F), indicating that S-phase fraction is 4.3 fold higher in DNA damaged retinal cells than in the total retinal cells, in *banp*^{rw337} mutants. Thus, DNA double-strand breaks are abnormally induced or fail to be repaired in retinal progenitor cells undergoing S phase. Next, we examined the fraction of ath5:EGFP+ cells (Fig. 4-figure supplement 1G). There was no significant difference in ath5:EGFP+ cell fraction between the total retinal cells and γ -H2AX+ retinal cells in *banp*^{rw337} mutants (Fig. 4-figure supplement 1H). Finally, we determined the fraction of mitotic cells by labeling with anti-pH3 and anti- γ -H2AX antibodies in *banp*^{rw337} mutant retinas at 48 hpf (Fig. 4-figure supplement 1I). There is no significant difference in pH3+ cell fraction between the total retinal cells and γ -H2AX+ retinal cells (Fig. 4-figure supplement 1J). Although DNA damage may occur in post mitotic neurons and mitotic cells in *banp*^{rw337} mutants, it is likely that most DNA damage is introduced in S-phase of retinal progenitor cells in the absence of Banp.

It is generally accepted that tp53 promotes DNA damage repair or eliminates DNA-damaged cells via apoptosis, so we expected that tp53 knockdown may enhance accumulation of DNA damage in *banp*^{rw337} mutants. However, compared with standard-MO injection control (n=120.8±60.6/section), the injection of FL tp53-MO significantly reduced the number of γ -H2AX+ cells in *banp*^{rw337} mutant retinas (n=55.9±15.9/section) at 48 hpf (Fig. 4C, 4F). The injection of Δ 113tp53-MO mildly reduced the number of γ -H2AX+ cells (n=85.4±15.2/section); however, the difference was not significant (Fig. 4C, 4F). It is likely that tp53 or Δ 113tp53 does not repair DNA damage in *banp*^{rw337} mutants, but rather it may enhance DNA damage in *banp*^{rw337} mutants. Thus, Banp normally suppresses DNA replication stress upstream of tp53, or promotes DNA damage repair in concert with tp53 (Fig. 4G).

In addition, we investigated whether retinal apoptosis and mitotic arrest in *banp*^{rw337} mutants depend on FL tp53-MO or Δ 113tp53-MO. Compared with Standard-MO injection control (n=174.7±30.5/section), retinal apoptosis was completely

inhibited by FL tp53-MO in *banp*^{rw337} mutant retinas (n=0.50±0.58/section) at 48 hpf (Fig. 4C, 4D). However, inhibition of apoptosis in *banp*^{rw337} mutant retinas by Δ113tp53-MO was significant, but ineffective (n=58.00±11.0/section) (Fig. 4C, 4D). This result is consistent with previous reports proposing that Δ113tp53 preferentially activates transcription of target genes related to cell-cycle arrest rather than apoptosis (Gong et al., 2015; Gong et al., 2020). However, compared with Standard-MO injection controls (n=376.6±74.8/section), the number of pH3+ cells in *banp*^{rw337} mutants was significantly decreased by FL tp53-MO (n=262.4±68.3/section) (Fig. 4C, 4E). However, the number of pH3+ cells was still higher in *banp*^{rw337} mutant retinas injected with FL tp53-MO than in wild-type control retinas injected with FL tp53-MO. The injection of Δ113tp53-MO did not significantly rescue mitotic arrest in *banp*^{rw337} mutants (n=303.8±46.6/section) (Fig. 4C, 4F). These data suggest that mitotic arrest in *banp*^{rw337} mutants is mostly mediated by a tp53-independent mechanism.

377

378 **Banp is required for the expression of chromatin segregation genes**

Mitotic arrest in *banp*^{rw337} mutants is independent of tp53. RNA-seq analysis revealed that two chromosomal segregation regulators, *cenpt* and *ncapg*, are downregulated in *banp*^{rw337} mutants at 48 hpf (Fig. 3B). Cenpt and Ncapg are essential for chromatin segregation (Hung et al., 2017; Seipold et al., 2009; Zhang et al., 2018). Quantitative RT-PCR confirmed their downregulation in *banp*^{rw337} mutants and *banp* morphants (Fig. 5A, 5C). Furthermore, ATAC-seq revealed that chromatin accessibility is decreased near the transcription start site (TSS) of both genes in *banp*^{rw337} mutants, leading to reduction of transcription activity (Fig. 5B, 5D). Thus, Banp is required for transcriptional activation of *cenpt* and *ncapg* genes. Interestingly, two independent chip-sequencing studies using Banp antibody revealed a specific binding site within 100 bp of the TSS of *cenpt* and *ncapg* genes in mice and humans (Grand et al., 2021; Mathai et al., 2016), suggesting that the requirement for Banp in *cenpt* and *ncapg* transcription is conserved in zebrafish, mice, and humans. Furthermore, using motif discovery software (HOMER), we discovered that TCTCGCGAGA, which was reported to be the Banp motif (Grand et al., 2021), is the most enriched motif in the closed chromatin region of *banp*^{rw337} mutants (Fig. 5E, Fig. 5-figure supplement 1A). Indeed, among 81 down-regulated genes in *banp*^{rw337} mutants (Fig. 3A), we identified the Banp motif near the

396 TSS of 31 genes (Fig. 5F and Supplementary table 1), 26 % of which was cell-cycle
397 regulators (Fig. 5G, 5H) including *cenpt* and *ncapg* (Fig. 5I and 5J). However, we
398 could not find the Banp motif in 258 upregulated genes. Furthermore, the Banp motif
399 occurs upstream of the TSS of both *cenpt* and *ncapg* in mice and humans (Fig. 5-figure
400 supplement 1B, 1C). Their Banp motif position is consistent with the previous report,
401 that the Banp motif is not located at a constant distance from TSSs, but rather between
402 20 and 70 nucleotides upstream on both strands, and that the frequency of the Banp
403 motif occurrence upstream of TSSs may correlate to a transcription rate of target genes
404 (Mahpour et al., 2018) (Supplementary table 1). Taken together, it is likely that Banp
405 directly regulates transcription of *cenpt* and *ncapg* via the Banp motif.

406

407 **Chromosomal segregation is abnormal in *banp* mutants**

408 Knockdown of *Cenpt* and *Ncapg* causes defects in chromosomal segregation during
409 mitosis in zebrafish (Hung et al., 2017; Seipold et al., 2009). So, we conducted time-
410 lapse imaging of mitosis in *banp*^{rw337} mutant retinas for 1-2 h from 48 to 54 hpf, using a
411 transgenic line *Tg[h2afv:GFP; EF1a:mCherry-zGem]*, which visualizes cell-cycle
412 phases (Mochizuki et al., 2014). In wild-type retinas injected with control *banp*
413 5misMO, chromatin starts to be condensed in prophase, kinetochores and a mitotic
414 spindle appear in prometaphase, chromosomes are centrally aligned and start to be
415 pulled toward the opposite poles by mitotic spindles in metaphase, sister chromatids
416 move toward the opposite poles in anaphase, and chromosomes arrive at the opposite
417 poles in telophase, whereupon cytokinesis starts (Fig. 6A, Video 1). The duration from
418 prophase to telophase was 22.9±5.8 minutes (Fig. 6B). On the other hand, mitosis did
419 not proceed smoothly in *banp* morphant retinas, especially from prophase to anaphase
420 (Fig. 6A, Video 2). It took longer from chromosomal condensation in prophase to
421 chromosomal alignment at the division plane in anaphase in *banp* morphant retinas
422 (t=15 to t=46 in *banp*-MO, Fig. 6A). Aligned chromosomes were often dispersed
423 during chromosomal segregation by the mitotic spindle (t=46 to t=64 in *banp*-MO, Fig.
424 6A), although all 28 cell divisions eventually completed cytokinesis in *banp* morphants.
425 The duration from prophase to telophase was 49.2± 17.0 minutes and significantly
426 longer in *banp* morphants (Fig. 6B). Thus, chromosomal segregation is abnormal during
427 mitosis in the absence of Banp. Furthermore, we labeled 48-hpf retinas with anti-α-

428 tubulin and anti-pH3 antibodies to visualize the mitotic spindle and prometaphase
 429 chromosomes, respectively. In *banp*^{rw337} mutants, mitotic spindles are irregularly
 430 attached to less condensed and misaligned chromosome (Fig. 6C). The percentage of
 431 cells with abnormal mitosis was significantly higher in *banp*^{rw337} mutant retinas than
 432 those of wild-type siblings (Fig. 6D). These data suggest that the interaction between
 433 chromosomes and mitotic spindles is compromised in the absence of Banp, probably
 434 due to kinetochore attachment defects caused by lack of Cenpt and Ncapg. Thus, Banp
 435 regulates chromosomal segregation during mitosis.

436 Taken together, Banp plays essential roles in cell-cycle regulation in the
 437 zebrafish developing retina. During the S phase, Banp suppresses DNA replication
 438 stress and promotes DNA damage repair in concert with tp53-mediated DNA damage
 439 response. In the M phase, Banp promotes mitosis by activating the transcription of two
 440 chromosome segregation regulators, *cenpt* and *ncapg*, most likely via their Banp motifs
 441 (Fig. 7).

442

443 Discussion

444 Banp was initially identified as a nuclear matrix-associated protein and reported to
 445 function as a tumor suppressor (Malonia et al., 2011). Very recently, it was reported that
 446 Banp binds to the Banp motif enriched near the TSS of CpG island promoters and
 447 regulates transcription of metabolic genes (Grand et al., 2021). However, physiological
 448 functions of Banp during embryonic development remain to be discovered. In this study,
 449 we identified zebrafish *banp* mutants, in which retinal progenitor cells show mitotic
 450 arrest and subsequent apoptosis during embryonic development. RNA-seq analysis
 451 revealed that expression of tp53-dependent DNA damage response regulators is
 452 upregulated in *banp* mutants. In a non-stress condition, tp53 is degraded through
 453 ubiquitination by an E3-ubiquitin ligase, Mdm2, and subsequent actions of proteasomes,
 454 keeping its protein level very low (Oliner et al., 1993). On the other hand, under stress,
 455 such as DNA replication stalling or DNA double-strand breaks, ATR and ATM are
 456 activated and phosphorylate the N-terminal serine of tp53 (Saito et al., 2002; Tibbetts et
 457 al., 1999). This phosphorylation reduces the binding affinity of Mdm2 for tp53,
 458 resulting in stabilization of tp53 protein (Yadahalli et al., 2019). Indeed, the tp53 protein
 459 level is increased in *banp* mutants, and mRNA expression of ATR and ATM is also
 460 elevated. tp53 knockdown effectively suppresses retinal apoptosis in *banp* mutants.
 461 These data suggest that DNA replication stress is elevated in *banp* mutants, resulting in
 462 activation of tp53-dependent DNA damage response, which subsequently induces
 463 apoptosis of retinal progenitor cells.

464 It is generally accepted that tp53 has dual, but opposing roles: cell survival
 465 versus cell death in response to DNA replication stress and DNA damage (Chen, 2016).
 466 In the initial phase of the DNA damage response, tp53 binds to its alternative promoter
 467 located in intron 4 and activates transcription of an N-terminal truncated isoform $\Delta 113$ -
 468 tp53, which preferentially activates transcription of target genes related to cell-cycle
 469 arrest for promoting DNA repair and cell survival (Chen et al., 2009). However, if DNA
 470 damage fails to be repaired, but accumulates chronically, FL tp53, in turn, activates
 471 transcription of apoptotic genes for eliminating DNA-damaged cells. Previous *in vitro*
 472 studies of human *banp* suggest that Banp physically interacts with tp53 to promote
 473 transcription of cell-cycle arrest genes and suppresses transcription of apoptotic genes,
 474 leading to promotion of its cell survival role (Jalota et al., 2005; Sinha et al., 2010).

475 Indeed, our quantitative RT-PCR showed that transcription of $\Delta 113$ -tp53 is significantly
476 elevated in *banp* mutants, whereas the mRNA level of FL tp53 is very low. RNA-seq
477 and ATAC-seq also confirmed a higher level of transcription and chromatin
478 accessibility of $\Delta 113$ tp53 in *banp* mutants. These data suggest that $\Delta 113$ tp53-mediated
479 transcription is selectively activated in *banp* mutants. Thus, it is important to clarify the
480 role of tp53, including $\Delta 113$ tp53 in mitotic arrest in *banp* mutants, because pH3⁺ cells
481 are markedly accumulated in *banp* mutant retinas prior to apoptosis. However, tp53-MO
482 mildly inhibited accumulation of pH3⁺ cells in *banp* mutants. There is no significant
483 difference in pH3⁺ cell accumulation between tp53-MO and $\Delta 113$ tp53-MO, or between
484 wild-type siblings and $\Delta 113$ tp53-MO. So, these data suggest that the contribution of
485 tp53 and $\Delta 113$ tp53 to mitotic arrest in *banp* mutants seems to be minor, and that a tp53-
486 independent pathway is responsible for mitotic arrest phenotypes. Our live imaging of
487 mitosis in *banp* morphant retinas reveals that mitosis is significantly longer due to
488 chromosome segregation defects; however, importantly, almost all mitoses are
489 eventually completed in *banp* mutants, suggesting that accumulation of pH3⁺ cells in
490 *banp* mutants is transient and depends mostly on prolonged mitosis, which is caused by
491 chromosome segregation defects. At present, we have not observed S-phase arrest in
492 *banp* mutants, because the fraction of BrdU⁺ cells in the total retinal region is not
493 increased in *banp* mutants, although mRNA expression of a cdk inhibitor, *cdkn1a* (*p21*),
494 is higher in *banp* mutants than in wild-type siblings. Taken together, we conclude that
495 tp53 mainly mediates retinal apoptosis, and that its role in cell-cycle arrest is minor in
496 *banp* mutants.

497 Labeling with anti- γ -H2AX antibody revealed that DNA damage is increased in
498 retinal progenitor cells in *banp* mutants, suggesting that DNA damage is abnormally
499 introduced or fails to be repaired in the absence of Banp. Importantly, the level of DNA
500 damage in *banp* mutant retinas is not increased by either tp53-MO and $\Delta 113$ tp53-MO,
501 suggesting that tp53 does not repair DNA damage without Banp. There are two possible
502 functions of Banp in DNA damage regulation. Banp may maintain the integrity of DNA
503 replication. Banp functions as a transcription factor to regulate expression of essential
504 metabolic genes in pluripotent stem cells and differentiating neurons (Grand et al.,
505 2021). Therefore, Banp may promote transcription of DNA replication regulators. Our
506 RNA-seq data identified 81 downregulated genes in *banp* mutants, compared to wild-

type siblings. Among them, 31 genes have a Banp motif near their TSSs and contain eight cell-cycle regulatory genes. It will be interesting to examine whether these Banp targets are involved in the activation of DNA replication stress in *banp* mutants. Another possibility is that Banp is required for DNA repair. A previous study using human *in vitro* culture cells revealed that Banp promotes HDAC6-mediated deacetylation of a DNA end joining protein Ku70, which is required for non-homologous end-joining repair (Chaudhary et al., 2014). Since Ku70 is recruited to the double-strand damage site after γ -H2AX accumulation, Ku70 is an interesting candidate. Since knockdown of Ku70 along with radiation-induced DNA damage markedly induces apoptosis in zebrafish embryos (Bladen et al., 2007), it will be interesting to investigate whether this scenario is the case in zebrafish.

In addition to the role of Banp in DNA replication stress and DNA repair mechanisms, mRNA expression of two important regulators of chromosome segregation, *ncapg* and *cenpt*, is significantly downregulated in *banp* mutants. ATAC-seq and RNA-seq confirmed that chromatin accessibility is decreased near the TSSs of these genes in *banp* mutants, suppressing transcription of these mRNAs. Our *de novo* motif enrichment study identified the Banp motif sequence, TCTCGCGAGA, as the most enriched motif of closed chromatin regions of downregulated genes. Thus, it is highly likely that Banp directly binds to the Banp motifs of both *ncapg* and *cenpt*, opens their chromatin, and promotes their transcription. Consistently, as reported in zebrafish *ncapg* and *cenpt* knockdown embryos (Hung et al., 2017; Seipold et al., 2009), chromosome segregation is compromised in mitosis of retinal progenitor cells in *banp* mutants, suggesting that Banp is an essential regulator of mitosis. Since a Banp motif is found in the promoter region of *ncapg* and *cenpt* in mice and humans, and Chip sequencing with Banp antibody validated TSS peaks of both *ncapg* and *cenpt* genes in humans and mice (Grand et al., 2021; Mathai et al., 2016), it will be interesting to confirm whether Banp regulates chromosome segregation during mitosis in mammals.

Lastly, we identified 26 candidates of Banp direct target genes, including *ncapg* and *cenpt*, which carry the Banp motifs near their TSSs, and show markedly decreased chromatin accessibility and transcription in the absence of Banp. This gene list will provide a useful starting point to investigate the Banp-mediated transcriptional network. It was reported that the binding of Banp to the Banp motif is dependent on DNA

539 methylation of the Banp motifs (Grand et al., 2021), suggesting that an epigenetic
 540 mechanism regulates Banp-mediated transcription. Thus, zebrafish *banp* mutants
 541 provide an excellent new model in which to study how epigenetic factors regulate
 542 cellular processes for embryonic development via the Banp motifs of CpG island
 543 promoter genes. In addition, Banp has been proposed as a tumor suppressor (Malonia et
 544 al., 2011). It is generally accepted that DNA methylation of essential genes is altered in
 545 cancer cells (Baylin et al., 2001), which is expected to modify a pattern of gene
 546 transcription through Banp motifs (Grand et al., 2021). It will be interesting to
 547 investigate how the Banp-mediated transcription profile is changed in cancer models of
 548 zebrafish. Future research on zebrafish *banp* mutants will provide more in-depth
 549 understanding of cancer growth and metastasis, as well as new insights into therapy
 550 development of tumors in human.

551

552

553

554 **Material and Methods**

555

556

557 **Zebrafish strains and maintenance**

558 Zebrafish (*Danio rerio*) were maintained using standard procedures (Westerfield, 1993).
 559 RIKEN Wako (RW) was used as a wild-type strain for mutagenesis in which *banp*^{rw337}
 560 was generated. Mapping of the *rw337* mutation and analysis of retinal phenotypes of
 561 *banp*^{rw337} mutants were carried out in the genetic background of WIK and Okinawa
 562 wild-type (oki), respectively. Two *banp* mutant alleles, *banp*^{rw337} and *banp*^{sa12976} were
 563 used. *banp*^{sa12976} was obtained from the Zebrafish International Resource Center (ZIRC,
 564 Eugene, OR, USA). Zebrafish transgenic lines, *Tg[h2afv:GFP]^{kca6/kca66}* (Pauls et al.,
 565 2001), *Tg[EF1α:mCherry-zGem]^{oki011}* (Mochizuki et al., 2014) and *Tg[EF1α:mCherry-
 566 CAAX]^{oki049}* (Mochizuki et al., 2017) were used to visualize chromatin, S/G2/M phases
 567 and plasma membranes, respectively.

568

569 **Ethics statement**

570 All zebrafish experiments were carried out in accordance with the Okinawa Institute of
 571 Science and Technology Graduate School's Animal Care and Use Program, which is
 572 based on the National Research Council of the National Academy's Guide for the Care
 573 and Use of Laboratory Animals and has been accredited by the Association for
 574 Assessment and Accreditation of Laboratory Animal Care (AAALAC International).
 575 The OIST Institutional Animal Care and Use Committee approved all experimental
 576 protocols.

577

578 **Zebrafish mutagenesis, mapping and cloning of the *banp* mutant gene**

579 Mutagenesis, mapping, and cloning were performed as previously described (Masai et
 580 al., 2003). The polymorphic markers, 070702B and zC93F2D, shown below were
 581 designed and used for restricting the genomic region covering the *banp*^{rw337} mutation.
 582 070702B forward: 5'-ACTTCTTATCAGGGCTGTGC-3'
 583 070702B reverse: 5'-TCAGTCAAGAGCAGTGAGAG-3'
 584 zC93F2D forward: 5'-TGGGATCTCTTTAAGTGAGTGAG-3'
 585 zC93F2D reverse: 5'-TCCAACATATGTGGGTCAAACC-3'

586

587 **Genotyping of *banp*^{rw337} mutant embryos**

588 *banp*^{rw337} mutant phenotypes were difficult to distinguish until 2 dpf. Identification of
589 homozygous mutant embryos at earlier time points was performed using PCR
590 amplification of genomic regions using specific primers that enabled restriction
591 digestion of only mutated sequence using MboII. Primers used, forward: 5'-
592 CGATGTTGATATCCATCAGTCAGGCGATC-3'; reverse primer: 5'-
593 GGTGCTGGTGTATAAATCACATGACCTATGGTCCTCTT-3'.

594

595 **Acridine Orange staining**

596 Larvae were incubated at 28°C, in 5 mg/mL Acridine Orange (AO) in E3 embryonic
597 medium (5mM NaCl, 0.17mM KCl, 0.33mM CaCl₂, 0.33mM MgSO₄) for 30 min,
598 protected from light. Embryos were rinsed in E3 medium and viewed with an
599 epifluorescence microscope (SteREO Discovery, Zeiss) to identify live mutant embryos
600 with dead cell accumulation.

601

602 ***In situ* hybridization**

603 Whole-mount *in situ* hybridization of zebrafish embryos was performed as previously
604 described (Xu et al., 1994). To prepare RNA probes, full-length cDNAs of zebrafish
605 *banp* gene were amplified with PCR and sub-cloned into pBluescript II SK(+) vector
606 (Stratagene/Agilent Technologies). Plasmids were digested with an appropriate
607 restriction enzyme and used for DNA templates to make antisense or sense RNA probes.
608 Digoxigenin-labeled RNA probes were generated by *in vitro* transcription using a DIG
609 RNA Labeling Kit (Roche) and purified using Micro Bio-Spin® Chromatography
610 Columns (BioRad). *In situ* hybridization signals in whole-mount embryos were detected
611 using anti-DIG alkaline phosphatase (AP)-conjugated antibody (Roche) with an AP
612 substrate, BM-Purple (Roche) and imaged using SteREO Discovery.V12 (ZEISS)
613 microscope.

614

615 **Rescue experiment of *banp* mutants with overexpression of wild-type Banp**

616 The full-length coding region of the *banp* gene was amplified with PCR. To
617 overexpress wild-type Banp protein, a DNA construct encoding zebrafish wild-type

618 Banp with an EGFP tag at the N-terminus or C-terminus, EGFP-banp(wt) or banp(wt)-
619 EGFP, was prepared. A DNA construct encoding an N-terminal EGFP-tagged *rw337*
620 mutant form of Banp, EGFP-Banp(*rw337*), was prepared as a negative control. These
621 DNA constructs were subcloned into the pCS2 expression vector. Capped mRNA was
622 generated in a standard *in vitro* transcription reaction using an SP6 polymerase
623 (mMESSAGE mMACHINE™ SP6 Transcription Kit, Invitrogen). We performed
624 mRNA injections in one-cell-stage embryos obtained from pair-wise crosses of
625 *banp^{rw337}* heterozygous mutant male and female fish. At 2 dpf, embryos were used to
626 determine their genotype with PCR and to identify homozygous mutants. Plastic
627 sections were obtained to evaluate retinal phenotypes of *banp^{rw337}* mutant embryos
628 injected with mRNA encoding banp(wt)-EGFP, or EGFP-banp(*rw337*). We also
629 evaluated protein stability and nuclear localization of wild-type and *rw337* mutant form
630 of Banp by live confocal imaging of wild-type retinas expressing banp(wt)-EGFP,
631 EGFP-banp(wt), or EGFP-banp(*rw337*) at 24 hpf.

632

633 **Morpholino injection**

634 ATG morpholino antisense oligos for *banp* (banp MO) and its 5 mismatch *banp* MO
635 (banp 5misMO) were designed as 5'-CCACTAAATCTTGCTCTGACATCAT-3' and
636 5'-CCtCaAAATgTTcCTCTcACATCAT-3', respectively. Morpholino antisense oligos
637 for tp53 (tp53 MO) (5'-GCGCCATTGCTTTGCAAGAATTG-3') (Langheinrich et al.,
638 2002), standard MO (STD MO) (5'-CCTCTTACCTCAGTTACAATTTATA-3') were
639 used. To knock down $\Delta 113$ tp53, morpholino antisense oligos for $\Delta 113$ tp53 ($\Delta 113$ tp53
640 MO; also referred to as MO6-tp53 in zebrafish database ZFIN) (5'-
641 GCAAGTTTTTGCCAGCTGACAGAAG-3') was used (Chen et al., 2009). All MOs
642 were injected into one-cell-stage eggs at 1 mM.

643

644 **Histology**

645 Plastic sectioning, immunostaining of cryosections, paraffin sectioning, and BrdU
646 incorporation were performed as described previously (Imai et al., 2010), except that we
647 injected BrdU solution into the yolk of embryos at 48 hpf instead of soaking embryos in
648 10mM BrdU solution. Anti- pH3 antibody (Cell signalling technology, 6G3, 9706;
649 Sigma-Aldrich, 06-570), anti-Pax6 antibody (Covance; PRB-278P), zpr1 antibody

(ZIRC, Eugene, Oregon), anti-Prox1 antibody (GeneTex, GTX128354), anti-glutamine synthetase (GS) (Millipore, MAB302), anti-BrdU antibody (abcam, Ab6326), anti-PCNA (Sigma-Aldrich, P8825) were used at 1:500, 1:500, 1:100, 1:500, 1:100, 1:200, 1:200 dilutions, respectively. Nuclear staining was performed using SYTOX Green (Molecular Probes). Filamentous actin (F-actin) was stained using 0.1 mM rhodamine-conjugated phalloidin (Molecular Probes). Terminal deoxynucleotide transferase-mediated dUTP nick-end labeling (TUNEL) was performed using the In Situ Cell Death Detection Kit (Roche).

Whole-mount immunostaining with anti- α -tubulin antibody (Sigma-Aldrich; #T5168) to label the mitotic spindle was performed as follows. Homozygous mutant embryos were selected by AO staining, and fixed in 2% trichloroacetic acid (TCA) at room temperature (RT) for 3 h. Then embryos were washed three times in 1X PBST (PBS, 0.1 % tritonx100), and incubated in 0.2% trypsin for 4 min at 4°C to increase antibody permeabilization through the skin. Then, embryos were rinsed thrice with PBS. After embryos were re-fixed with 4% PFA for 5 min at 4°C, they were incubated in blocking solution (10% Goat serum in PBST) for 1 h at RT and transferred to 1% blocking solution with diluted anti- α -tubulin antibody at 1:1000 dilution for 2 days at 4°C. Embryos were washed with PBST four times for 15 min and then incubated with fluorescent conjugated anti-mouse antibody in 1% blocking solution in the dark for 2 days at 4°C. Next, all samples were washed in PBST three times for 15 min followed by glycerol storage at 4°C until imaging. Samples were imaged with a confocal microscope LSM 880 (Zeiss) followed by image analysis using IMARIS software (ver.9.1.2 Bitplane). Whole-mount immunostaining for anti-pH3, anti- γ -H2AX (GeneTex, GTX127342) (Sidi et al., 2008), anti-active caspase3 (BD Pharmingen, Clone C92-605), anti-HuC/D (Thermo Fisher, A-21271) (Sorrells et al., 2013) were performed as described previously (Imai et al., 2010). Images were scanned using an LSM710 confocal laser scanning microscope (Zeiss). Number of fluorescent-positive cells and percentage of fluorescence-positive area relative to the region of interest was calculated using original scanning images with IMARIS (Bitplane) and Zen (Zeiss) or Image-J (NIH) software, respectively.

RT-PCR

682 Total RNA was extracted from embryo heads at the indicated developmental stages
683 using a Sepasol RNA I Super (Nacalai, Japan). cDNA synthesis was carried out from
684 500ng of RNA using Toyobo ReverseTra Ace® qPCR RT master mix with gDNA
685 remover according to the manufacturer's instructions. Expression profiles of the genes
686 listed below were analyzed with the specified primers using Luna® Universal qPCR
687 Master Mix (NEB). Quantitative RT-PCR was performed using StepOnePlus (Applied
688 Biosystems). The relative expression was calculated using $\Delta\Delta C_t$ method with the
689 reference gene *ef1a*. Primers used in qRT-PCR analysis; *mdm2* (Wilkins et al., 2013),
690 *p21* (Stiff et al., 2016), *FL tp53* and $\Delta 113$ *tp53* (Chen et al., 2016), *ef1a* (McCurley and
691 Callard, 2008).

692

<i>Gene</i>	RT PCR-Forward primer	RT PCR-Reverse primer
<i>ccng1</i>	ccctggagattgaggatcag	cacacaaaccaggtctccaa
<i>mdm2</i>	caggaggaggagaagcagt	agggaaaagctgtccgactt
<i>p21(cdkn1a)</i>	aagcgcaaacagaccaacat	tcagctactggccggattt
<i>FL tp53</i>	tggagaggaggtcggcaaaatcaa	gactgcgggaacctgagcctaaat
$\Delta 113$ <i>tp53</i>	atatcctggcgaaacatttggagggg	cctcctgggtcttgaatgtcac
<i>Puma (bbc3)</i>	ctgaggaggacccacact	tctccagttctgccagtgc
<i>cenpt</i>	tcatgaggagattgtggaagatg	ggtgagctctgcgagtattt
<i>ncapg</i>	ctgatgtgaggagcctattt	gagtctgtttggcctccatta
<i>atm</i>	cctcaaggctgtggagaact	aggggattttctttacaccactc
<i>atr</i>	aggaacccaatctgccagt	gatgtccagtgccagctctc
<i>ef1a</i>	cttctcaggctgactgtgc	ccgctagcattaccctcc

693

694 **Western blotting**

695 Total 21 embryos were divided into 3 groups (each n=7) for 48 hpf wild-type and
696 *banp*^{rw337} mutants, respectively. Heads were dissected in ice-cold E3 medium. Seven
697 heads removed from each group were homogenized together in sample buffer (125 mM
698 NaCl, 50 mM Tris-HCl pH 7.4, 0.5 mM EDTA, 1% Triton X-100, 1X Protease
699 inhibitor) and used as one sample. Appropriate volumes of samples were diluted to
700 provide equal protein amounts and used for SDS-PAGE (BIO-RAD, Mini-PROTEAN

701 TGX Gels). Western blot analysis was performed to 3 samples for wild-type and
702 *banp*^{rw337} mutants, respectively, according to standard protocols using anti-tp53
703 antibody (GeneTex, GTX128135) at a 1:1000 dilution. β -actin protein level was
704 evaluated by western blot analysis of rehybridized membranes with anti- β -actin
705 antibody (Sigma, A5441) at a 1:5000 dilution, and used to normalize tp53 protein level
706 for each sample. For secondary antibodies, horseradish peroxidase-conjugated anti-
707 rabbit or anti-mouse IgG antibodies (Amersham, NA931 and NA934) were used.
708 Enzyme activity was detected using Immunostar[®] LD (Wako) and luminescence was
709 imaged and quantified using iBright 1500 (Thermo Fisher Scientific).

710

711 **Live imaging**

712 Banp-MO was injected into one-cell-stage eggs of *Tg[h2afv:GFP; EF1a: mCherry-
713 zGem]* transgenic line. Banp-5misMO was used as a control. Two dpf embryos were
714 anesthetized using 5% (v/v) Tricane (3-amino benzoic acid-ethylester) and mounted
715 laterally using 1% low-melting agarose on a depression slide to image retinas without
716 left or right preference. Time-lapse images were obtained every 1.1 min using a
717 confocal laser scanning microscope LSM710 (Zeiss) with 40X0.8/1.0W Plane
718 Apochromat lens. Data were evaluated using IMARIS software (ver.9.1.2 Bitplane) to
719 calculate the time taken to complete each cell division.

720

721 **RNA sequencing**

722 Embryo heads were homogenized using a Polytron 1200E homogenizer
723 (KINEMATICA) with TRIzol reagent (Invitrogen). RNA was isolated using a Direct-
724 zol RNA Miniprep Kit (ZYMO RESEARCH) from three replicates of *banp*^{rw337} mutant
725 and wild-type embryos at 48 hpf. Each sample contained a pool of 7 embryo heads.
726 RNA isolation was followed by library preparation for sequencing using an NEBNext[®]
727 Ultra[™] II Directional RNA Library Prep Kit for Illumina[®] according to the
728 manufacturer's instructions. 151-base paired-end sequencing was performed using a
729 NovaSeq6000 SP. The quality of sequenced reads was evaluated with FastQC (Andrews,
730 2010) and adaptor trimming and quality filtering were done with fastp (Chen et al.,
731 2018). Cleaned reads were then mapped to the zebrafish reference genome (GRCz11)

732 using hisat2.1.0 (Kim et al., 2019) and mapped reads were counted with FeatureCounts
733 from the Subread package (Liao et al., 2014) using annotation from Ensembl
734 (Danio_rerio.GRCz11.95.gtf). Differentially expressed gene (DEG) analysis was
735 performed using the EdgeR package (Robinson and Oshlack, 2010). Genes with false
736 discovery rates (FDR) < 0.01 were considered significant DEGs. The EnhancedVolcano
737 package (Blighe et al., 2018) was used to create a volcano plot with Log₂FoldChange
738 (wildtype – mutant) values and FDR calculated with EdgeR. Differences in alternative
739 splicing between mutant and wild type was tested using the DEXseq package (Anders et
740 al., 2012). Adjusted p-value < 0.01 was considered as significant.

741

742 **ATAC sequencing**

743 48-hpf embryos were used for ATAC sequencing. We used the Kaestner Lab ATAC seq
744 protocol (Ackermann and Kaestner, 2019) until library preparation, followed by
745 sequencing using a NovaSeq6000 SP. We generated reads for three independent
746 *banp*^{rw337} mutant samples, as well as three independent *banp*^{rw337} wild-type sibling
747 samples. Each sample contained a pool of 3 embryo heads. A quality check of reads was
748 performed using FastQC. Reads from each sample were adaptor trimmed and aligned
749 using fastp and Bowtie2 to the zebrafish reference genome (GRCz11) (Langmead and
750 Salzberg, 2012) respectively. For quality control, aligned reads were filtered for low-
751 quality reads, non-unique alignments, and PCR duplicates using samtools (Li et al.,
752 2009; Yan et al., 2020). Moreover, alignment files were peak-called using Genrich, with
753 removal of mitochondrial reads (Gaspar, 2019). Differences in differentially enriched
754 peaks in wild-type and mutant embryos were identified using the DiffBind R package
755 (Stark and Brown, 2021). The obtained peaks were annotated using the annotatePeaks
756 tool of HOMER software (Heinz et al., 2010). The findMotifsGenome tool of HOMER
757 (Hypergeometric Optimization of Motif EnRichment) was used to find enriched motifs
758 in differentially expressed peaks. To compare coverage tracks across genes of interest in
759 *banp*^{rw337} mutants and wild-type fish in RNA-seq and ATAC-seq data, distributions of
760 mapped reads in each replicate were averaged and plotted with SparK (Kurtenbach and
761 Harbour, 2019).

762

763 **Statistics**

764 Significance of differences between groups (wild type vs mutant) was tested using
 765 GraphPad Prism (GraphPad Software Version 9.1.2). The number of samples used per
 766 experiment is represented within figures or figure legends. Tests used to calculate
 767 statistical significance are mentioned in figure legends. All data are presented as means
 768 $\pm$ SDs. A p value of 0.05 was considered statistically significant. In the histogram, $p^* <$
 769 0.05, $p^{**} < 0.01$, $p^{***} < 0.001$, $p^{****} < 0.0001$, or ns (not significant) is indicated.
 770
 771

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781 **Competing interests**

782 The authors declare no competing or financial interests.

783

784 **Author contributions**

785 Conceptualization: SB, YT, IM; Methodology: SB, YT, IM; Software: SB, YT, IM;
786 Validation: SB, YT, IM; Formal analysis: SB, YT, IM; Investigation: SB, YT, IM;
787 Resources: SB, YT, IM; Data curation: SB, YT, IM; Writing - original draft: SB, IM;
788 Writing - review & editing: SB, IM; Visualization: SB, IM; Supervision: IM; Project
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794

795 **Data availability**

796 Raw RNA-seq and ATAC-seq datasets of *banp*^{rw337} mutant and wild-type siblings are
797 available at DDBJ Sequence Read Archive (DRA012572).

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1055

1056 **Figure Legends**

1057 **Figure 1. Zebrafish *banp*^{rw337} mutants show mitotic cell accumulation and cell** 1058 **death in the developing retina.**

1059 (A) Morphology of wild-type and *rw337* mutant embryos at 4 dpf. *rw337* mutant
1060 embryos show smaller eyes compared to wild type at 4 dpf (red arrow). The black
1061 arrow shows the cloudy tectum in *rw337* mutant embryos, representing cell death.
1062 Scale bars: 100 μ m.

1063 (B) Plastic sections of wild-type and *rw337* mutant retinas during development. Higher
1064 magnification images of green squares in the left columns are shown in the right
1065 columns. Retinal neuroepithelium is normal in *rw337* mutants until 2 dpf. However,
1066 many round cells reminiscent of mitotic cells are accumulated in the apical region
1067 of *rw337* mutant retinas at 2 dpf (open green arrowheads), suggesting mitotic arrest
1068 of retinal progenitor cells. In wild-type retinas, three nuclear layers and two
1069 plexiform layers are form at 2.5 dpf. However, only RGCs appear near the lens and
1070 the outer region was occupied by pyknotic nuclei, indicating dead cells in *rw337*
1071 mutants. At 3 dpf, INL is formed, but the outer region is still occupied by pyknotic
1072 nuclei in *rw337* mutants. At 4 dpf, pyknotic nuclei are reduced in *rw337* mutants;
1073 however, ONL and OPL are absent despite the small number of photoreceptor-like
1074 columnar cells in putative INL (PR, asterisks). At 3 and 4 dpf, the CMZ is
1075 maintained in *rw337* mutants (green double asterisks).

1076 (C) The *rw337* mutation is mapped in the genomic region flanked by two polymorphic
1077 markers, 070702B and zC93F2D, on chromosome 25. This genomic region contains
1078 four genes including *banp*.

1079 (D) *banp* cDNA consists of 13 exons. In *rw337* mutants, T at the donor splice site of the
1080 intron between exons 4 and 5 (intron4/5) is converted to G, leading to the insertion
1081 of 52 nucleotides (red region) from intron 4/5. A new stop codon appears within
1082 this insertion.

1083 (E) Wild-type Banp protein has a coiled-coil domain, a BEN domain and a DNA-
1084 binding domain. An NLS domain, an RS-rich domain, and MAR-binding domain
1085 are shown. A truncated protein missing BEN and DNA-binding domains is
1086 predicted in *rw337* mutants.

1087 (F) Retinal sections of wild-type, wild-type embryos injected with Banp-5misMO and
 1088 banp MO. *banp* morphant retinas have similar phenotypes to *banp^{rw337}* mutants,
 1089 with increased cell death and loss of the ONL. Yellow open arrowheads show
 1090 pyknotic nuclei.

1091 (G) Retinal section of wild-type retinas, *banp^{rw337}* mutant retinas, and *banp^{rw337}* mutant
 1092 retinas overexpressing Banp(wt)-EGFP and EGFP-Banp(rw337). Overexpression of
 1093 Banp(wt)-EGFP inhibits cell death and recovers retinal lamination in *banp^{rw337}*
 1094 mutants, whereas overexpression of EGFP-Banp(rw337) does not inhibit retinal
 1095 defects in *banp^{rw337}* mutants. Yellow open arrowheads indicate pyknotic cells in
 1096 *banp^{rw337}* mutant retinas and *banp^{rw337}* mutant retinas overexpressing EGFP-
 1097 Banp(rw337).

1098 Scale bars: 20 μ m for (B, F, G).

1099 RGC, retinal ganglion cell; AC, amacrine cell, BP, bipolar cell; HC, horizontal cell; PR,
 1100 photoreceptor; OPL, outer plexiform layer; IPL, inner plexiform layer; INL, inner
 1101 nuclear layer; CMZ, ciliary marginal zone.

1102 **Figure Supplement 1.** Retinal cell differentiation in *banp^{rw337}* mutants at 4 dpf.

1103 **Figure Supplement 2.** Amino acid conservation of Banp protein between zebrafish,
 1104 mice and humans.

1105 **Figure Supplement 3.** *banp^{sa12976}* homozygous mutants phenocopy *rw337* mutants.

1106 **Figure Supplement 4.** Confirmation of specificity of banp MO and nuclear localization
 1107 of zebrafish Banp protein.

1108 **Figure Supplement 5.** Spatio-temporal pattern of zebrafish *banp* mRNA expression.

1109

1110 **Figure 2. *banp* mutant retinas show mitotic arrest and apoptosis.**

1111 (A) TUNEL of wild-type (upper) and *banp^{rw337}* mutant (lower) retinas. In *banp^{rw337}*
 1112 mutants, apoptotic cells start to be observed in the neural retinas at 53 hpf, and
 1113 increase in the apical region of retinas (white arrowheads) and at the interface
 1114 between the CMZ and the central retina (yellow open arrowheads) at 77 and 101
 1115 hpf.

1116 (B) Labeling of wild-type (upper) and *banp^{rw337}* mutant (lower) retinas with anti-pH3
 1117 antibody. Mitotic cells are accumulated at the apical surface of *banp^{rw337}* mutant

retinas at 53 hpf (white open arrowheads) and restricted in the apical region of the CMZ (yellow open arrow) at 77 and 101 hpf.

(C) The number of TUNEL-positive cells per retina. Apoptosis is significantly increased in *banp*^{rw337} mutants after 53 hpf. Unpaired t-test, two-tailed, Mean ± SD. [n=3, p**< 0.01]

(D) The number of pH3-positive cells per retina. The number of mitotic cells is significantly increased in *banp*^{rw337} mutants at 53 hpf. The number is decreased, but still significantly higher in *banp*^{rw337} mutants than in wild-type siblings after 77 hpf. Unpaired t-test, two-tailed, Mean ± SD. [n=3, p**< 0.01]

(E) Schematic diagram of wild-type retinas from 48 to 77 hpf. Neuronal differentiation proceeds from the central to the peripheral retina. At 48 hpf, the proliferating area is gradually restricted in the CMZ; however, there are still retinal progenitor cells that proliferate in the central retina. Daughter cells start to exit from the cell cycle to produce differentiated neurons (light green color). At 77 hpf, most progenitor cells exit from the cell cycle, except at the CMZ. BrdU-labeled cells and pH3-positive cells are indicated in blue and green, respectively.

(F) Schematic diagram of *banp*^{rw337} mutant retinas from 48 to 77 hpf. At 48 hpf, mitotic cells are significantly accumulated at the apical surface of the neural retina and apoptosis starts in the intermediate region of the central retina. At 77 hpf, the apoptotic area is more restricted to the apical region of the retina and at the interface between the central retina and the CMZ. Mitotic cells accumulate in the apical region of the CMZ at 77 hpf. TUNEL-positive cells are indicated in magenta.

Scale bars: 20 μm for (A, B).

Source data 1. Data for Figure 2CD.

Figure Supplement 1. Apoptosis is induced in retinal progenitor cells and newly differentiating neurons in *banp*^{rw337} mutants.

Figure 3. tp53-mediated DNA damage response is activated in the absence of Banp.

(A) A volcano plot indicating gene differential expression of 48 hpf wild-type versus *banp*^{rw337} mutant embryos. Red dots show genes that were upregulated ≥2-fold in mutants, while blue dots represent genes downregulated ≥ 2-fold.

(B) Heat map of the top 15 differentially expressed genes.

- 1150 (C) Relative mRNA expression levels for *bbc3*, *ccng1*, *mdm2*, *cdkn1a* in wild type and
1151 *banp^{rw337}* mutants. Quantitative RT-PCR confirmed the up-regulation of p53
1152 pathway genes in *banp^{rw337}* mutants at 48 hpf. Unpaired t-test (two-tailed), Mean $\pm$
1153 SD. [n=3, $p^* < 0.05$, $p^{**} < 0.01$, $p^{***} < 0.001$, $p^{****} < 0.0001$].
- 1154 (D) Western blotting of wild-type and *banp^{rw337}* mutants with anti-tp53 antibody. Full-
1155 length tp53 protein is stabilized in *banp^{rw337}* mutants, compared to wild type. β -actin
1156 protein is an endogenous control and used for normalization of tp53 protein level.
- 1157 (E) Quantification of tp53 protein level relative to a control protein, β -actin, in wild-
1158 type and *banp^{rw337}* mutants. The tp53 protein level is significantly higher in
1159 *banp^{rw337}* mutants than in wild type at 48 hpf. Unpaired t-test (two-tailed), Mean $\pm$
1160 SD. [n=3, $p^* < 0.05$].
- 1161 (F) Relative mRNA expression levels for *atm* and *atr* in wild type and *banp^{rw337}*
1162 mutants. Both mRNAs are significantly up-regulated in *banp^{rw337}* mutants at 48 hpf.
1163 Unpaired t-test (two-tailed) [n=3, $p^* < 0.05$].
- 1164 (G) TUNEL of wild-type and *banp^{rw337}* mutant retinas injected with STD MO and tp53
1165 MO at 48 hpf and 72 hpf. Scale bars: 20 μ m.
- 1166 (H) Histogram of the number of TUNEL-positive cells per retinal section in wild-type
1167 and *banp^{rw337}* mutant retinas injected with STD MO and tp53 MO at 48 hpf and 72
1168 hpf. Two way ANOVA, Turkey's multiple comparisons test, Mean $\pm$ SD. [n=3 for
1169 STD-MO at 48 hpf and tp53-MO at 72 hpf; n=4 for tp53-MO at 48 hpf and STD-
1170 MO at 72 hpf, $p^{****} < 0.0001$].

1171 **Source data 1.** Data for Figure 3CEFh.

1172 **Source data 2.** Data for Figure 3D.

1173

1174 **Figure 4. Banp is required for the integrity of DNA replication and DNA damage**
1175 **repair.**

- 1176 (A) Quantitative RT-PCR-based validation of FL tp53 and Δ 113tp53 mRNA
1177 expression at 48 hpf. Δ 113tp53 mRNA expression is markedly higher in *banp^{rw337}*
1178 mutants than in wild-type siblings, whereas FL tp53 mRNA is not significantly
1179 different between mutants and wild-type siblings. Unpaired t-test (two-tailed),
1180 Mean $\pm$ SD. [n=3, $p^{**} < 0.01$, ns (not significant)].

1181 (B) ATAC-seq- and RNA-seq-based validation of chromatin accessibility (upper panel)
1182 and transcription level (bottom panel) of the genomic region covering both tp53
1183 and $\Delta 113\text{tp53}$ transcription units. Data of wild-type and *banp*^{rw337} mutants are
1184 indicated in blue and red color, respectively. Light blue indicates overlapping level.
1185 The red box in ATAC-seq shows open chromatin in *banp*^{rw337} mutants in intron 4,
1186 signifying differential transcription of $\Delta 113\text{tp53}$ using an internal promoter.

1187 (C) TUNEL (top), anti-pH3 antibody labeling (middle), and anti- γ -H2AX antibody
1188 labeling (bottom) of wild-type and *banp*^{rw337} mutant retinas injected with STD MO,
1189 tp53 MO, and $\Delta 113\text{tp53}$ MO. tp53 MO-mediated knockdown completely rescues
1190 apoptosis in *banp*^{rw337} mutant retinas at 48 hpf. However, $\Delta 113\text{tp53}$ MO-mediated
1191 knockdown mildly inhibits apoptosis in *banp*^{rw337} mutant retinas. Neither tp53 MO
1192 nor $\Delta 113\text{tp53}$ MO effectively rescued mitotic arrest and accumulation of γ -H2AX-
1193 positive cells (white arrowheads) in *banp*^{rw337} mutant retinas. Scale bars: 20 μm .

1194 (D–F) Histogram of the number of TUNEL-positive cells (D), pH3-positive cells (E),
1195 and γ -H2AX-positive cells (F) per retinal section in wild-type and *banp*^{rw337} mutants
1196 injected with STD-MO, tp53 MO, and $\Delta 113\text{tp53}$ MO. Two way ANOVA with Tukey's
1197 multiple comparisons test, Mean $\pm$ SD. [n= in graph, $p^* < 0.05$, $p^{**} < 0.01$, $p^{***} < 0.001$,
1198 $p^{****} < 0.0001$, ns (not significant)].

1199 (G) Possible role of Banp in tp53-mediated the DNA damage response pathway. DNA
1200 replication stress and subsequent DNA damage activate ATR and ATM, respectively,
1201 which subsequently inhibit Mdm2-mediated tp53 degradation. Stabilized tp53 mainly
1202 promotes transcription of the $\Delta 113\text{tp53}$ isoform, which activates cell-cycle arrest genes
1203 and promotes DNA damage repair. Chronic activation of tp53 activates apoptotic genes
1204 to induce apoptosis. Banp may normally suppress DNA replication stress and also
1205 promote DNA damage repair.

1206 **Source data 1.** Data for Figure 4ADEF.

1207 **Figure supplement 1.** DNA damage is induced in retinal progenitor cells in *banp*
1208 knockdown.

1209

1210 **Figure 5. Banp promotes transcription of chromosome segregation regulators,**
1211 ***cenpt* and *ncapg*.**

- 1212 (A) Quantitative RT-PCR of *ncapg* expression at 48 hpf. *ncapg* mRNA expression is
1213 down-regulated in *banp*^{rw337} mutants and *banp* morphants. Unpaired t-test (two-
1214 tailed), Mean $\pm$ SD. [n=3, p**< 0.01].
- 1215 (B) ATAC-seq and RNA-seq analysis of *ncapg* mRNA transcription. In *banp*^{rw337}
1216 mutants, chromatin access is reduced at the TSS of *ncapg* (ATAC-seq) and
1217 transcript expression is markedly decreased (RNA seq).
- 1218 (C) Quantitative RT-PCR of *cenpt* expression at 48 hpf. *cenpt* mRNA expression is
1219 down-regulated in *banp*^{rw337} mutants and *banp* morphants. Unpaired t-test (two-
1220 tailed), Mean $\pm$ SD. [n=3, p**< 0.01].
- 1221 (D) ATAC-seq and RNA-seq analysis on *cenpt* mRNA transcription. In *banp*^{rw337}
1222 mutants, chromatin access is reduced at TSS of *cenpt* (ATAC-seq) and transcript
1223 expression is markedly decreased (RNA seq).
- 1224 (E) The motif enriched in genomic regions in which chromatin accessibility is reduced
1225 in *banp*^{rw337} mutants. This sequence shows a high similarity to the Banp motif.
- 1226 (F) A schematic diagram representing the flow of RNA-sequencing combined with
1227 ATAC-sequencing to find candidates for zebrafish Banp direct target genes.
1228 HOMER was used to scan for Banp motifs in downregulated candidate genes, from
1229 RNA- and ATAC-sequencing in *banp*^{rw337} mutants. RNA sequencing yielded 31
1230 candidates, whereas ATAC sequencing yielded 72, with 26 target genes consistent
1231 in both. These 26 candidate genes are the most likely to be target genes of Banp.
- 1232 (G) STRING interactome analysis showing the interaction of Banp motif-containing
1233 genes downregulated in RNA sequencing. Black arrows specify genes related to
1234 cell-cycle regulation.
- 1235 (H) Banp target candidate genes related to cell-cycle regulation. Gene ontology (GO)
1236 analysis was applied to 31 Banp motif-containing genes downregulated in RNA
1237 sequencing. 26% of these genes are engaged in the cell-cycle regulation. Others
1238 participate in a variety of non-categorized pathways.
- 1239 (I) Banp motif upstream to the 5'-UTR of *ncapg*.
- 1240 (J) Banp motif upstream to the 5'-UTR of *cenpt*.
- 1241 **Source data 1.** Data for Figure 5AC.
- 1242 **Figure supplement 1.** Motifs enriched in the closed and open chromatin area in
1243 *banp*^{rw337} mutants.

1244

1245 **Figure 6. Banp is required for chromosomal segregation during mitosis.**

1246 (A) Time-lapse images of mitosis of retinal progenitor cells injected with banp 5misMO
 1247 (upper) and banp MO (lower) at 2 dpf using *Tg [h2afv: GFP; EF1α: mCherry-
 1248 zGem]* transgenic zebrafish. In retinas injected with banp 5misMO, mitosis proceeds
 1249 sequentially from prophase (t=5~10 min), metaphase (t=16 min), anaphase (t=21
 1250 min), and telophase (t=24 min). On the other hand, in *banp* morphant retinas, the
 1251 duration of mitosis from prophase to telophase was 67 min, significantly longer than
 1252 that of the control MO (24 min). Furthermore, a prophase-like chromosome
 1253 arrangement appeared abnormally (t=59) after several metaphase-like alignments
 1254 (t=36 and 46), followed by anaphase (t=64) and telophase. Scale bar: 5μm.

1255 (B) Violin plot of the duration of mitosis from prophase to telophase in retinas injected
 1256 with banp 5misMO and banp MO. Mitosis in *banp* morphants is significantly longer
 1257 than that of banp 5misMO injected embryos. Mann Whitney test, Mean ± SD. [n=3,
 1258 p****< 0.0001]

1259 (C) Confocal images of metaphase cells in zebrafish retinas at 48 hpf. The mitotic
 1260 spindle is labeled with anti-α-tubulin antibody, whereas metaphase chromosomes
 1261 are labeled with anti-pH3 antibody. The mitotic spindle is correctly attached to
 1262 metaphase chromosomes in wild-type retinal cells (left panel). On the other hand,
 1263 mitotic cells in *banp^{rw337}* mutants exhibit inefficient mitotic spindle attachment and
 1264 poorly orientated metaphase chromosomes (right panels). Dotted circles indicate the
 1265 outline of metaphase cells. Scale bar: 2μm.

1266 (D) Percentage of cells with normal and abnormal spindle attachment in wild-type and
 1267 *banp^{rw337}* mutant embryos. Two way ANOVA with Šídák's multiple comparisons
 1268 test, Mean ± SD. [n=3, p****< 0.0001].

1269 **Source data 1.** Data for Figure 6BD.

1270

1271 **Figure 7. A possible model of Banp functions in DNA damage repair and**
 1272 **chromosome segregation during mitosis.**

1273 Our study reveals two novel roles of Banp in cell-cycle regulation of retinal progenitor
 1274 cells. First, Banp suppresses DNA replication stress and promotes DNA damage repair.

1275 In the absence of Banp, a DNA replication stress sensor, ATR, is activated, leading to
 1276 accumulation of DNA double-strand breaks, which activate ATM and the tp53-
 1277 mediated DNA damage response pathway. tp53 activates the apoptotic pathway and
 1278 promotes transcription of $\Delta 113$ tp53, both of which promote cell-cycle arrest and DNA
 1279 repair. Second, Banp is required for chromosome segregation during mitosis by
 1280 promoting transcription of two mitotic regulators, *cenpt* and *ncapg*. Since a Banp motif
 1281 is found near the TSSs of these genes, it is likely that *cenpt* and *ncapg* are direct targets
 1282 of Banp.
 1283

Supplementary Figure Legends

1285

Figure 1-figure supplement 1. Retinal cell differentiation in *banp*^{rw337} mutants at 4 dpf.

(A, B) *ath5*:EGFP expression in wild-type (A) and *rw337* mutant (B) retinas at 4 dpf. *ath5*:EGFP is strongly expressed in RGCs and weakly expressed in amacrine cells and photoreceptor precursors. In *rw337* mutants, *ath5*:EGFP expression in RGCs, amacrine cells and photoreceptor precursors is detected.

(C, D) Wild-type (C) and *rw337* mutant (D) retinas labeled with anti-Pax6 antibody at 4 dpf. Pax6 is strongly expressed in amacrine cells and weakly expressed in RGCs. In *rw337* mutants, amacrine cells seem to be decreased in number.

(E, F) Wild-type (E) and *rw337* mutant (F) retinas labeled with anti-Prox1 antibody at 4 dpf. Prox1 is expressed in bipolar cells and horizontal cells. In *rw337* mutants, both bipolar-like round nuclei and horizontal-like flat nuclei are observed, but horizontal-like cells are very few.

(G, H) Wild-type (G) and *rw337* mutant (H) retinas labeled with *zpr1* antibody at 4 dpf. *zpr1* antibody labels double-cone-type photoreceptors in zebrafish. In *rw337* mutants, only a small cluster of *zpr1*-positive cells is observed in the ventro-nasal retina, suggesting that a majority of photoreceptors fail to differentiate in *rw337* mutants.

(I, J) Wild-type (I) and *rw337* mutant (J) retinas labeled with anti-GS antibody at 4 dpf. Anti-GS antibody labels Müller cells. In *rw337* mutants, Müller cells are decreased in number.

(K, L) Wild-type (K) and *rw337* mutant (L) retinas labeled with anti-PCNA antibody at 4 dpf. Anti-PCNA antibody labels retinal progenitor cells in the CMZ at 4 dpf. In *rw337* mutants, retinal progenitor cells are maintained in the CMZ.

(M, N) Phalloidin labeling of wild-type (M) and *rw337* mutant (N) retinas. Phalloidin visualizes retinal synaptic layers, the IPL and OPL. In *rw337* mutants, the IPL is detected, but not fully formed on the ventral side. Furthermore, there is no OPL, which is consistent with the lack of photoreceptors in *rw337* mutants. Scale bars: 20µm.

RGC, retinal ganglion cell; AC, amacrine cell; BP, bipolar cell; HC, horizontal cell; PR, photoreceptor; MG, Müller cell; RPC, retinal progenitor cell; OPL, outer plexiform layer; IPL, inner plexiform layer.

1316

1317 **Figure 1-figure supplement 2. Amino acid conservation of Banp protein between**
1318 **zebrafish, mice and humans.**

1319 Alignment of Banp protein of humans (upper, ENSP00000376902), mice (middle,
1320 ENSMUSP000000026) and zebrafish (bottom, ENSDARP000000023) using a multiple
1321 sequence alignment program, PRALINE (Simossis and Heringa, 2005). Amino acid
1322 conservation is indicated by scoring and color coding from the least conserved (0, blue)
1323 to the most conserved (10, red). The BEN domain is indicated.

1324

1325 **Figure 1-figure supplement 3. *banp*^{sa12976} homozygous mutants phenocopy *rw337***
1326 **mutants.**

1327 (A) Another *banp* mutant allele, *sa12976*, carries a nonsense mutation in exon 4.
1328 (B) Comparison of phenotypes between *banp* mutant alleles. *sa12976* homozygous
1329 *banp* mutant alleles and trans-heterozygous *rw337/sa12976* mutants show similar
1330 phenotypes to *rw337*: small eyes (left, red arrowheads) and retinal phenotypes:
1331 apoptosis and no ONL (right, open red arrowheads). Scale bars: 100 µm (left panel).
1332 Scale bars: 20 µm (right panel).

1333

1334 **Figure 1-figure supplement 4. Confirmation of specificity of *banp* MO and nuclear**
1335 **localization of zebrafish Banp protein.**

1336 (A) Experimental design to confirm the specificity of *banp* MO and *banp* 5misMO
1337 (control MO). Co-injection of MO and RNA encoding Banp(wt)-EGFP to one-cell-
1338 stage eggs enables us to examine EGFP expression in gastrula stage-embryos at 6
1339 hpf.
1340 (B) *banp* MO effectively inhibits translation of Banp(wt)-EGFP protein in zebrafish
1341 embryos at 6 hpf and the retina at 24 hpf. On the other hand, *banp* 5misMO did not
1342 inhibit the translation of Banp(wt)-EGFP protein, suggesting that *banp* MO
1343 specifically inhibits Banp protein translation. Yellow dotted line indicates
1344 blastoderm cells covering yolk, in which GFP fluorescence was detected only in
1345 5mismatch morphant embryos. Scale bars: 20µm.

1346 (C) Time-lapse confocal scanning of mitosis of *Tg[EF1α:mCherry-CAAX]* transgenic
1347 wild-type retinal cells expressing Banp(wt)-EGFP. EGFP fluorescence is indicated
1348 in green, whereas mCherry-CAAX fluorescence labels plasma membrane and is
1349 indicated in magenta. White arrowheads indicate time-lapse change of a retinal
1350 progenitor cell undergoing mitosis. Banp(wt)-EGFP is localized in the nucleus in
1351 the interphase, whereas it spreads into the whole cell region during mitosis. Scale
1352 bars: 4μm.

1353 (D) Histogram of intensity of Banp(wt)-EGFP during cell division shown in (A). Signals
1354 decline from mitosis to cytokinesis.

1355 (E) Expression of Banp(wt)-EGFP, EGFP-Banp(wt) and EGFP-Banp(rw337) in
1356 zebrafish retina at 24 hpf. Both N-terminal and C-terminal EGFP-tagged wild-type
1357 Banp show nuclear localization, indicating that nuclear localization is conserved in
1358 zebrafish Banp. However, EGFP-Banp(rw337) is unstable and not maintained,
1359 suggesting that EGFP-Banp(rw337) is degraded, probably by the nonsense mutation
1360 decay mechanism. Scale bar: 10μm.

1361 **Source data 1.** Data for Figure 1-figure supplement 4D.

1362

1363 **Figure 1-figure supplement 5. Spatio-temporal pattern of zebrafish *banp* mRNA**
1364 **expression.**

1365 (A) Whole-mount *in situ* hybridization of zebrafish *banp* mRNA probe. *banp* mRNA is
1366 expressed in the 4-cell stage, suggesting maternal expression. *banp* mRNA is
1367 ubiquitously expressed during development from the sphere stage until the 21-
1368 somite stage. mRNA expression is restricted in the brain including the retina at 30
1369 hpf. At 48 hpf, mRNA is prominently expressed in the brain, fins, and notochord.
1370 At 72 hpf and later, mRNA expression appears in neuromasts of the lateral lines
1371 (red open arrowheads). br, brain; re, retina; LL, neuromasts of the lateral line; n,
1372 notochord. Scale bars: 200 μm

1373 (B) Frontal sections of zebrafish head labeled with *banp* RNA probe at 24 hpf and 48
1374 hpf. *banp* mRNA is expressed in the entire neural retina at 24 hpf and in the CMZ
1375 at 48 hpf, suggesting the expression of *banp* mRNA in retinal progenitor cells. OT,
1376 optic tectum; CMZ, ciliary marginal zone. Scale bars: 50 μm

(C) Whole-mount *in situ* hybridization of *banp* mRNA expression in wild-type and *banp^{rw337}* mutant embryos at 72 hpf. *banp* mRNA expression is absent in *banp^{rw337}* mutants, suggesting non-sense mutation decay of mRNA. Scale bars: 200 μ m

Figure 2-figure supplement 1. Apoptosis is induced in retinal progenitor cells and newly differentiating neurons in *banp^{rw337}* mutants.

- (A) BrdU labeling of wild-type and *banp^{rw337}* mutant retinas at 48 hpf.
- (B) ath5:EGFP expression of wild-type and *banp^{rw337}* mutant retinas at 48 hpf.
- (C) Percentage of BrdU-positive area or ath5:EGFP-positive area relative to the total retinal area. There is no significant difference in BrdU-positive area between wild-type and *banp^{rw337}* mutant retinas. The fraction of ath5:EGFP-positive area is slightly, but significantly reduced in *banp^{rw337}* mutants compared with wild-type siblings. Unpaired t test (two tailed), Mean $\pm$ SD. [n=6, $p < 0.05$, ns (not significant)].
- (D) Labeling of wild-type and *banp^{rw337}* mutant retinas with anti-activated caspase 3 antibody at 48 hpf.
- (E) Labeling of wild-type and *banp^{rw337}* mutant retinas with anti-pH3 antibody at 48 hpf.
- (F) The number of caspase 3-positive or pH3-positive cells per section of retina. The number of caspase 3-positive cells and pH3-positive cells is significantly higher in *banp^{rw337}* mutants than in wild-type siblings. Unpaired t-test (two tailed), Mean $\pm$ SD. [n= 3, $p < 0.05$, $p^{**} < 0.01$].
- (G) The relative position of caspase 3-positive cells or pH3-positive cells along the apico-basal axis of the neural retina: Positional value from 0 to 1 corresponds to the range from apical to basal position, respectively. In wild-type retinas (upper panel), pH3-positive cells are located in the apical region of the neural retina (peak of positional value is 0.2), whereas only one caspase 3-positive cell is located in the intermediate position of the neural retina (peak of positional value is 0.4). On the other hand, in *banp^{rw337}* mutant retina, pH3-positive cells are located in the apical region of the neural retina (peak of positional value is 0.1), whereas caspase3-positive cells are increased in number and most of them are located in the intermediate zone (peak of positional value is 0.3). Although pH3- and caspase3-positive positional ranges are slightly overlapped in *banp^{rw337}* mutant retinas, these

- 1409 data suggest that apoptosis occurs in S phase of retinal progenitor cells or newly
1410 differentiating neurons migrating toward the basal region in *banp*^{rw337} mutants.
- 1411 (H) Double labeling of wild-type and *banp*^{rw337} mutant retinas with ath5:EGFP
1412 transgene and anti-caspase 3 antibody.
- 1413 (I) Percentage of ath5:EGFP-positive area relative to the total retinal area (left) and
1414 percentage of ath5:EGFP- and caspase 3-double positive cells relative to the total
1415 number of caspase 3-positive cells (right). Mann Whitney test, Mean $\pm$ SD. [n=7,
1416 p**< 0.01].
- 1417 (J) Double labeling of wild-type and *banp*^{rw337} mutant retinas with anti-HuC/D
1418 antibody and anti-caspase 3 antibody.
- 1419 (K) Percentage of HuC/D-positive area relative to the total retinal area (left) and
1420 percentage of HuC/D- and caspase 3-double positive cells relative to the total
1421 number of caspase 3-positive cells (right). Mann Whitney test, Mean $\pm$ SD. [n=7 for
1422 wild-type, n=6 for *banp*^{rw337} mutants, p* < 0.05].
- 1423 (L) A possible model on how apoptosis is induced during retinal progenitor cell
1424 proliferation and retinal neurogenesis in *banp*^{rw337} mutants.
- 1425 Scale bar: 20 μ m for (A, B, D, E, H, J)
- 1426 **Source data 1.** Data for Figure 2-figure supplement 1CFGIK
- 1427
- 1428 **Figure 4-figure supplement 1. DNA damage is induced in retinal progenitor cells in**
1429 ***banp* knockdown.**
- 1430 (A) Labeling of 48 hpf wild-type retinas injected with STD MO and *banp* MO with anti-
1431 γ -H2AX antibody. γ -H2AX-positive cells are increased in *banp* morphant retinas.
1432 Scale bars: 20 μ m.
- 1433 (B) The number of γ -H2AX-positive cells in 48 hpf wild-type retinas injected with STD
1434 MO and *banp* MO. The number of γ -H2AX-positive cells is significantly higher in
1435 *banp* morphant retinas than in wild-type retinas injected with STD MO. Mann
1436 Whitney test, Mean $\pm$ SD. [n=5, p**< 0.01].
- 1437 (C) Labeling of 48 hpf wild-type and *banp*^{rw337} mutant retinas with anti-BrdU antibody
1438 (red), anti- γ -H2AX antibody (blue), and ath5:EGFP transgene (green). Two
1439 independent *banp*^{rw337} mutant retinas are shown. Scale bars: 20 μ m.

- 1440 (D) The fraction of γ -H2AX-positive cells in the total retinal area in wild-type and
 1441 *banp*^{rw337} mutants. Mann Whitney test, Mean $\pm$ SD. [n=6, p < **0.01].
- 1442 (E) Double labeling of retinal CMZ in *banp*^{rw337} mutants with anti-BrdU antibody (red),
 1443 anti- γ -H2AX antibody (blue). Higher magnification images of square in *banp*^{rw337}
 1444 mutant retinas shown in (C).
- 1445 (F) The fraction of BrdU-positive cells in the total retinal cells and γ -H2AX-positive
 1446 cells in *banp*^{rw337} mutants. Mean of difference between the total retinal cells and γ -
 1447 H2AX-positive cells is shown in the right side. The fraction is significantly higher
 1448 in γ -H2AX-positive cells than in the total retinal cells. Paired t-test. [n=6, p
 1449 < ***0.001]. Correlation plot (F') indicates significantly effective pairing [p < *0.05].
- 1450 (G) Double labeling of retinal CMZ in *banp*^{rw337} mutants with ath5:EGFP transgene
 1451 (green), anti- γ -H2AX antibody (blue). Higher magnification images of square in
 1452 *banp*^{rw337} mutant retinas shown in (C).
- 1453 (H) The fraction of ath5:EGFP-positive cells in the total retinal cells and γ -H2AX-
 1454 positive cells in *banp*^{rw337} mutants. Mean of difference between the total retinal cells
 1455 and γ -H2AX-positive cells is shown in the right side. There is no significant
 1456 difference between the total retinal cells and γ -H2AX-positive cells. Paired t-test.
 1457 [n=6, ns]. Correlation plot (H') indicates no significant pairing. [ns].
- 1458 (I) Labeling of 48 hpf wild-type and *banp*^{rw337} mutant retinas with anti-pH3 antibody
 1459 (magenta), anti- γ -H2AX antibody (green). Top and bottom right-side panels of
 1460 *banp*^{rw337} mutant retinas indicate original fluorescent images and their Imaris
 1461 surface-rendered images, respectively. Scale bars: 20 μ m.
- 1462 (J) The fraction of pH3-positive cells in the total retinal cells and γ -H2AX-positive
 1463 cells in *banp*^{rw337} mutants. Mean of difference between the total retinal cells and γ -
 1464 H2AX-positive cells is shown in the right side. There is no significant difference
 1465 between the total retinal cells and γ -H2AX-positive cells. Paired t-test [n=10, ns].
 1466 Correlation plot (J') indicates no significant pairing. [ns].

1467 **Source data 1.** Data for Figure 4-figure supplement 1BDFHJ.

1469 **Figure 5-figure supplement 1. Motifs enriched in the closed and open chromatin**
 1470 **area in *banp*^{rw337} mutants.**

1471 (A) List of top6 motifs enriched in genomic regions of decreased (downregulated) and
 1472 increased (upregulated) accessibility in *banp*^{rw337} mutants. We found that the Banp
 1473 motif (TCTCGCGAGA) is the most enriched sequence in regions with reduced
 1474 chromatin accessibility in *banp*^{rw337} mutants (large arrowhead).
 1475 (B, C) A putative Banp motif with a conserved CGCG element upstream of the 5' UTR
 1476 of *ncapg* (B) and *cenpt* (C) in zebrafish, mice and humans.
 1477

1478 **Video File Legends**

1479 **Video 1. Time-lapse movie of mitosis of wild-type retinal progenitor cells.**

1480 *Tg [h2afv: GFP; EF1α: mCherry-zGem]* transgenic embryos were injected with banp
1481 5misMO and mitosis of retinal progenitor cells was scanned with confocal LSM.
1482 Mitosis proceeds sequentially from prophase (t=5~10 min), metaphase (t=16 min),
1483 anaphase (t=21 min) and telophase (t=24 min). Green indicates GFP fluorescence,
1484 representing chromatin. On the other hand, magenta indicates fluorescence from
1485 mCherry, representing S/G2/M phase cells.

1486

1487 **Video 2. Time-lapse movie of mitosis of *banp* morphant retinal progenitor cells.**

1488 *Tg [h2afv: GFP; EF1α: mCherry-zGem]* transgenic embryos were injected with banp
1489 MO and mitosis of retinal progenitor cells was scanned with confocal LSM. Mitosis did
1490 not proceed smoothly, and it took longer from chromosome condensation in prophase
1491 (t=15 min) to chromosome alignment at the division plane in anaphase (t=46 min).
1492 Aligned chromosomes were often dispersed during chromosome segregation by mitotic
1493 spindle (t=46~64 min). Green indicates fluorescence from GFP, representing chromatin.
1494 On the other hand, magenta indicates fluorescence from mCherry, representing S/G2/M
1495 phase cells.

1496

1497

1498 **Supplementary Information**

1499 **Supplementary Figures**

1500 **Figure 1-figure supplement 1.** Retinal cell differentiation in *banp*^{rw337} mutants at 4 dpf.

1501 **Figure 1-figure supplement 2.** Amino acid conservation of Banp protein between
1502 zebrafish, mice and humans.

1503 **Figure 1-figure supplement 3.** *banp*^{sa12976} homozygous mutants phenocopy *rw337*
1504 mutants.

1505 **Figure 1-figure supplement 4.** Confirmation of specificity of banp MO and nuclear
1506 localization of zebrafish Banp protein.

1507 **Figure 1-figure supplement 5.** Spatio-temporal pattern of zebrafish *banp* mRNA
1508 expression.

1509 **Figure 2-figure supplement 1.** Apoptosis is induced in retinal progenitor cells and
1510 newly differentiating neurons in *banp*^{rw337} mutants.

1511 **Figure 4-figure supplement 1.** DNA damage is induced in retinal progenitor cells in
1512 *banp* knockdown.

1513 **Figure 5-figure supplement 1.** Motifs enriched in the closed and open chromatin area
1514 in *banp*^{rw337} mutants.

1515

1516 **Supplementary Table file**

1517 **Supplementary Table 1.**

1518

1519 **Source data files**

1520 **Figure 2-Source data 1.** Data for Figure 2CD.

1521 **Figure 3-Source data 1.** Data for Figure 3CEFH.

1522 **Figure 3-Source data 2.** Data for Figure 3D.

1523 **Figure 4-Source data 1.** Data for Figure 4ADEF.

1524 **Figure 5-Source data 1.** Data for Figure 5AC.

1525 **Figure 6-Source data 1.** Data for Figure 6BD.

1526 **Figure 1-figure supplement 4-Source data 1.** Data for Figure 1-figure supplement 4D.

1527 **Figure 2-figure supplement 1-Source data 1.** Data for Figure 2-figure supplement

1528 1CFGIK

1529 **Figure 4-figure supplement 1-Source data 1.** Data for Figure 4-figure supplement
1530 1BDFHJ.

1531

1532 **Video files**

1533 **Video 1.** Time-lapse movie of mitosis of wild-type retinal progenitor cells.

1534 **Video 2.** Time-lapse movie of mitosis of *banp* morphant retinal progenitor cells.

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Appendix

Key Resources Table				
Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
strain, strain background (Danio Rerio)	<i>banp</i> ^{rw337}	This paper		
strain, strain background (Danio Rerio)	<i>banp</i> ^{sa12976}	ZIRC	ZFIN: ZDB-ALT-130411-2647	
strain, strain background (Danio Rerio)	<i>Tg[EF1α:mCherry-zGem]</i> ^{oki011}	PMID: 25260917	ZFIN: ZDB-ALT-150128-2	
strain, strain background (Danio Rerio)	<i>Tg[EF1α:mCherry-CAAX]</i> ^{oki049}	PMID: 28196805	ZFIN: ZDB-TGCONSTRCT-181026-1	
strain, strain background (Danio Rerio)	<i>Tg[h2afv:GFP]</i> ^{kca6/kc a66}	PMID: 11819118	ZFIN: ZDB-ALT-020918-4, ZDB-ALT-020717-2	
strain, strain background (Danio)	<i>Tg[ath5:EGFP]</i> ^{rw021}	PMID: 15728672	ZFIN: ZDB-ALT-050627-2	
antibody	pH3 (Mouse ,monoclonal)	Cell signaling technology	6G3, 9706	1:500 (IHC)
antibody	pH3 (Rabbit, polyclonal)	Sigma-Aldrich (Merk)	06-570	1:500 (IHC)

antibody	Caspase3 (Rabbit, monoclonal)	BD Pharmingen	Clone C92-605	1:200 (IHC)
antibody	Pax6 (Rabbit, polyclonal)	Covance	PRB-278P	1:500 (IHC)
antibody	zpr1 (Mouse, monoclonal)	ZIRC, Eugene, Oregon	ZFIN: ZDB-ATB-081002-43	1:100 (IHC)
antibody	glutamine synthetase (GS) (Mouse, monoclonal)	Millipore	MAB302, clone GS-6	1:100 (IHC)
antibody	PCNA (Mouse, monoclonal)	Sigma-Aldrich	Clone PC10,P8825	1:200 (IHC)
antibody	Prox1 (Rabbit, polyclonal)	Gene Tex	GTX128354	1:500 (IHC)
antibody	Alpha tubulin (Mouse, monoclonal)	Sigma-Aldrich	T5168, clone B512	1:1000 (IHC)
antibody	γ -H2AX (Rabbit, polyclonal)	Gene Tex	GTX127342	1:500 (IHC)
antibody	BrdU (Rat, monoclonal)	abcam	Ab6326	1:200 (IHC)
antibody	HuC/D (Mouse, monoclonal)	Thermo Fisher	A-21271	1:200 (IHC)

antibody	β -actin (Mouse, monoclonal)	Sigma-Aldrich	A5441	1:5000 (western blot)
antibody	tp53 (Rabbit, polyclonal)	Gene Tex	GTX128135	1:1000 (western blot)
antibody	Secondary antibodies, horseradish peroxidase-conjugated (Mouse)	Amersham	NA931	1:5000 (western blot)
antibody	secondary antibodies, horseradish peroxidase-conjugated (Rabbit)	Amersham	NA934	1:5000 (western blot)
recombinant DNA reagent	pBluescript II SK(+) (vector)	Stratagene/Agilent Technologies		<i>in vitro</i> transcription (In situ hybridization probe synthesis)
recombinant DNA reagent	pCS2 (expression vector)	PMID: 7926732		<i>in vitro</i> transcription (Capped mRNA synthesis)
sequence-based reagent (mutation mapping)	Polymorphic marker to link with banp mutation locus	This paper	PCR primers	70702B forward: 5'- ACTTCTTATCAGGGC TGTGC-3' 070702B reverse: 5'- TCAGTCAAGAGCAG TGAGAG-3'
sequence-based reagent (mutation mapping)	Polymorphic markers to link banp mutation locus	This paper	PCR primers	zC93F2D forward: 5'- TGGGATCTCTTTAA GTGAGTGAG-3' zC93F2D reverse:

				5'- TCCAACATGTGGG TCAAACC-3'
sequence- based reagent (Morpholino)	banp MO	This paper	Morpholi no antisense oligos	5'- CCACTAAATCTT GCTCTGACATCA T-3'
sequence- based reagent (Morpholino)	banp 5misMO	This paper	Morpholi no antisense oligos	5'- CCtCaAAATgTTc CTCTcACATCAT- 3'
sequence- based reagent (Morpholino)	tp53 MO	PMID: 12477391	Morpholi no antisense oligos	5'- GCGCCATTGCTT TGCAAGAATTG- 3'
sequence- based reagent (Morpholino)	Δ113 tp53 MO	PMID: 19204115	Morpholi no antisense oligos	5'- GCAAGTTTTTGC CAGCTGACAGAA G-3'
sequence- based reagent (Morpholino)	STD MO	PMID: 30322969	Morpholi no antisense oligos	5'- CCTCTTACCTCAG TTACAATTATA- 3'
sequence- based reagent (primer)	ccng1	This paper	Primers for qRT- PCR	Forward primer: ccctggagattgaggatc ag Reverse primer: cacacaaaccaggtctc caa
sequence- based reagent (primer)	mdm2	PMID: 24147052	Primers for qRT- PCR	Forward primer: caggaggaggagaagca gtg Reverse primer: agggaaaagctgtcc gactt

sequence-based reagent (primer)	p21(cdkn1a)	PMID: 26908596	Primers for qRT-PCR	Forward primer: aagcgcaaacagaccaa cat Reverse primer: tcagctactggccgga t
sequence-based reagent (primer)	FL tp53	PMID: 27539857	Primers for qRT-PCR	Forward primer: tggagaggagggtcggcaa aatcaa Reverse primer: gactgcgggaacctgag cctaaat
sequence-based reagent (primer)	Δ 113 tp53	PMID: 27539857	Primers for qRT-PCR	Forward primer: atatcctggcgaaacattg gaggg Reverse primer: cctcctggtcttgaatg tcac
sequence-based reagent (primer)	Puma (bbc3)	This paper	Primers for qRT-PCR	Forward primer: ctgaggaggacccacac t Reverse primer: tctccagttctgccagtg c
sequence-based reagent (primer)	cenpt	This paper	Primers for qRT-PCR	Forward primer: tcatgaggagattgtgga agatg Reverse primer: ggtgagctctgcgagtt att
sequence-based reagent (primer)	ncapg	This paper	Primers for qRT-PCR	Forward primer: ctgatgtgaggagacctat tt Reverse primer: gagtctgtttggcctcca tta

sequence-based reagent (primer)	atm	This paper	Primers for qRT-PCR	Forward primer: cctcaaggctgtggagaa ct Reverse primer: aggggattttctttacac cactc
sequence-based reagent (primer)	atr	This paper	Primers for qRT-PCR	Forward primer: aggaacccaatctgccag t Reverse primer: gatgtccagtgccagct ctc
sequence-based reagent (primer)	ef1 α	PMID: 19014500	Primers for qRT-PCR	Forward primer: cttctcaggctgactgtgc Reverse primer: ccgctagcattaccctcc
sequence-based reagent (sequencing primer)	Genotyping primers for <i>banp</i> ^{sa12976}	This paper	Primers	Forward primer: TGTTGATATCCATCA GTCAG Reverse primer: GGTGTATAAATCAC ATGACC
sequence-based reagent (primer)	Genotyping primers for <i>banp</i> ^{rw337}	This paper	Primers	forward: 5'- CGATGTTGATATCC ATCAGTCAGGCGAT C-3'; reverse primer: 5'- GGTGCTGGTGATA AATCACATGACCTAT GGTCCTCTT-3'.
commercial assay or kit	DIG RNA Labelling Kit	Roche	11175025 910	In situ hybridization

commercial assay or kit	mMESSAGE mACHINE™ SP6 Transcription Kit	Invitrogen	AM1340	Capped mRNA synthesis
commercial assay or kit	In Situ Cell Death Detection Kit, TMR red	Roche	12156792910	apoptosis
commercial assay or kit	ReverTra Ace® □ aPCR master mix with gDNA remover	Toyobo	FSQ-301	cDNA synthesis
commercial assay or kit	Luna® Universal qPCR Master Mix	NEB	M3003L	qRT-PCR
commercial assay or kit	Direct-zol RNA Miniprep Kit	ZYMO RESEARCH	R2050	RNA-sequencing
commercial assay or kit	NEBNext® Ultra™ II Directional RNA Library Prep Kit	NEB	E7760	Illumina RNA-sequencing
chemical compound, drug	Acridine orange	WALDECK (CHROMA)	1B-307	Live cell death detection
software	IMARIS	Bitplane	ver.9.1.2	http://www.bitplane.com/imaris ; RRID: SCR_007370
software	Image J	NIH	Version 2.1.0/1.53c	Percentage area calculation
software	Zen	Zeiss	LSM 710	Percentage area calculation
software	GraphPad Prism	GraphPad Software	Version 9.1.2	https://www.graphpad.com/scientific-software/prism/
other	SYTOX Green	Molecular Probes	S34862	Nuclear stain 1:1000 (IHC)
other	rhodamine-conjugated	Molecular Probes	R415	Filamentous actin (F-actin) stain

	phalloidin			1: 40 (IHC)
other	Restore™ PLUS Western Blot Stripping Buffer	Thermo Scientific	46430	Remove high- affinity antibodies from membranes (western blot)

Figure 1

Figure 3

Figure 5

A **B** **C** **D** **Figure 6**

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Figure 7