The Nup107–160 multiprotein subcomplex is essential for the assembly of nuclear pore complexes. The developmental functions of individual constituents of this subcomplex in vertebrates remain elusive. In particular, it is unknown whether Nup107 plays an important role in development of vertebrate embryos. Zebrafish nup107 is maternally expressed and its zygotic expression becomes prominent in the head region and the intestine from 24 h postfertilization (hpf) onward. In this study, we generate a zebrafish mutant line, nup107tsu068Gt, i n which the nup107 locus is disrupted by an insertion of Tol2 transposon element in the first intron and as a result it fails to produce normal transcripts. Homozygous nup107mutant embryos exhibit tissue-specific defects after 3 days postfertilization (dpf), including loss of the pharyngeal skeletons, degeneration of the intestine, absence of the swim bladder, and smaller eyes. These mutants die at 5–6 days. Extensive apoptosis occurs in the affected tissues, which is partially dependent on p53 apoptotic pathways. In cells of the defective tissues, FG-repeat nucleoporins are disturbed and nuclear pore number is reduced, leading to impaired translocation of mRNAs from the nucleus to the cytoplasm. Our findings shed new light on developmental function of Nup107 in vertebrates.

Nuclear pores are large multiprotein structures, also called nuclear pore complexes (NPCs), across the nuclear envelope of eukaryotic cells. NPCs function to control the exchange of various molecules including mRNAs and proteins between the cytoplasm and the nucleoplasm during interphase, but some of their components play roles in mitosis. Recent studies have indicated that NPCs are important for regulating signaling pathways in development and homeostasis, and their dysfunction can lead to abnormal development of tissues and organs (1, 2) as well as human diseases (3–6).

Nucleoporins (Nups), a class of NPC proteins including more than 30 members, are the main components of the NPCs and some Nups form subcomplexes of the NPCs (7). The nup107–160 complex, consisting of Nup160, Nup133, Nup107, Nup96/Nup98, Nup85, Nup43, Nup37, Sec13, Seh1, and ELYS, are the largest and essential building block of the NPC in metazoa (8, 9). Recently, efforts have been made to understand developmental roles of components of the nup107–160 complexes in metazoan species (10). It has been demonstrated that depletion of Nup98 in mice leads to retarded embryonic development during gastrulation but Nup98-deficient cells have a normal number of NPCs with inefficient integration of some cytoplasmically oriented Nups (11). Similarly, depletion of mouse Nup96, an isoform of Nup98, also causes embryonic lethality (12). The absence of Nup133 in mouse embryos results in lethality at midgestation with an impediment of neural differentiation, but does not impair NPC formation (13). Interestingly, fly seh1 homozygous mutants are viable but mutant females have reduced fecundity (18). These reports suggest that some constituents of the nup107–160 complex are essential for embryonic development while others have tissue-specific functions.

The first genetic mutant line of metazoan nup107 has been reported in Caenorhabditis elegans (19). The mutant embryos, when maternal nup107 is depleted, display temperature-dependent lethality without failure of incorporation of most Nups...
into the NPCs. It remains unknown whether and how Nup107 plays roles in development of vertebrate embryos. In this study, we generated a zebrafish nup107 mutant line by Tol2 transposon-based gene trap approach as described before (20–22). Homozygous mutants show missing of the pharyngeal cartilages, degeneration of the intestine, disappearance of the swim bladder, and smaller eyes after 3 dpf. These defective tissues undergo widespread apoptosis. Cells in the defective tissues have a reduced number of the nuclear pores with abnormal distribution of FG repeat Nups, which leads to impaired nuclear export of mRNAs. Thus, our findings present the first example of tissue-specific function of nup107 in vertebrate embryos.

EXPERIMENTAL PROCEDURES

Zebrafish Strains and Transgenesis—The AB strain was used for transgenesis. For transposon-mediated insertional mutagenesis, the transposon vector TSG was constructed as previously described (22). Co-injection of TSG DNA and transposase mRNA, screening of transgenic lines and identification of trapped mutants were performed as previously described (20, 21). tp53M214K mutagenesis was originally described by Berghmans et al. (23). Tg(hsp70:grhl2b-EGFP) was described previously (22).

Genotyping, RT-PCR, and Real-time PCR—Genotyping, RT-PCR, and Real-time PCR were done as previously described (21). Sequence information of primers used in this study will be provided upon request.

Morpholino (MO) Microinjection—Nup107 MO (5’TCTGACTCCATTCCATATTGTC-3’) was designed to target the translational start region of zebrafish nup107. Morpholino was injected into the one-cell stage embryos at the indicated doses.

Cryosectioning—Embryos at desired stages were fixed by 4% paraformaldehyde at 4 °C overnight and incubated in 30% sucrose dissolving in PBS until embryos sunk to the bottom. Then, a single embryo was embedded using JUNG tissue-freezing medium (Leica) and snap-frozen in liquid nitrogen. Sagittal sections of embryos were cut in 8–20 μm thickness by Leica CM1900. Sections were transferred onto superfrost plus slides and stored at -80 °C for subsequent use.

In Situ Hybridization and Immunostaining—Whole-mount in situ hybridization and whole-mount immunostaining were performed as previously described with modifications (21). For detection of nup107 expression, a sequence, consisting of a part of coding region at 3’-end and 3’-untranslated region (UTR), was subcloned and used for making antisense RNA probe. To repair antigens, the fixed embryos were bathed in 1 mM EDTA (pH 8.0) at 94–100 °C for 15 min in a microwave, cooled down to room temperature, followed by blocking and antibody incubation. When cryosections were used, frozen slides stored in -80 °C were thawed for 30 min at room temperature, followed by washing with PBS three times in a coplin jar, each for 5 min. The slides were then incubated in blocking solution (10% fetal calf serum, 1% dimethyl sulfoxide in PBS, 0.3% Triton X-100) for 1 h at room temperature and covered with parafilm in a humidification chamber. Primary antibody was added and incubated at 4 °C overnight, followed by washing with PBST three times for 15 min each. Next, the slides were incubated in the presence of Rhodamine-conjugated secondary antibody (Jackson ImmunoResearch) at 1:200 for 1 h at room temperature and then washed three times for 15 min each in PBST. Finally, DAPI was added (1 μg/ml) and incubated for 5 min at room temperature. Following wash three times with PBST, the slides were mounted and observed under a laser scanning confocal microscope (Zeiss LSM710). Mouse monoclonal anti-tubulin antibody was purchased from Sigma (T6199) and used at a dilution of 1:500; rabbit anti-caspase 3 active form antibody from BD Biosciences (559565), 1:500 dilution; monoclonal antibody mAb414 from Covance (MMS-120P), 1:5000 dilution; rabbit anti-phosphohistone H3 from Cell Signaling (9701), 1:100 dilution; Zn5 from ZFIN (111605), 1:50 dilution.

TUNEL Assay and BrdU Incorporation—TUNEL assay was performed to detect apoptotic cells using ApopTag® Red In Situ Apoptosis Detection Kit (Millipore, S7165). BrdU incorporation assay was performed as previously described (24).

Flow Cytometry—Mutants and their wild-type embryos at 48 h and 60 h, 150 embryos for each group, were anesthetized, and their heads were then dissected at the position anterior to the yolk. Single-cell suspension was achieved by incubating embryos in 1 ml of 0.25% trypsin (Jingke, China) at 28 °C for 1 h and triturated repetitively using a 1-ml tip. Then, 1 ml DMEM with 10% fetal bovine serum was added to stop the activity of trypsin. The cells were collected by centrifugation and resuspended with 500 μl of PBS. The homogenates were passed through a 5-ml polystyrene round-bottom tube with a cell strainer cap (12 × 75 mm, BD, #352235) and the outflow was collected. Following fixation with an equal volume of cold ethanol at 4 °C for at least 20 min, the cells were precipitated and resuspended with 500 μl of propidium iodide solution (0.1 mg/ml propidium iodide, 0.1% sodium citrate, 100 μg/ml RNase A, 0.0002% Triton X-100). The suspension was incubated at 4 °C in the dark for at least 20 min and analyzed using the BD FACS Aria II flow cytometer.

Transmission Electron Microscopy—Embryos were fixed with 2.5% glutaraldehyde (pH 7.2), osmicated in 1% osmium tetroxide (OsO4), and dehydrated with graded ethanol and acetone. Next, the embryos were embedded in the Spur resin and polymerized. The regions containing pharyngeal arches and intestine were cut sagittally. Sections were stained with lead citrate and imaged under a HITACHI H-7650 electron microscope.

mRNA Accumulation Assay on Cryosections—Poly[A]+ RNA accumulation assay on cryosections of embryos was performed according to a previously described protocol for cell culture with modifications (25). Briefly, embryos at 60 h were cryosectioned in 10 μm thickness in the sagittal plane as mentioned above, and the slides were stored at -80 °C. When used, the slides were washed with 1× cold PBS and fixed with 4% paraformaldehyde for 20 min on ice. Following permeabilization with 0.5% Triton X-100 in PBS for 10 min on ice, the slides were incubated in 2× SSC for 5 min, and prehybridized in 50% formamide, 10% dextran sulfate, 2× SSC, 10 mg/ml tRNA, 1 μM ribonucleoside vanadyl complexes (VRC) (GIBCO BRL), 1% BSA at 37 °C for 1 h. Next, hybridization with Cy3-oligo[dT]90 (Invitrogen) at 1000 pg/μl was carried out in the same hybridization buffer at 37 °C for 16 h in a humidification chamber.
covered with parafilm, followed by washing with 2× SSC at 37 °C three times, each 5 min. The slides were stained with DAPI at room temperature for 5 min and washed with PBS three times, 10 min each. The slides were mounted and imaged under a laser scanning confocal microscope (Zeiss LSM710).

**RESULTS**

**Nup107 Expression Is Ubiquitous at Early Stages and Becomes Restricted to Specific Tissues at Later Stages**—We obtained a transgenic zebrafish line, nup107tsu068Gt, using the Tol2-transposon gene trap vector TSG (22). We first observed, by fluorescence microscopy, the spatiotemporal expression pattern of GFP in the transgenic embryos at indicated stages. Except one-cell stage embryo with an unidentified genotype (A), the others were heterozygotes. I, the inserting position of the transposon element in the nup107 locus. Arrowheads were positions of primers used for genotyping and RT-PCR analyses. J, genotyping of individual embryos with different GFP levels. The primers f1, r1, and r2 were mixed for use. M, molecular weight markers. K–O, nup107 expression pattern in wild-type embryos using a nup107 antisense probe.

![FIGURE 1. EGFP expression pattern and genotyping of nup107tsu068Gt transgenic embryos. A–H, fluorescent images of EGFP expression in the transgenic embryos at indicated stages. Except one-cell stage embryo with an unidentified genotype (A), the others were heterozygotes. I, the inserting position of the transposon element in the nup107 locus. Arrowheads were positions of primers used for genotyping and RT-PCR analyses. J, genotyping of individual embryos with different GFP levels. The primers f1, r1, and r2 were mixed for use. M, molecular weight markers. K–O, nup107 expression pattern in wild-type embryos using a nup107 antisense probe.](image)
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1) Whole-mount in situ hybridization using antisense probe for nup107 mRNA in wild-type embryos revealed spatiotemporal expression patterns similar to GFP patterns in nup107\(^{+/-}\) transgenic embryos (Fig. 1, K–O). These results support the idea that the transposon is inserted into the nup107 locus in the nup107\(^{+/-}\) embryos and GFP expression is controlled by the nup107 promoter.

nup107 Expression Is Interrupted in nup107\(^{+/-}\) Transgenic Embryos—We expected that the transposon insertion in nup107\(^{+/-}\) transgenic embryos would interrupt production of normal nup107 transcripts due to the utilization of the splicing acceptor within the Tol2 transposon vector, producing a fused transcript (Fig. 2A). RT-PCR analysis revealed that the amount of wild-type nup107 transcripts was not eliminated, but markedly reduced in strong GFP embryos (with homozygous transposon insertion) at 24 hpf compared with that in their siblings with weak GFP (without transposon insertion) (Fig. 2A). In contrast, the expression level of nup107 exon1-GFP fusion transcript was high in strong GFP embryos at 24 hpf, but it was very low in weak GFP embryos. Similar changes were also observed at 36 hpf and 48 hpf. The wild-type transcripts in strong GFP embryos and the fusion transcripts in weak GFP embryos should be derived from their heterozygous mother.

To further confirm the above results, we performed in situ hybridization to examine the levels of nup107 wild-type transcripts in 24-hpf and 36-hpf embryos, which were derived from crosses between heterozygous nup107\(^{+/-}\) females and males, using the nup107 antisense probe that would not bind to the nup107 exon1-GFP fusion transcript. As shown in Fig. 2, B and C, nup107 wild-type transcripts were undetectable in strong GFP embryos and reduced in moderate GFP embryos. Therefore, we conclude that the transposon insertion in nup107\(^{+/-}\) embryos results in the expression of nup107 exon1-GFP fusion transcript at the expense of nup107 normal transcript.

nup107\(^{+/-}\) Homozygous Embryos Exhibit Abnormal Pharyngeal Arches, Eyes, and Intestine—nup107\(^{+/-}\) homozygous embryos, which had strong GFP fluorescence, looked morphologically normal until 3 days postfertilization (dpf), after which they showed obvious morphological defects including a thin pharyngeal region, smaller intestine without folds, smaller eyes and disappearance of the swim bladder (Fig. 2D). These embryos died at 5–6 dpf. Whole-mount skeleton staining with Alcian blue indicated that all of seven pharyngeal skeletons were missing in 4-dpf mutants but the neurocranium appeared unaffected (Fig. 2E). The expression of fabp2/fiabp gene, which labels enterocytes (28), was markedly reduced in mutants at 3 dpf (Fig. 2F) and further shrunk at 4 dpf (data not shown), suggestive of intestine degeneration. These results substantiate the defects in pharyngeal arches and intestine of mutants.

To further verify the accountability of Nup107 deficiency for the mutant phenotype, we knocked down nup107 expression using an antisense morpholin, nup107-MO, which efficiently blocked nup107–5’-UTR-EGFP reporter expression in wild-type embryos and zygotic GFP expression in nup107\(^{+/-}\) transgenic embryos (supplemental Fig. S1). Wild-type embryos injected with 5 ng nup107-MO displayed anomalies in eyes, pharyngeal arches and intestine (Fig. 2, G–I), which were similar to nup107\(^{+/-}\) mutants, supporting the notion that the phenotype in nup107\(^{+/-}\) mutants is caused by insufficient amount of Nup107.

Chondrogenic Differentiation Is Affected in nup107 Mutants—The formation of pharyngeal skeletons involves multiple steps, including specification of cranial neural crest cells (CNCCs), migration of CNCCs into the pharyngeal pouches, prechondrogenic condensation formation, and chondrocytes differentiation (29, 30). We investigated the steps at which nup107 deficiency exerted an effect by examining the expression of corresponding markers. All embryos produced by nup107\(^{+/-}\) heterozygote intercrosses, which had a genotype nup107\(^{+/-}\), nup107\(^{+/-}\), or nup107\(^{+/-}\), showed indistinguishable expression patterns of the early crest markers sox9a, sox10, and foxd3 at the 5-somites stage (data not shown), suggesting that CNCCs in mutants were correctly specified. The expression pattern of the postmigratory neural crest marker dlx2a (31) was unperturbed in mutants at 24 hpf and 36 hpf (Fig. 3A), which is indicative of normal migration of CNCCs. sox9a is expressed in CNCC mesenchymal and prechondrogenic cartilage condensations in the pharyngeal arches (32, 33). We found that sox9a expression was normal in nup107 mutants at and before 60 hpf but only retained in ventral parts of the first and second arches at 72 hpf (Fig. 3B). col2a1a expression marks prechondrogenic and chondrogenic cells in the pharyngeal arches (34), and its expression was reduced in the anterior two arches and absent in the posterior arches of mutants (Fig. 3C). Sagittal sections of 4-dpf mutants staining with hematoxylin and eosin showed few chondrocytes in the mutant arches while wild-type arches had more, well-ordered chondrocytes (Fig. 3D). These results indicate that nup107 deficiency leads to an impediment of prechondrogenic condensation and chondrogenic differentiation during pharyngeal arch formation.

Pharyngeal pouches of endoderm origin are required for CNCC patterning and cartilage development (35). Immunostaining with Zn5 antibody, which labels pharyngeal pouches (33), disclosed that pharyngeal pouches formed normally in nup107 mutants (Fig. 3E). This suggests that loss of pharyngeal cartilage in nup107 mutants is unattributable to defective pouches.

Extensive Apoptosis Occurs in the Affected Tissues of nup107 Mutants—We next asked whether loss of pharyngeal arches in nup107 mutants is related to cell death. Using TUNEL assay, we detected more apoptotic cells in the head, including the pharyngeal region, eyes and optic tectum (Fig. 4A) as well as in the intestine (data not shown) of mutants at and after 48 hpf compared with wild-type siblings. Apoptosis in these tissues of mutants occurs well before the appearance of morphological defects. Immunostaining with anti-active caspase 3 antibody also showed more positive signals in the head of the mutant at 60 hpf (Fig. 4B). We quantified the expression levels of a set of apoptosis-related genes by real-time RT-PCR, and found that mutants had already expressed higher levels of tp53/p53, p21, mdm2, bcl2 and caspase 8 since 48 hpf and also showed an elevated level of gadd45a expression from 60 hpf onward while fiabp level exhibited little changes (supplemental Fig. S2).
hybridization confirmed higher levels of tp53, mdm2, bbc3, and gadd45al in the pharyngeal region, eyes and intestine of mutants (Fig. 4C). We conclude that nup107 deficiency can trigger intrinsic and extrinsic apoptotic pathways in the pharyngeal arches, eyes, and intestine.

As p53 plays a critical role in the intrinsic apoptotic pathway (36), we tested whether p53 deficiency could inhibit apoptosis in nup107 mutants using tp53^{M214K} mutant strain (23). TUNEL assay revealed that the number of apoptotic cells in the head of nup107;p53 double mutants was reduced compared with that...
in nup107 mutants, but slightly higher than that in wild-type siblings (Fig. 4D), which suggests a partial rescuing effect. Similar effect was observed in the intestine (data not shown). Morphological observation and examination of fabp2 expression also indicated that Nup107 deficiency-induced defects in pharyngeal arches and intestine could not be completely rescued by p53 depletion (Fig. 4E). Thus, extensive apoptosis in the affected tissues of nup107 mutants is induced partially through activation of p53-dependent apoptotic signaling pathway, while p53-independent cell death signaling pathways could also be implicated in that process.

**Nup107 Mutation Does Not Affect Cell Proliferation**—To investigate whether cell proliferation in nup107 mutants is hindered, we performed BrdU incorporation assay and phospho-histone 3 (pH3) immunostaining. Results showed that the number of BrdU- or pH3-positive cells in the pharyngeal region and retinas of mutants was comparable to that in wild-type siblings at 48 hpf (supplemental Fig. S3, A–D), being indicative of unpaired cell proliferation in mutants. Fluorescence-activated cell sorting analysis using cells disassociated from the heads unveiled that the percentage of cells in the G0/G1, S or G2/M phase was comparable between mutants and their wild-type siblings at 48 hpf or 60 hpf (supplemental Fig. S3E), implying that mitotic cycle in mutants can proceed normally. We also examined spindle assembly and chromosome segregation in mitotic cells by DAPI and anti-tubulin antibody staining. As shown in supplemental Fig. S4, the percentage of pharyngeal or intestinal mitotic cells with abnormal spindles and chromosome segregation was comparable between mutants and their wild-type siblings. Taken together, these data strongly suggest that cell proliferation in nup107 mutants is unaffected.

**Nuclear Pores Are Disrupted in nup107 Mutants**—Nup107 is a component of the Nup107-Nup160 complexes and is required for building nuclear pore complexes as demonstrated in cultured mammalian cells (9, 37). We wondered whether nuclear pores are normally formed in nup107 mutants. To
address this question, we first examined the distribution of Nups by immunostaining tissue sections with mAb414 antibody, which can recognize FG-repeat Nups including Nup62, Nup153, Nup214, and Nup358 (37, 38). In all examined tissues of wild-type siblings, the recognized Nups were nicely located at the nuclear rim in a punctuated pattern (Fig. 5, A–E). In nup107 mutants, the immunofluorescence signal was reduced in most parts of the nuclear rim with a few bright aggregates in pharyngeal cartilage precursors, retinal, optic tectum and intestine epithelial cells (Fig. 5, A’–D’); however, the signal distribution appeared normal in mutant skeletal muscles (Fig. 5E’). These results suggest that the assembly of nuclear pore complexes is disturbed in specific tissues due to insufficient amount of Nup107. We then observed nuclear pores by transmission electron microscopy. The number of nuclear pores in the pharyngeal cartilage precursors, retinal, optic tectum and intestine epithelial cells was
dramatically reduced in nup107 mutants and nuclear pores appeared structurally abnormal (Fig. 6, A–I). Therefore, we conclude that Nup107 deficiency in zebrafish embryos disrupts the formation of nuclear pores in specific tissues.

**Transportation of mRNAs Is Impaired in nup107 Mutants**—
Nuclear pores play important roles in translocation of RNA and proteins between the nucleus and the cytoplasm. As nuclear pores are disrupted in cells in the affected tissues of nup107 mutants, we asked whether the transportation of macromolecules is deterred in the mutants. We detected the distribution of mRNAs by *in situ* hybridization on cryosections with Cy3-oligo(dT)₅₀ followed by confocal microscopy. As shown in Fig. 7,
the Cy3-oligo[dT]50-bound mRNAs showed a diffused pattern in pharyngeal arch cells, retinal and intestinal epithelial cells in wild-type embryos at 60 hpf; in contrast, the bound mRNAs appeared to be accumulated in nuclei of many cells in \textit{nup107} mutants, forming some clusters. This phenomenon was not observed in skeletal muscles (data not shown). These results suggest that nuclear pores are dysfunctional in exporting nuclear mRNAs into the cytoplasm in specific tissues of \textit{nup107} mutants, which may be related to observed tissue defects.

**DISCUSSION**

In this study, we generated the transgenic zebrafish line \textit{nup107tsu068Gt} using a \textit{Tol2} transposon-based gene trap approach, in which the \textit{nup107} locus is interrupted and its expression is disrupted. Embryos homozygous (mutant) for the insertion lack pharyngeal skeletons and die at 5–6 dpf. Mutant embryos suffer from extensive apoptosis in the pharyngeal arches, optic tectum, retinas and intestine. In the affected tissues, nucleoporin proteins are dislocated and nuclear pores are disrupted, resulting in an impairment of nuclear export of mRNAs. Our findings provide evidence for the first time in vertebrates that Nup107 is required for nuclear pore formation and for development of specific tissues.

\textit{Nup107} is an essential component of the Nup107–160 complexes of NPCs. Depletion of Nup107 in mammalian cells impairs NPCs assembly and nuclear export of mRNAs (9, 37). However, deficiency of NPP-5/Nup107 in \textit{Caenorhabditis elegans} does not block the NPCs assembly (19). We demonstrate that, in zebrafish, a vertebrate model system, loss of Nup107 can cause abnormal distribution of FG repeat-containing Nups (Fig. 5) and disrupt nuclear pores (Fig. 6). Therefore, Nup107 is essential for nuclear pore formation in vertebrate organisms.
The requirement of the Nup107–160 complex components for development of specific tissues has been demonstrated in a few cases. For example, zebrafish flo/elys mutant embryos show defects in the intestine, liver, pancreas, and eyes (16, 17); Fly Seh1 mutant females have some of oocytes developed as pseudo-nurse cells but their somatic tissues are unaffected (18). We have demonstrated in this study that zygotic deficiency of Nup107 in zebrafish embryos results in loss of pharyngeal skeletons in addition to degeneration of intestinal and retinal epithelia. This is the first example of Nup implication in cartilage/bone formation. It seems that zebrafish elys, also regarded as a component of the Nup107–160 complexes, is expressed in pharyngeal region during embryogenesis (see Fig. 3B in (16)), and elys/flo mutant embryos might have defects in pharyngeal arches (comparing Fig. 1C to 1D in (17)). It is possible that the other components of the Nup107–160 complexes play a similar role in the formation of the pharyngeal arches.

We observed extensive apoptosis in the affected tissues of nup107 mutants (Fig. 4A). In the present, we do not know the direct causes for this phenomenon. We suspected that apoptosis in nup107 mutants is associated with the genome instability due to insufficient amount of Nup107. We examined, by Western blotting using the extracts from the heads at 48 hpf and 54 hpf, the levels of phospho-H2ax/γ-H2A.X, a marker for DNA double-strand breaks (DSBs) (39), as well as its upstream activators and downstream signal transducers/effectors, including ataxia-telangiectasia-mutated (ATM), ATM-Rad3-related (ATR), Chk1 and Chk2. Results indicated that γ-H2A.X, p-ATM, p-Chk2, and p-p53 were elevated markedly in nup107 mutant heads (supplemental Fig. S5, A–D), while p-ATR and p-chkl stayed unchanged (supplemental Fig. S5, E–F). This suggests that insufficient amount of Nup107 in mutants causes extensive DSBs and overactivation of ATM-Chk2-p53 DNA damage response. The generation of DSBs may be ascribed to the displacement of specific mRNA species.

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