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Profiling ATM regulated genes in *Drosophila* at physiological condition and after ionizing radiation

Jun Liu¹, Tianyu Jin², Lanxi Ran², Ze Zhao², Rui Zhu², Gangcai Xie^{1*}  and Xiaolin Bi^{1,2*} 

Abstract

Background: ATM (ataxia-telangiectasia mutated) protein kinase is highly conserved in metazoan, and plays a critical role at DNA damage response, oxidative stress, metabolic stress, immunity, RNA biogenesis etc. Systemic profiling of ATM regulated genes, including protein-coding genes, miRNAs, and long non-coding RNAs, will greatly improve our understanding of ATM functions and its regulation.

Results: 1) differentially expressed protein-coding genes, miRNAs, and long non-coding RNAs in *atm* mutated flies were identified at physiological condition and after X-ray irradiation. 2) functions of differentially expressed genes in *atm* mutated flies, regardless of protein-coding genes or non-coding RNAs, are closely related with metabolic process, immune response, DNA damage response or oxidative stress. 3) these phenomena are persistent after irradiation. 4) there is a cross-talk regulation towards miRNAs by ATM, E2f1, and p53 during development and after irradiation. 5) knock-out flies or knock-down flies of most irradiation-induced miRNAs were sensitive to ionizing radiation.

Conclusions: We provide a valuable resource of protein-coding genes, miRNAs, and long non-coding RNAs, for understanding ATM functions and regulations. Our work provides the new evidence of inter-dependence among ATM-E2F1-p53 for the regulation of miRNAs.

Keywords: ATM, X-ray irradiation, miRNA, lncRNA

Introduction

The ataxia-telangiectasia mutated (ATM) protein kinase is a member of the Phosphoinositide-3-Kinase (PI3K)-like Kinase (PIKK) family, and plays a master role to regulate DNA damage response (DDR), especially at double strand breaks (DSBs) repair, to maintain genome integrity [1, 2]. Besides of DNA damage, ATM activation has been shown in connection with other stimuli, such as oxidative stress [3], ATP depletion, mitotic spindle, or metabolic stress, specifically inhibition of glycolysis, which is independent of DNA damage or oxidative stress [1, 2].

Mutation of *atm* gene in humans can cause a rare genetic disorder ataxia telangiectasia (A-T), which is characterized by radiation sensitivity, neurodegeneration, immunodeficiency, cancer predisposition, premature aging, and metabolic dysfunctions [4]. While complete loss of ATM kinase activity or functions does not cause lethality in humans, most of A-T patients die at 20 s or 30 s of age [4], mice with kinase-dead *atm* mutations are embryonic lethal [5]. In *Drosophila*, *atm/tefu* mutated flies have aberrant small head, significant telomere-telomere fusions in neuroblasts, and are sensitive to irradiation [6–9]. While loss of function *atm* allele, *atm^{stg}*, is pupal lethal [10], and *atm^s* is a heat sensitive loss of function allele [7].

ATM resides predominantly in the nucleus and is activated rapidly by DSBs in the Mre11-Rad50-Nbs1 (MRN)

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dependent manner. ATM and ATR (A-T and Rad3-related, another member of PIKK family) phosphorylate more than 700 proteins in response to DNA damage [11]. When ATM is activated by oxidative stress, it can phosphorylate a specific set of proteins that only partially overlap with the phosphorylated proteins in response to the canonical DSBs [12]. In response to replication stress induced by mitomycin C (MMC) and hydroxyurea (HU) treatment, ATM and ATR regulate SUMOylation modification [13]. Thousands of SUMOylation sites and hundreds of phosphorylation sites were identified after treatment, and proteins SUMOylated and phosphorylated are in the overlapping network.

ATM is required for both adaptive and innate immunity, and plays a supportive role in V(D)J-recombination, the core of adaptive immunity [4, 14]. The V(D)J-recombination generates programmed breaks, these breaks are held together in a synapse by the Recombination Activating Genes (RAG) protein. ATM is recruited to RAG-induced DSBs and activated, cooperates with MRN complex, γ H2AX, and 53BP1, to stabilize DSBs during V(D)J-recombination, to ensure correct end processing and ligation [14].

Unscheduled transcription and alternative pre-mRNA splicing induced by DNA damage signify great threat to genome integrity. R-loop misregulation can lead to DNA damage, transcription elongation defects and genomic instability. R-loop activates ATM signaling, impedes spliceosome organization, and enhances UV-triggered alternative splicing [15, 16]. ATM and PIKK members influence DNA damage induced transcription through post-translational modification of RNA polymerase II (RNA pol II), transcription elongation factors, and multiple transcription factors, to regulate RNA-dependent DNA damage repair [17, 18]. ATM and ATR phosphorylate components of core spliceosome, promote displacement of spliceosome [1, 18]. Moreover, ATM modulates miRNAs biogenesis by regulating miRNAs transcription, nuclear exporting, processing, and maturation. On the other side, ATM is regulated by miRNAs at post-transcriptional level. ATM-miRNAs work coordinately to regulate DNA damage response and tissue homeostasis [19]. ATM kinase interacts and phosphorylates the KH-type splicing regulatory protein KSRP, a key component in both Drosha and Dicer miRNA-processing complex, enhances KSRP-pri-miRNAs interaction and miRNAs maturation [20].

ATM is required for the maintenance of glucose metabolism. Most A-T patients succumb to metabolic syndromes in early age, show the tendency towards insulin resistance, glucose tolerance, high blood pressure etc. Mutation of *atm* gene causes loss of redox sensing, increased ROS level, and altered redox balance, which

leads to mitochondrial dysregulation and changes of glucose metabolism [1, 4]. A-T patients also develop early-onset cerebellar neurodegeneration, the neurological defect which might be due to loss of redox homeostasis and dysregulation in DNA damage response, however its molecular basis is still not clear [1, 4].

Due to the large number of ATM substrates with different functional features, ATM affects many cellular processes. A systematic genome-wide analysis of genes that may be regulated by ATM will provide a valuable reference for the study of ATM. In this study, we investigated differentially expressed (DE) protein-coding genes, miRNAs, and lncRNAs in *atm* mutated flies at physiological condition and after ionizing radiation. We found that biological functions of DE genes are diversified, regardless of protein-coding genes or non-coding RNAs, and a lot of DE genes are closely related with metabolic process, immune response, DNA damage response or oxidative stress. These phenomena are persistent even after irradiation treatment. We identified miRNAs regulated by ATM, E2f1 and p53 during development and after irradiation, and provided evidence of a cross-talk regulation among them. We proved that most of irradiation induced miRNAs in *atm* mutated flies are closely related with DNA damage response.

Materials and methods

Fly genetics

All flies were maintained at 25 °C on standard corn meal unless specified. Fly lines used in this study were: *w*¹¹¹⁸ (*wild-type*, Bloomington Drosophila Stock Center 3605), *atm*^{stg} (*atm*) [6, 10], *miR-274*^{KO} (Bloomington Drosophila Stock Center 58904); *miR-956*^{KO} (Bloomington Drosophila Stock Center 58941) [21], *miR-963*^{sp} (Bloomington Drosophila Stock Center 61449); *miR-1007*^{sp} (Bloomington Drosophila Stock Center 61490) [22], *tubulin-Gal4*.

Ionizing radiation

Actively crawling third instar larvae of *w*¹¹¹⁸ and *atm*^{stg} flies were collected and irradiated with 40 Gy, at a dose rate of 340 cGy/min, with X-RAD 320 iX at 320 kV (Precision X-Ray, Inc., North Branford, CT). The irradiated L3 larvae were recovered for 1 h before further experiments.

RNA-seq

Actively crawling third instar larvae of *w*¹¹¹⁸ and *atm*^{stg} flies were collected. There are three replicates in each group with about 30 larvae in each replicate. RNA was purified from irradiated and non-irradiated flies. Total RNA was extracted using Trizol with DNase treatment at the Beijing Genomics Institute (BGI Co., Ltd., China).

The integrity of extracted RNA was assessed by Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). The mRNA sequencing libraries were constructed with Illumina TruSeq Stranded mRNA Sample Prep Kit (Illumina, Cat. No.RS-122–2101), and paired-end 50 bp sequencing was performed using Illumina HiSeq 2000. The small RNA libraries were constructed with Illumina's TruSeq Small RNA Sample Prep kit (Illumina, Cat. No. RS-200–0012), and single-end 50 bp sequencing was performed using Illumina HiSeq 2000. All RNA-Seq experiments encompass three biological replicates.

RNA-seq data analysis

For mRNAs and lncRNAs analysis, raw sequencing data quality was assessed with FastQC (v2.0.1) (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and trim_galore (v0.6.6) [23], and aligned to *Drosophila melanogaster* reference genome (dmel_r6.41_FB2021_04) using STAR (v2.7.9a). Annotated genes were assigned using annotations from Flybase (dmel-all-r6.41.gtf) [24] and featureCounts (v2.0.1) [25]. For miRNAs analysis, raw sequencing data quality was assessed using FastQC (v2.0.1). The expression level of annotated miRNAs was quantified by miRDeep2 [26] using the quantifier.pl script with the following parameters: quantifier.pl -m mature.fa -p hairpin.fa -r reads.fa -t dme_index -y filename. Sequence files (mature.fa, hairpin.fa) of annotated miRNAs were retrieved from the miRBase Release 22.1 [27]. Differentially expressed genes (DEGs) were identified by comparison of genes expression in *atm*^{sg} flies with *w*¹¹¹⁸ flies at same experimental condition using R package DESeq2 (version 1.25.12) [28], and a gene is considered differentially expressed when the absolute log₂-fold change is ≥ 1 and the adjusted *p*-value is < 0.05 .

Reverse transcription-quantitative PCR

Total RNA was extracted using Trizol and reverse transcribed with Evo M-MLV RT Kit with gDNA Clean for qPCR (Accurate Biology, AG11705) using Oligo dT(18 T) primer 5' d(TTTTTTTTTTTTTTTTTT) 3'. Gene transcripts were quantified by quantitative PCR (qPCR) with SYBR Green Premix Pro Taq HS (Accurate Biology, AG11701) on a Roche LightCycler 96 with comparative Cq method, *rp49* was used as a reference gene, and

relative expression change was calculated from three independent experiments.

Function enrichment analysis

The Gene Ontology (GO) annotation, KEGG pathway annotation and enrichment analysis were conducted for differentially expressed protein-coding genes and experimentally validated miRNAs targeted genes, based on Gene Ontology database (<http://www.geneontology.org/>), and KEGG (Kyoto Encyclopedia of Genes and Genomes) (<http://www.genome.jp/kegg/>) with PANTHER [29] and DAVID (The Database for Annotation, Visualization and Integrated Discovery, <https://david.ncifcrf.gov/>) [30]. For GO analysis, biological processes (BP) and molecular functions (MF) were applied, GO terms with adjusted *p*-value < 0.05 were considered significant. Significantly enriched biological processes were displayed with R software ggplot2 package and Cytoscape software [31].

Viability assay

Actively crawling third instar larvae were irradiated with the dosage of 0, 10, 20, 30, and 40 Gy, respectively, at a dose rate of 1.3 Gy/min in a X-Ray Biological Irradiator (X-RAD 320iX, Precision X-ray Inc). At least 100 larvae were treated for each genotype at each dosage, and experiments were repeated three times. Survival percentage was calculated as the number of viable adults divided by the number of irradiated third instar larvae. Significant differences between the values under designated experimental conditions were subjected to two-tailed Student's *t*-tests. For all tests, a *P* value less than 0.05 was considered statistically significant.

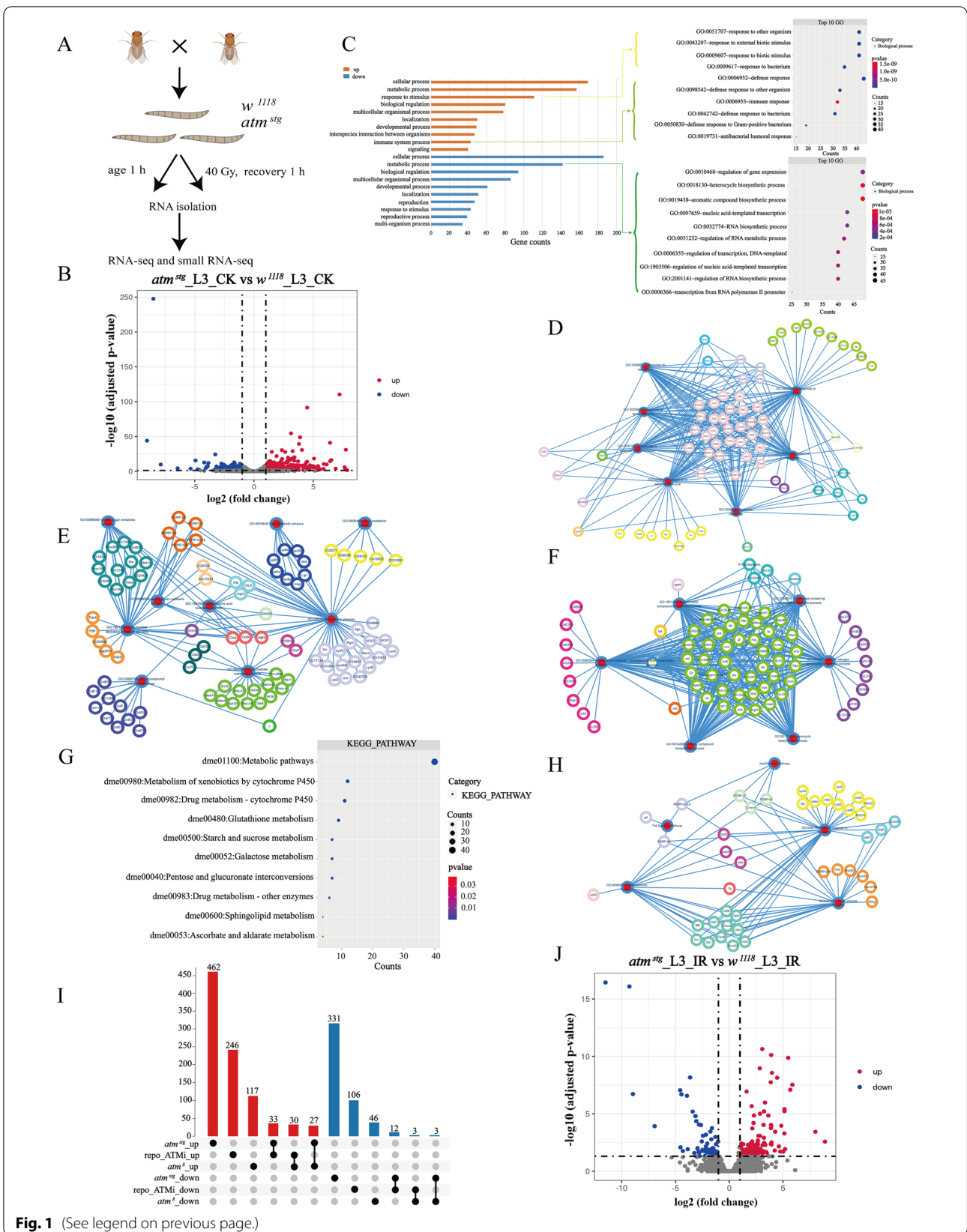
Results

To explore potential protein-coding genes and non-coding RNAs regulated by ATM in *Drosophila*, we performed RNA-sequencing (RNA-seq) and small RNA-sequencing (miRNA-seq) on third instar larvae (L3) of *wild-type* (*wt*) and *atm* mutated flies (Fig. 1A). Raw sequencing data of RNA-seq was passed through stringent quality control, and aligned to the *Drosophila melanogaster* BDGP6 (dmel_r6.41_FB2021_04) using STAR (2.7.9a). In total, 1,025 million reads were collected at an average of 51.25 million reads per sample, range from

(See figure on next page.)

Fig. 1 Differentially expressed (DE) protein-coding genes in *atm* mutated flies at physiological condition (CK) and after ionizing radiation (IR).

A Experimental scheme. **B** Volcano plots of protein-coding genes differentially expressed between *atm* mutated flies and *wild-type* flies. up, up-regulated genes in *atm* mutant compared with *wild-type* flies; down, down-regulated genes in *atm* mutant compared with *wild-type* flies. **C** The top 10 GO terms of upregulated and downregulated protein-coding genes. **D** Functional gene clusters in response to stimulus. **E** Functional gene clusters in metabolic processes of upregulated genes. **F** Functional gene clusters in metabolic processes of downregulated genes. **G** KEGG pathways analysis. **H** Functional gene clusters in immune response. **I** UpSet plot of the number of overlapping genes between depicted genotypes of flies. **J** Volcano plots of protein-coding genes differentially expressed between *atm* mutated flies and *wild-type* flies after ionizing radiation



42.3 to 59.7 million reads, and mapped loci range from 68 ~ 96%. We identified 12,584 protein-coding genes with at least 10 reads using featureCounts (v2.0.1) (Table S1), which accounts for 95% of protein-coding genes in *Drosophila*. Differential expression of the protein-coding gene between the *atm* and *wild-type* flies was assessed using DESeq2 (Table S2), and a gene expressed with significant difference (fold change $\geq \pm 2$, adjusted $p < 0.05$) between the *atm* and *wild-type* flies in 3 replicates was regarded as differentially expressed. We identified 462 upregulated and 331 downregulated protein-coding genes in *atm* mutated flies when compared with *wild-type* flies (Fig. 1B, Table S3). The expression profile of differentially expressed genes was further verified by RT-qPCR for 10 randomly selected DE genes, including 5 upregulated genes and 5 downregulated genes (Figure S1). We also analyzed the tissue expression characteristics of these differentially expressed genes using tissue expression data from FlyAtlas2 (Figure S2) [32].

To investigate the functional annotations of differentially expressed genes, we conducted Gene Ontology enrichment analysis with DAVID. In the GO analysis, the top 10 significantly enriched biological processes were identified in both upregulated and downregulated genes. The highly related biological processes that DE genes involved are cellular process, metabolic process, response to stimulus, developmental process, immune system process, reproduction etc. (Fig. 1C, Table S3). The cellular process and metabolic process are the top 2 biological processes in either upregulated or downregulated DE genes, there are more than 140 DE genes in each of them. And response to stimulus is the number 3 biological process in upregulated DE genes with 111 genes, and number 8 in downregulated DE genes with 43 genes, respectively (Fig. 1C, Table S3). Among DE genes in response to stimulus, they are highly enriched in biological processes such as immune response, stress, bacterial or biotic stimulus etc., and forms several highly connected functional gene clusters (Fig. 1D). Moreover, some DE genes are closely related with DNA damage response or oxidative stress. In upregulated genes, *agt* is required for DNA dealkylation [33], *tie* negatively regulate X-ray irradiation induced cell death [34], *TotC* is induced by oxidation, UV and bacterium treatment [35], *timeless* is involved into DNA replication checkpoint [36], and *corp* negatively regulate p53-dependent apoptosis [37]. While in downregulated genes, *eya* positively regulate DNA repair [36], *tfb4* is in response to UV and involved in nucleotide excision repair (NER) pathway [38]. Interestingly, some DE genes play roles in response to light stimulus, including *timeless* [36], *laza* [39] and *tfb4* [38] in upregulated genes, and *glass* [40], *inaC* [41], *ptth* [42], and *rh6* [36] in downregulated genes (Table S3).

As loss of human *atm* gene causes metabolic syndromes and neurodegeneration, we further investigated their functions in manually curated DE genes. In DE genes related with metabolic process, the top 3 biological processes in upregulated genes are protein metabolic process, cellular aromatic compound metabolic process, and carbohydrate derivative metabolic process. And in downregulated genes, they are cellular aromatic compound metabolic process, protein metabolic process, and nucleic acid metabolic process, respectively (Fig. 1E-F, Table S3). Many DE genes are involved in carbohydrate, lipid, amino acid, and RNA metabolic processes, suggesting the critical role that ATM protein kinase plays at metabolism. We also analyzed signaling pathways with KEGG of differentially expressed genes involved, and found that both upregulated genes and downregulated genes are highly enriched in metabolic pathways (Fig. 1G, Table S3). We further analyzed DE genes related with immunity, and found that some of them play important roles directly in humoral immune response, or innate immune response (Fig. 1H), in which *Charon*, *PGRP-LF*, *PGRP-SC1b*, and *PGRP-LC* are members in the Imd signaling pathway, and *gd*, *PGRP-SA*, and *PGRP-SC1b* are in the Toll signaling pathway, respectively (Table S3) [43–45]. *Charon* is an important mediator of PARP-1-dependent transcription in the innate immune pathway [46]. The *gd* gene is required for the Toll ligand produced by the proteolytic reaction [47]. A previous study performed microarray analysis using adult heads of a temperature sensitive *atm* allele (*atm*⁸), and identified 117 upregulated and 46 downregulated genes respectively, and found that decreased ATM kinase activity increases expression of innate immune response genes. Moreover, *atm* knockdown in glial cells resulted in neuronal and glial cell death, decreased mobility and lifespan. When a short hairpin RNA of *atm* gene was expressed by *repo-Gal4* to knock-down *atm* gene in glial cells, genes expression in 3 ~ 5 days old adult flies were analyzed, there are 246 upregulated genes and 106 downregulated genes respectively, and innate immune response is the most significantly changed biological process [48]. We performed the comparative analysis. In upregulated genes, we identified 33 overlapping genes when compared with *repo-ATMi* allele, and 27 overlapping genes when compared with *atm*⁸ allele, respectively. While in downregulated genes, there are 12 overlapping genes when compared with *repo-ATMi* allele, and 3 overlapping genes when compared with *atm*⁸ allele (Fig. 1I), and most of overlapping genes are functional related with immune response.

To study ATM regulated genes in response to DNA damage, we treated the third instar larvae of both *wild-type* and *atm* flies with irradiation, and identified 129 upregulated genes and 61 downregulated genes (Fig. 1),

Table S4). We also analyzed the tissue expression characteristics of differentially expressed genes after irradiation (Figure S3). Among DE genes after irradiation, many of them are related with biological processes such as cellular process, metabolic process, developmental process, reproduction etc., although the number of differentially expressed genes was significantly reduced when compared with that of no irradiation treatment, which strengthen the previous suggestion that the fine tune of metabolic demands by communicating with surrounding milieu in response to stress is required in multicellular organisms [49].

ATM regulates miRNAs biogenesis at both physiological condition and after ionizing radiation

As ATM plays an important role at regulating miRNAs biogenesis, we further investigated differentially expressed miRNAs in *atm* flies at physiological condition (NP) and in response to ionizing radiation (IR). Total RNA was prepared and small RNA-seq was performed. Raw sequencing data was processed with cutadapt to remove adapter sequence and generate the clean miRNA data. The miRNA reads were ranged from 38.4 to 54.6 million (Table S5), and mapped to genome with miRBase Release 22.1 using mirdeep2 (2.0.1.3). In total, we identified 281 annotated mature miRNAs with at least 10 reads (Table S5). The differential expression of miRNAs was assessed using DESeq2, and a miRNA gene expressed with significant difference (fold change $\geq \pm 2$, adjusted $p < 0.05$) between the *atm* and *wild-type* flies in 3 replicates was regarded as differentially expressed.

Compared with *wild-type* flies, we identified 6 upregulated miRNAs, including *miR-274-5p*, *miR-956-5p*, *miR-956-3p*, *miR-980-5p*, *miR-986-5p*, and *miR-1007-5p*, and 11 downregulated miRNAs in *atm* flies, including *let-7-5p*, *miR-210-5p*, *miR-310-5p*, *miR-311-5p*, *miR-311-3p*, *miR-963-3p*, *miR-981-3p*, *miR-982-3p*, *miR-983-5p*, *miR-984-5p*, and *miR-4944-5p* (Fig. 2A, Table S6). In upregulated miRNAs with annotated functions, *miR-274-5p* coordinates nervous and vascular development in glia, regulates circadian behavior in astrocytes, and plays a role in response to hypoxia [50, 51]; *miR-956-5p* is related with muscular dystrophy and has the pro-virulence function [52, 53]; *miR-980-5p* regulates axon guidance and is related with muscular dystrophy, suppresses memory in adult brain, and is downregulated upon starvation and metabolic stress [52, 54, 55]. In downregulated miRNAs with annotated functions, *let-7-5p* forms a cluster with *miR-100* and *miR-125*, they play critical role during metamorphosis [56] and have diversified functions; *miR-210-5p* modulates circadian rhythms, locomotion, and lipid metabolism, and prevents neurodegeneration in retina [57–59]; *miR-310-5p*, *miR-311-5p* and *miR-311-3p*

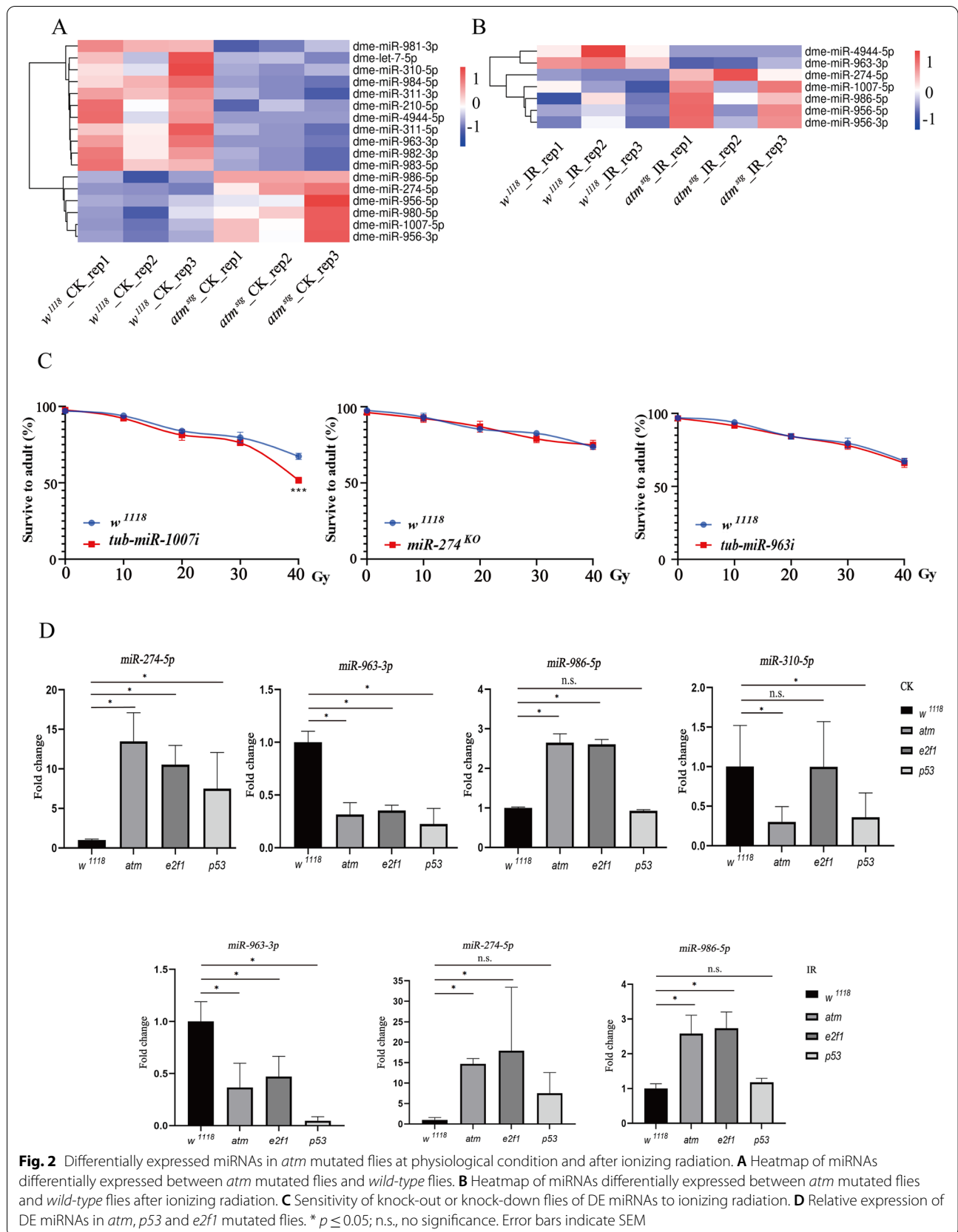
in the *miR-310/313* cluster transduce nutritional signals as a metabolic regulator, are required for normal synaptic transmission, and involved into Toll pathway mediated immune response by regulating expression of the antimicrobial peptide Drosomycin [60–62]; *miR-981-3p* negatively regulates anti-bacterial defense by reducing dipterin in IMD pathway [62]. While *miR-986-5p* and *miR-1007-5p* in upregulated genes, and *miR-963-3p*, *miR-982-3p*, *miR-983-5p*, *miR-984-5p*, and *miR-4944-5p* in downregulated genes have no annotated functions, their functions might be closely connected with ATM, which needs further investigation.

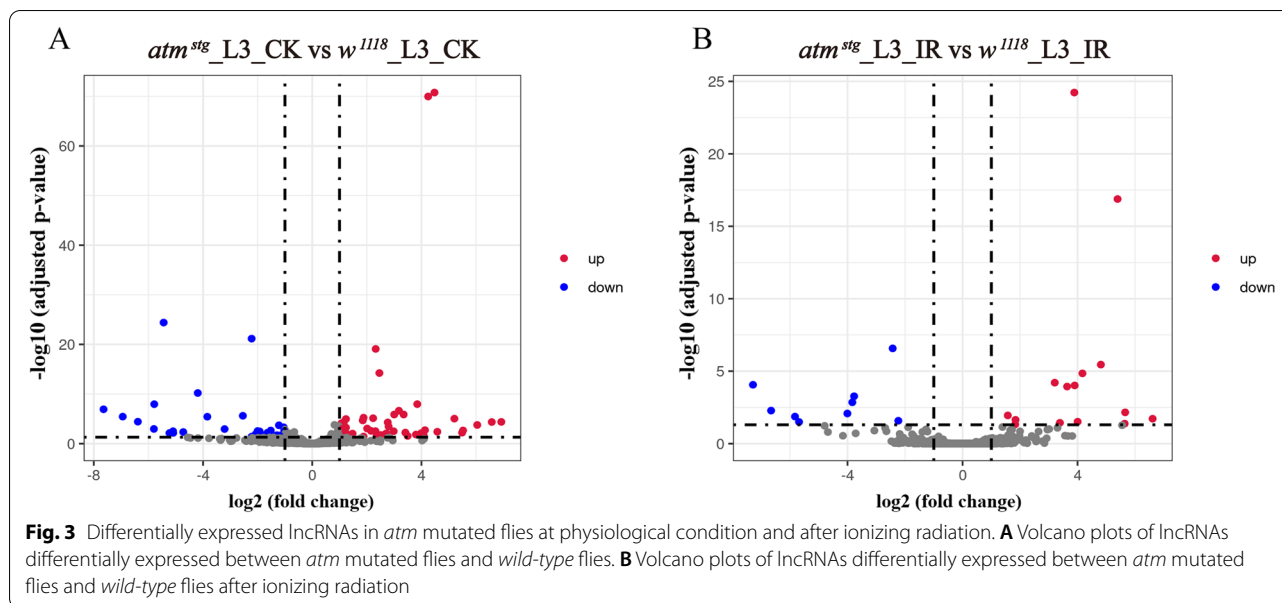
Furthermore, we identified 5 upregulated miRNAs, including *miR-274-5p*, *miR-956-5p*, *miR-956-3p*, *miR-986-5p*, and *miR-1007-5p*, and 2 downregulated miRNAs, including *miR-963-3p* and *miR-4944-5p*, in *atm* flies after irradiation (Fig. 2B, Table S7). We treated miRNA knockout or knock-down flies with X-ray irradiation, looked at their survival rates to investigate whether they are sensitive to ionizing radiation. Our previous result has shown that *miR-956^{KO}* and *miR-986^{KO}* flies are highly sensitive to irradiation [63], while *miR-274^{KO}* flies are not sensitive to irradiation, indicating that although *miR-274-5p* expression was increased after irradiation, it may not play a role at DNA damage response. We used *tubulin-Gal4* to drive ubiquitous expression of *miR-963^{sp}* and *miR-1007^{sp}*, and found that *tublin-miR-1007i* (*tub-miR-1007i*) flies with *miR-1007-5p* knock-down are sensitive to irradiation, while *tublin-miR-963i* (*tub-miR-963i*) flies with *miR-963-3p* knock-down are not sensitive to irradiation (Fig. 2C). As miRNAs play a role to fine tune genes expression and activities, residual expression level of *miR-963-3p* may influence the DNA damage sensitivity, a knock-out mutant of *miR-963-3p* is required to test whether it plays a role at DNA damage response. We did not test *miR-4944-5p* as no allele is available.

We performed comparison analysis with DE miRNAs in *p53* or *e2f1* mutated flies [63]. At physiological condition, *miR-274-5p* was upregulated in *atm*, *p53* and *e2f1* mutated flies, *miR-963-3p* was downregulated in *atm*, *p53* and *e2f1* mutated flies, *miR-986-5p* was upregulated in *atm* and *e2f1* mutated flies, and *miR-310-5p* was downregulated in *atm* and *p53* mutated flies. After irradiation, *miR-963-3p* was downregulated in *atm*, *p53*, and *e2f1* mutated flies, *miR-274-5p* and *miR-986-5p* were upregulated in *atm* and *e2f1* mutated flies (Fig. 2D), suggesting a cross-talk regulation to miRNAs among ATM, E2f1 and p53 during development and after irradiation.

Differentially expressed lncRNAs in *atm* mutated flies

We further investigated differentially expressed lncRNAs in *atm* flies at physiological condition and in response to ionizing radiation. RNA preparation, data processing,





and identification of differentially expressed lncRNAs were performed as protein-coding genes. We identified 1128 lncRNAs with at least 10 reads (Table S8). Compared with *wild-type* flies, we identified 45 upregulated lncRNAs and 32 downregulated lncRNAs in *atm* flies at physiological condition (Fig. 3A, Table S9), and 15 upregulated lncRNAs and 9 downregulated lncRNAs in *atm* flies after irradiation (Fig. 3B, Table S10). Until now, very few lncRNAs in *Drosophila* have been studied, differentially expressed lncRNAs in *atm* mutated flies might play diversified roles as ATM kinase does. Three differentially expressed lncRNAs, including *CR31781*, which is upregulated in *atm* flies at physiological condition, and *CR43282* and *CR43356*, which are downregulated in *atm* flies at physiological condition, have been studied in systemic analysis. *CR31781* is involved in muscle lateral inhibition [64], and *CR43282* and *CR43356* knock-out flies have reduced male fertility [65]. These three functional DE lncRNAs play different functions in *Drosophila*, such as in development and production, which is consistent with that of differentially expressed protein-coding genes and microRNAs.

Discussion

The ATM protein kinase plays an important role and has diversified functions during development and in response to stress. Study of ATM regulated genes had been focused on post-translational modifications such as phosphorylation, somoylation etc. In this study, we systematically profiled differentially expressed genes in *Drosophila*, including protein-coding genes, miRNAs and lncRNAs, in *atm* mutated flies at physiological condition

and after irradiation. We identified hundreds of differentially expressed protein-coding genes, and multiple miRNAs and lncRNAs. For those protein-coding genes and miRNAs with annotated functions, their functions are diversified and complex, span metabolism, immune response, multicellular organismal process, response to stimulus etc., which is consistent with our current understanding of the complex functions of ATM kinase.

The DNA damage response is highly conserved from invertebrates to mammals. Activation of systemic response is executed in response to DNA damage, including metabolic change, immune response etc., which plays very important role for the physiological adjustments for remodeling or regeneration of tissues and organs. Due to its simpler yet conserved genetics and physiology, *Drosophila* is of particular importance to investigate local and systemic interactions in response to stimulus. Moreover, compared with previous studies that showing proteins phosphorylated by ATM, our study provides a new angle of view to understand ATM functions and its downstream targets. Furthermore, by doing comparison analysis with our previous work studying miRNAs regulated by p53 and E2F1 [63], we identified miRNAs differentially expressed in *atm* mutated flies, *p53* mutated flies or *e2f1* mutated flies, and provide new evidence of inter-dependent regulation of miRNAs among ATM-E2F1-p53.

Conclusions

Differentially expressed genes, including protein-coding genes and non-coding RNAs, in *atm* mutated flies have diversified functions and are highly

related with metabolism, immune response, multicellular organismal process, response to stimulus etc. These phenomena are persistent after irradiation. There is a cross-talk regulation towards miRNAs by ATM, E2f1 and p53 during development and after irradiation.

Abbreviations

ATM: Ataxia-telangiectasia mutated; DDR: DNA damage response; DE: Differentially expressed; DEGs: Differentially expressed genes; DSB: Double strand breaks; GO: Gene Ontology; IR: Ionizing radiation; KEGG: Kyoto Encyclopedia of Genes and Genomes; L3: Third instar larvae; NP: Physiological condition; PI3K: Phosphoinositide-3-Kinase (PI3K)-like Kinase; RNA-seq: RNA-sequencing.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s41065-022-00254-9>.

Additional file 1: Figure S1. RT-qPCR validation of DE protein-coding genes identified by RNA-seq. Validation of 5 upregulated and 5 downregulated protein-coding genes in *atm* mutated flies. Error bars indicate SEM.

Additional file 2: Figure S2. Tissue expression of differentially expressed protein-coding genes in *atm* mutated flies at physiological condition.

A Heatmap of differentially expressed genes expressed in larval tissues.

B Heatmap of differentially expressed genes expressed in adult female tissues. **C** Heatmap of differentially expressed genes expressed in adult male tissues.

Additional file 3: Figure S3. Tissue expression of differentially expressed protein-coding genes in *atm* mutated flies after ionizing radiation. **A** Heatmap of differentially expressed genes expressed in larval tissues.

B Heatmap of differentially expressed genes expressed in adult female tissues. **C** Heatmap of differentially expressed genes expressed in adult male tissues.

Additional file 4: Table S1. mRNA counts.

Additional file 5: Table S2. Protein-coding genes sequenced.

Additional file 6: Table S3. Differentially expressed protein-coding genes in *atm* mutated flies.

Additional file 7: Table S4. Differentially expressed protein-coding genes in *atm* mutated flies after X-ray irradiation.

Additional file 8: Table S5. miRNA counts.

Additional file 9: Table S6. Differentially expressed miRNAs in *atm* mutated flies.

Additional file 10: Table S7. Differentially expressed miRNAs in *atm* mutated flies after X-ray irradiation.

Additional file 11: Table S8. lncRNA counts.

Additional file 12: Table S9. Differentially expressed lncRNAs in *atm* mutated flies.

Additional file 13: Table S10. Differentially expressed lncRNAs in *atm* mutated flies after X-ray irradiation.

Additional file 14: Table S11. Primers.

Acknowledgements

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Authors' contributions

X.B. conceived project and designed experiments; J.L. performed bioinformatic analysis; T.J., L.R., Z.Z., and R.Z. performed experiments; J.L., G.X., and X.B. analyzed data, and X.B. wrote the manuscript. The author(s) read and approved the final manuscript.

Authors' information

Not applicable.

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Availability of data and materials

The raw sequencing data generated in this study has been submitted to the NCBI BioProject database (<https://www.ncbi.nlm.nih.gov/bioproject/>) under accession number PRJNA809845. The custom code used is available at <https://github.com/Liu970101/atm>.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agreed for this publication.

Competing interests

The authors declare that they have no conflict of interest.

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References

- Lee JH, Paull TT. Cellular functions of the protein kinase ATM and their relevance to human disease. *Nat Rev Mol Cell Biol.* 2021;22(12):796–814.
- Paull TT. Mechanisms of ATM Activation. *Annu Rev Biochem.* 2015;84:711–38.
- Guo Z, Kozlov S, Lavin MF, Person MD, Paull TT. ATM activation by oxidative stress. *Science.* 2010;330(6003):517–21.
- Zaki-Dizaji M, Akrami SM, Abolhassani H, Rezaei N, Aghamohammadi A. Ataxia telangiectasia syndrome: moonlighting ATM. *Expert Rev Clin Immunol.* 2017;13(12):1155–72.
- Daniel JA, Pellegrini M, Lee BS, Guo Z, Filsuf D, Belkina NV, You Z, Paull TT, Sleckman BP, Feigenbaum L, et al. Loss of ATM kinase activity leads to embryonic lethality in mice. *J Cell Biol.* 2012;198(3):295–304.
- Bi X, Wei SC, Rong YS. Telomere protection without a telomerase; the role of ATM and Mre11 in *Drosophila* telomere maintenance. *Curr Biol.* 2004;14(15):1348–53.
- Silva E, Tiong S, Pedersen M, Homola E, Royou A, Fasulo B, Siriaco G, Campbell SD. ATM is required for telomere maintenance and chromosome stability during *Drosophila* development. *Curr Biol.* 2004;14(15):1341–7.
- Song YH, Mirey G, Betson M, Haber DA, Settleman J. The *Drosophila* ATM ortholog, dATM, mediates the response to ionizing radiation and to spontaneous DNA damage during development. *Curr Biol.* 2004;14(15):1354–9.
- Bi X, Gong M, Srikanta D, Rong YS. *Drosophila* ATM and Mre11 are essential for the G2/M checkpoint induced by low-dose irradiation. *Genetics.* 2005;171(2):845–7.
- Gong M, Bi X, Rong YS. Targeted mutagenesis of *Drosophila atm* and *mre11* genes. *Drosoph Inf Serv.* 2005;88:79–83.
- Matsuoka S, Ballif BA, Smogorzewska A, McDonald ER 3rd, Hurov KE, Luo J, Bakalarski CE, Zhao Z, Solimini N, Lerenthal Y, et al. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science.* 2007;316(5828):1160–6.
- Yan S, Sorrell M, Berman Z. Functional interplay between ATM/ATR-mediated DNA damage response and DNA repair pathways in oxidative stress. *Cell Mol Life Sci.* 2014;71(20):3951–67.

13. Munk S, Sigurethsson JO, Xiao Z, Batth TS, Franciosa G, von Stechow L, Lopez-Contreras AJ, Vertegaal ACO, Olsen JV. Proteomics Reveals Global Regulation of Protein SUMOylation by ATM and ATR Kinases during Replication Stress. *Cell Rep.* 2017;21(2):546–58.
14. Weitering TJ, Takada S, Weemaes CMR, van Schouwenburg PA, van der Burg M. ATM: Translating the DNA Damage Response to Adaptive Immunity. *Trends Immunol.* 2021;42(4):350–65.
15. Niehrs C, Luke B. Regulatory R-loops as facilitators of gene expression and genome stability. *Nat Rev Mol Cell Biol.* 2020;21(3):167–78.
16. Tresini M, Warmerdam DO, Kolovos P, Snijder L, Vrouwe MG, Demmers JA, van Ijcken WF, Grosveld FG, Medema RH, Hoeijmakers JH, et al. The core spliceosome as target and effector of non-canonical ATM signalling. *Nature.* 2015;523(7558):53–8.
17. Burger K, Ketley RF, Gullerova M. Beyond the Trinity of ATM, ATR, and DNA-PK: Multiple Kinases Shape the DNA Damage Response in Concert With RNA Metabolism. *Front Mol Biosci.* 2019;6:61.
18. Angarola BL, Anczukow O. Splicing alterations in healthy aging and disease. *Wiley Interdiscip Rev RNA.* 2021;12(4):e1643.
19. Rezaeian AH, Khanbabaei H, Calin GA. Therapeutic Potential of the miRNA-ATM Axis in the Management of Tumor Radioresistance. *Cancer Res.* 2020;80(2):139–50.
20. Zhang X, Wan G, Berger FG, He X, Lu X. The ATM kinase induces microRNA biogenesis in the DNA damage response. *Mol Cell.* 2011;41(4):371–83.
21. Chen YW, Song S, Weng R, Verma P, Kugler JM, Buescher M, Rouam S, Cohen SM. Systematic study of Drosophila microRNA functions using a collection of targeted knockout mutations. *Dev Cell.* 2014;31(6):784–800.
22. Fulga TA, McNeill EM, Binari R, Yelick J, Blanche A, Booker M, Steinkraus BR, Schnall-Levin M, Zhao Y, DeLuca T, et al. A transgenic resource for conditional competitive inhibition of conserved Drosophila microRNAs. *Nat Commun.* 2015;6:7279.
23. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics.* 2013;29(1):15–21.
24. Thurmond J, Goodman JL, Strelets VB, Attrill H, Gramates LS, Marygold SJ, Matthews BB, Millburn G, Antonazzo G, Trovisco V, et al. FlyBase 2.0: the next generation. *Nucleic Acids Res.* 2019;47(D1):D759–65.
25. Liao Y, Smyth GK, Shi W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics.* 2014;30(7):923–30.
26. Friedlander MR, Mackowiak SD, Li N, Chen W, Rajewsky N. miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades. *Nucleic Acids Res.* 2012;40(1):37–52.
27. Kozomara A, Birgaoanu M, Griffiths-Jones S. miRBase: from microRNA sequences to function. *Nucleic Acids Res.* 2019;47(D1):D155–62.
28. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 2014;15(12):550.
29. Mi H, Ebert D, Muruganujan A, Mills C, Albou LP, Mushayama T, Thomas PD. PANTHER version 16: a revised family classification, tree-based classification tool, enhancer regions and extensive API. *Nucleic Acids Res.* 2021;49(D1):D394–403.
30. Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA. DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol.* 2003;4(5):P3.
31. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 2003;13(11):2498–504.
32. Krause SA, Overend G, Dow JAT, Leader DP. FlyAtlas 2 in 2022: enhancements to the Drosophila melanogaster expression atlas. *Nucleic Acids Res.* 2022;50(D1):D1010–5.
33. Kooistra R, Zonneveld JB, Watson AJ, Margison GP, Lohman PH, Pastink A. Identification and characterisation of the Drosophila melanogaster O6-alkylguanine-DNA alkyltransferase cDNA. *Nucleic Acids Res.* 1999;27(8):1795–801.
34. Bilak A, Uyetake L, Su TT. Dying Cells Protect Survivors from Radiation-Induced Cell Death in Drosophila. *PLoS Genet.* 2014;10(3):e1004220.
35. Ekengren S, Hultmark D. A family of Turandot-related genes in the humoral stress response of Drosophila. *Biochem Biophys Res Commun.* 2001;284(4):998–1003.
36. Gaudet P, Livstone MS, Lewis SE, Thomas PD. Phylogenetic-based propagation of functional annotations within the Gene Ontology consortium. *Brief Bioinform.* 2011;12(5):449–62.
37. Chakraborty R, Li Y, Zhou L, Golic KG. Corp Regulates P53 in Drosophila melanogaster via a Negative Feedback Loop. *PLoS Genet.* 2015;11(7):e1005400.
38. Aoyagi N, Wassarman DA. Genes encoding Drosophila melanogaster RNA polymerase II general transcription factors: diversity in TFIIA and TFIID components contributes to gene-specific transcriptional regulation. *J Cell Biol.* 2000;150(2):F45–50.
39. Kwon Y, Montell C. Dependence on the Lazarop phosphatidic acid phosphatase for the maximum light response. *Current biology : CB.* 2006;16(7):723–9.
40. Klarsfeld A, Malpel S, Michard-Vanhee C, Picot M, Chelot E, Rouyer F. Novel features of cryptochrome-mediated photoreception in the brain circadian clock of Drosophila. *J Neurosci.* 2004;24(6):1468–77.
41. Wang N, Leung HT, Pak WL, Carl YT, Wadzinski BE, Shieh BH. Role of protein phosphatase 2A in regulating the visual signaling in Drosophila. *J Neurosci.* 2008;28(6):1444–51.
42. Yamanaka N, Romero NM, Martin FA, Rewitz KF, Sun M, O'Connor MB, Leopold P. Neuroendocrine control of Drosophila larval light preference. *Science.* 2013;341(6150):1113–6.
43. Tanji T, Ip YT. Regulators of the Toll and Imd pathways in the Drosophila innate immune response. *Trends Immunol.* 2005;26(4):193–8.
44. Hanson MA, Lemaître B. New insights on Drosophila antimicrobial peptide function in host defense and beyond. *Curr Opin Immunol.* 2020;62:22–30.
45. Lindsay SA, Wasserman SA. Conventional and non-conventional Drosophila Toll signaling. *Dev Comp Immunol.* 2014;42(1):16–24.
46. Ji Y, Thomas C, Tulin N, Lodhi N, Boamah E, Kolenko V, Tulin AV. Charon Mediates Immune Deficiency-Driven PARP-1-Dependent Immune Responses in Drosophila. *J Immunol.* 2016;197(6):2382–9.
47. Han JH, Lee SH, Tan YQ, E K LeMosy, Hashimoto C. Gastrulation defective is a serine protease involved in activating the receptor toll to polarize the Drosophila embryo. *Proc Natl Acad Sci USA.* 2000;97(16):9093–7.
48. Petersen AJ, Rimkus SA, Wassarman DA. ATM kinase inhibition in glial cells activates the innate immune response and causes neurodegeneration in Drosophila. *Proc Natl Acad Sci U S A.* 2012;109(11):E656–664.
49. Chatzidoukaki O, Goulielmaki E, Schumacher B, Garinis GA. DNA Damage Response and Metabolic Reprogramming in Health and Disease. *Trends Genet.* 2020;36(10):777–91.
50. Tsai YW, Sung HH, Li JC, Yeh CY, Chen PY, Cheng YJ, Chen CH, Tsai YC, Chien CT. Glia-derived exosomal miR-274 targets Sprouty in trachea and synaptic boutons to modulate growth and responses to hypoxia. *Proc Natl Acad Sci U S A.* 2019;116(49):24651–61.
51. You S, Fulga TA, Van Vactor D, Jackson FR. Regulation of Circadian Behavior by Astroglial MicroRNAs in Drosophila. *Genetics.* 2018;208(3):1195–207.
52. Marrone AK, Edeleva EV, Kucherenko MM, Hsiao NH, Shcherbata HR. Dg-Dys-Syn1 signaling in Drosophila regulates the microRNA profile. *BMC Cell Biol.* 2012;13:26.
53. Monsanto-Hearne V, Tham ALY, Wong ZS, Asgari S, Johnson KN. Drosophila miR-956 suppression modulates Ectoderm-expressed 4 and inhibits viral replication. *Virology.* 2017;502:20–7.
54. Guven-Ozkan T, Busto GU, Schutte SS, Cervantes-Sandoval I, O'Dowd DK, Davis RL. MiR-980 Is a Memory Suppressor MicroRNA that Regulates the Autism-Susceptibility Gene A2bp1. *Cell Rep.* 2016;14(7):1698–709.
55. Kucherenko MM, Shcherbata HR. Stress-dependent miR-980 regulation of Rbfox1/A2bp1 promotes ribonucleoprotein granule formation and cell survival. *Nat Commun.* 2018;9(1):312.
56. Belles X. MicroRNAs and the Evolution of Insect Metamorphosis. *Annu Rev Entomol.* 2017;62:111–25.
57. Cusumano P, Biscontin A, Sandrelli F, Mazzotta GM, Tregnago C, De Pitta C, Costa R. Modulation of miR-210 alters phasing of circadian locomotor activity and impairs projections of PDF clock neurons in Drosophila melanogaster. *PLoS Genet.* 2018;14(7):e1007500.
58. Niu Y, Liu Z, Nian X, Xu X, Zhang Y. miR-210 controls the evening phase of circadian locomotor rhythms through repression of Fasciclin 2. *PLoS Genet.* 2019;15(7):e1007655.
59. Lyu J, Chen Y, Yang W, Guo T, Xu X, Xi Y, Yang X, Ge W. The conserved microRNA miR-210 regulates lipid metabolism and photoreceptor maintenance in the Drosophila retina. *Cell Death Differ.* 2021;28(2):764–79.
60. Cicek IO, Karaca S, Brankatschk M, Eaton S, Urlaub H, Shcherbata HR. Hedgehog Signaling Strength Is Orchestrated by the miR-310 Cluster of MicroRNAs in Response to Diet. *Genetics.* 2016;202(3):1167–83.

61. Tsurudome K, Tsang K, Liao EH, Ball R, Penney J, Yang JS, Elazzouzi F, He T, Chishti A, Lnenicka G, et al. The *Drosophila* miR-310 cluster negatively regulates synaptic strength at the neuromuscular junction. *Neuron*. 2010;68(5):879–93.
62. Li S, Shen L, Sun L, Xu J, Jin P, Chen L, Ma F. Small RNA-Seq analysis reveals microRNA-regulation of the Imd pathway during *Escherichia coli* infection in *Drosophila*. *Dev Comp Immunol*. 2017;70:80–7.
63. Li D, Ge Y, Zhao Z, Zhu R, Wang X, Bi X. Distinct and Coordinated Regulation of Small Non-coding RNAs by E2f1 and p53 During *Drosophila* Development and in Response to DNA Damage. *Front Cell Dev Biol*. 2021;9:695311.
64. Mummery-Widmer JL, Yamazaki M, Stoeger T, Novatchkova M, Bhalerao S, Chen D, Dietzl G, Dickson BJ, Knoblich JA. Genome-wide analysis of Notch signalling in *Drosophila* by transgenic RNAi. *Nature*. 2009;458(7241):987–92.
65. Wen K, Yang L, Xiong T, Di C, Ma D, Wu M, Xue Z, Zhang X, Long L, Zhang W, et al. Critical roles of long noncoding RNAs in *Drosophila* spermatogenesis. *Genome Res*. 2016;26(9):1233–44.

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