

Inhibition of ATM kinase rescues planarian regeneration after lethal radiation

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Abstract

As stem cells divide, they acquire mutations that can be passed on to daughter cells. To mitigate potentially deleterious outcomes, cells activate the DNA damage response (DDR) network, which governs several cellular outcomes following DNA damage, including repairing DNA or undergoing apoptosis. At the helm of the DDR are three PI3-like kinases including Ataxia-Telangiectasia Mutated (ATM). We report here that knockdown of ATM in planarian flatworms enables stem cells to withstand lethal doses of radiation which would otherwise induce cell death. In this context, stem cells circumvent apoptosis, replicate their DNA, and recover function using homologous recombination-mediated DNA repair. Despite radiation exposure, *atm* knockdown animals survive long-term and regenerate new tissues. These effects occur independently of ATM's canonical downstream effector p53. Together, our results demonstrate that in planarians, ATM promotes radiation-induced apoptosis. This acute, ATM-dependent apoptosis is a key determinant of long-term animal survival. Our results suggest that inhibition of ATM in these organisms could, therefore, potentially favor cell survival after radiation without obvious effects on stem cell behavior.

Keywords apoptosis; ataxia-telangiectasia mutated; DNA damage; planarians; stem cells

Subject Categories Autophagy & Cell Death; DNA Replication, Recombination & Repair; Stem Cells & Regenerative Medicine

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Introduction

Stem cells fuel the production of new cells during homeostasis and tissue regeneration (Tanaka & Reddien, 2011), despite continual exposure to environmental and organismal insults (e.g., aging) that increase the probability of acquiring mutations with each round of cell division. Mutations can alter the output of stem cells, often adversely, by driving senescence, premature differentiation, or pro-

moting tumorigenesis (Blanpain *et al.*, 2011; Mandal *et al.*, 2011). Whether stem cells undergo cell death or repair their DNA depends on their cell cycle status, the intrinsic turnover rate of tissues, and the probability of entering quiescence (Borges *et al.*, 2008). To combat the accumulation of DNA damage and prevent tumorigenesis, adult stem cells engage different arms of the DNA damage response (DDR) pathway depending on the tissue where they reside (Mandal *et al.*, 2011; Soteriou & Fuchs, 2018). For example, mouse hair follicle stem cells rely on increased expression of DNA repair proteins and anti-apoptotic factors to repair and evade apoptosis (Sotiropoulou *et al.*, 2010), while stem cells in the small intestine exhibit increased radiation sensitivity due to reduced expression of anti-apoptotic proteins (Merritt *et al.*, 1995). The difference among stem cell types raises the question of how stem cells cope with DNA damage and the underlying mechanisms that determine their fates.

In many species, the choice to repair DNA or initiate apoptosis is coordinated by three apical kinases Ataxia-Telangiectasia Mutated (ATM), Ataxia-Telangiectasia and Rad3-related (ATR), and DNA-dependent protein kinase (DNA-pk), which are members of the phosphoinositide 3-kinase (PI3K)-related kinase family (Fig EV1A; Blackford & Jackson, 2017). ATM and ATR serve as important checkpoint regulators that stall the cell cycle upon DNA damage (Blackford & Jackson, 2017). Human cells lacking ATM are often hypersensitive to DNA damage induced by radiation (Xu & Baltimore, 1996; Shiloh & Ziv, 2013), resulting in the cellular accumulation of double-strand breaks. Cellular outcomes following DNA damage can vary depending on cell type. For example, *ATM*^{-/-} thymocytes and postmitotic neurons are resistant to radiation-induced apoptosis (Xu & Baltimore, 1996; Herzog *et al.*, 1998), while *ATM*^{-/-} hematopoietic stem cells fail to self-renew long term (Ito *et al.*, 2004; Fortin *et al.*, 2021). Collectively, these results suggest that tuning apical kinase activity in different cell types could be a strategy used to manipulate stem cell outcomes.

Planarian flatworms have an abundant population of adult stem cells that actively divide to maintain tissue homeostasis and fuel regeneration (Rink, 2013). Exposing planarians to ionizing radiation introduces double-strand breaks (DSBs) that induce apoptosis (Pellettieri *et al.*, 2010). High-dose radiation (60 Gray) depletes all the stem cells within 2 days (Reddien *et al.*, 2005b; Eisenhoffer *et al.*, 2008; Wagner *et al.*, 2012), while lower doses of radiation (20 Gray)

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allow a few stem cells to persist. Although these cells slowly resume dividing, the animals still succumb (Wagner *et al*, 2011; Lei *et al*, 2016), suggesting that irreversible damage has occurred. This eventual death suggests that despite survival at the cellular level, stem cells are unable to counterbalance DNA damage at sufficient rates to fuel long-term animal viability.

Even at radiation doses of 20 Gray (Gy), the fact that some stem cells survive while others perish within the same animal suggests that factors such as cell cycle state play an important role in driving radiation resistance. Despite conservation of key cell cycle regulators and components of the DDR pathway (Grohme *et al*, 2018; Sahu *et al*, 2021), molecular mechanisms that govern planarian stem cell responses to radiation are as yet unclear (Barghouth *et al*, 2019). Here, we capitalize on the radiation sensitivity of planarian stem cells to understand how these cells decide whether to repair their DNA or undergo apoptosis. We find that after radiation exposure, ATM drives these cells to undergo apoptosis, and that depletion of ATM prevents radiation-induced stem cell death. Those stem cells that persist can proceed into the S and G2 phase of the cell cycle. These cells subsequently re-enter the cell cycle, likely through homologous recombination-mediated repair, and successfully fuel animal regeneration and repair. Together, our data uncovers a specific role for ATM in driving apoptosis after radiation, and highlights how the function of this conserved protein might have evolved in planarians. The findings in this study could, therefore, reveal mechanisms underlying stem cell rejuvenation that operate in highly regenerative animals.

Results

ATM is required for radiation-induced apoptosis of stem cells

To determine whether apical kinases regulate stem cell responses to radiation in planarians, we used RNA interference (RNAi) to knock down key components of the DNA damage response, including orthologs of apical kinases (*atm*, *atr*, and *dna-pk*) and the MRN complex (*mre11*, *rad50*, and *nbs1*), which localizes ATM to double-strand breaks. Analysis of single-cell RNA-sequencing databases shows that expression of these genes is enriched in stem cells as compared to differentiated cells (Fig EV1B). Two days after exposure to 20 Gy, we examined stem cell distribution and abundance with *in situ* hybridization and qRT-PCR for the canonical stem cell marker *piwi-1* (Reddien *et al*, 2005b). Knockdown of *atm*, *atr*, and MRN complex components caused *piwi-1*⁺ cells to persist after radiation (Fig 1A and B), while virtually no stem cells remained in control animals. Among these genes, *atm* (RNAi) preserved the most stem cells, as shown by *piwi-1* expression levels in qRT-PCR (Fig 1B), regardless of the RNAi trigger (Fig EV2A–C). Based on these results, we conclude that the apical kinases *atm* and *atr*, but not *dna-pk*, regulate radiation-induced stem cell loss.

To evaluate if stem cells in radiated *atm*(RNAi) animals might be evading cell death, we purified stem cells (X1s) using fluorescence activated cell sorting (FACS) (Reddien *et al*, 2005b; Hayashi *et al*, 2006) and stained them with the cell death marker Annexin V (Peiris *et al*, 2016a; Shiroor *et al*, 2020). One day after radiation, *atm*(RNAi) animals had significantly more X1s as compared to

controls (9.5% vs. 3.8%; Fig 1C and D), which was accompanied by a reduction in Annexin V staining (Fig 1E and F). By contrast, in unirradiated RNAi animals, there was no difference in either stem cell abundance or Annexin-V-positive X1s (Figs 1D and F and EV2D–G), suggesting that ATM is specifically activated by ionizing radiation to induce apoptosis.

Radiation variably affects the persistence and responses of stem cells, quiescent stem cells, and transient amplifying cells. For example, some cell types accelerate apoptosis (Sotiropoulou *et al*, 2010), or will differentiate prematurely (Barazzuol *et al*, 2017), in order to prevent propagating mutations into daughter cells. To address the possibility that radiation-induced behavior of other cell types may be affected by *atm* knockdown, we examined several differentiated cell types and progenitor markers 2 days after radiation exposure in *atm*(RNAi) animals. A major output of planarian stem cells are cells of the epidermal lineage (Eisenhoffer *et al*, 2008), which mature without cell division by successive transcriptional activation of *p53*, *prog-1*, and *agat* (Wagner *et al*, 2012; van Wolfswinkel *et al*, 2014; Tu *et al*, 2015). We observed no change in *p53* or *agat* abundance after radiation (Fig EV3). Although we observed a slight preservation of *prog-1* cells in *atm*(RNAi) animals after radiation, the magnitude of difference was minimal compared to the *piwi-1*⁺ stem cells. Markers of strictly postmitotic intestinal or neural cells (*mat* and *chat*, respectively) were unchanged. These data, therefore, indicate that the abundant stem cells that persist after radiation in *atm* (RNAi) animals do not appear to differentiate prematurely as a protective mechanism.

To examine the transcriptional response that enables stem cell survival after radiation, we purified stem cells from *atm*(RNAi) animals and subjected them to RNA-sequencing (Fig 2A). Following radiation, 431 transcripts were upregulated in *atm*(RNAi) stem cells, while in unirradiated animals, the only differentially expressed transcript was *atm* (Fig 2B and C; Datasets EV1 and EV2, and Table EV1). Classification of upregulated transcripts using Gene Ontology (GO) analysis revealed that *atm*(RNAi) stem cells were enriched for terms related to cell cycle regulation, DNA replication, and gene expression (Fig EV4). To determine if these genes might function similarly to *atm*, we knocked down transcripts either significantly upregulated in *atm*(RNAi) stem cells or those associated with DDR terms (Fig 2D). Among these, only the conserved ATM effector, *checkpoint kinase 2* (*chk2*), phenocopied *atm*(RNAi) (Fig 2E and F; Thompson, 2012). Notably, knockdown of the classic ATM effector *p53* did not influence stem cell persistence following radiation. This finding is consistent with the function of planarian *p53* having diverged away from monitoring genotoxic stress (Pearson & Sánchez Alvarado, 2010; Cheng *et al*, 2018; Wendt *et al*, 2022). Together, these data indicate that the MRN-ATM-CHK2 axis controls stem cell abundance after radiation, independently of canonical *p53* activity.

Planarian ATM is required for the G1/S checkpoint

Based on ATM's known roles in activating cell cycle checkpoints (Waterman *et al*, 2020), we analyzed cell cycle progression in *atm* (RNAi) animals following radiation. First, to label cells undergoing DNA replication, we administered the thymidine analog F-ara-EdU (EdU) (Neef & Luedtke, 2011; Bohr *et al*, 2021) for 24 h on the first or second day after radiation exposure (Fig 3A). In stark contrast to

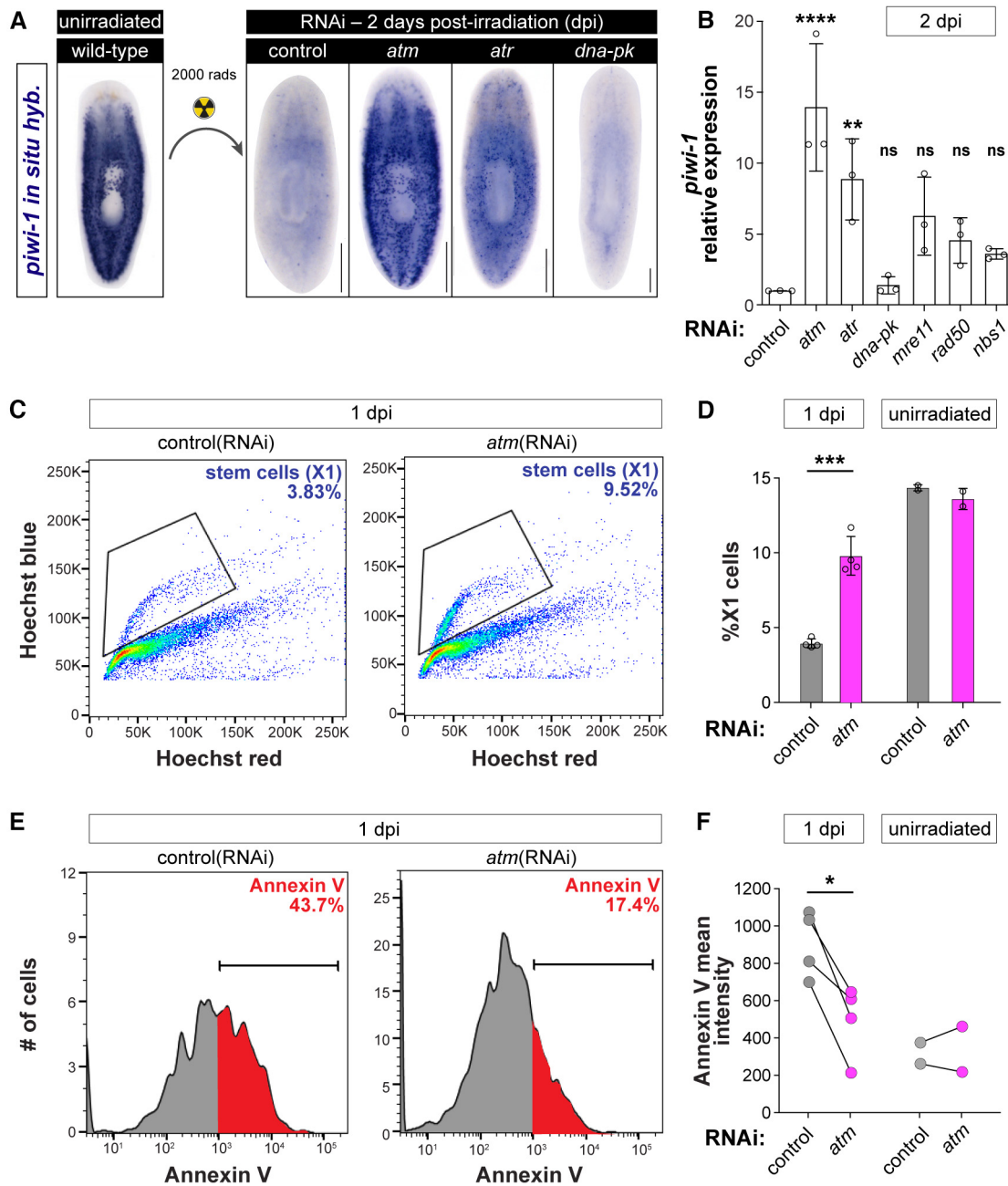


Figure 1. ATM drives stem cell apoptosis after radiation.

A Experimental design and *piwi-1* whole mount *in situ* hybridization (WISH) of animals after RNAi, 2 days post-irradiation (dpi). Scale bars = 250 μ m. Experiment repeated > 3 times for all conditions.

B qRT-PCR of *piwi-1* relative to GAPDH in RNAi animals as indicated, 2dpi. Circles represent individual biological replicates averaged from three technical replicates.

C FACS plots of Hoechst-stained X1 cells from control and *atm*(RNAi) animals, 1 day post-irradiation (dpi). Black polygon = X1 cells, percentage indicated in blue.

D Percentage of X1 cells from control and *atm*(RNAi) animals, 1 dpi or unirradiated (Fig EV2F).

E Histograms of X1s from (C) stained with Annexin V (percentage in red). Brackets and red shaded area = Annexin V mean fluorescent intensity staining > 10^3 .

F Mean fluorescent intensity of Annexin V calculated from the bracketed region in (E) or Fig EV2G; lines connect results from individual experiments.

Data information: For all panels, radiation dose = 20 Gy. Data are averages $\pm$ SD; circles represent biological replicates; ns, not significant. In (B), statistics represent ordinary one-way ANOVA compared to controls, **** P < 0.0001, ** P < 0.005, repeated twice. In (D) and (F), statistics represent unpaired t-test, **** P < 0.0001, * P < 0.02; radiated samples were repeated 4 times; unirradiated were repeated twice. Source data are available online for this figure.

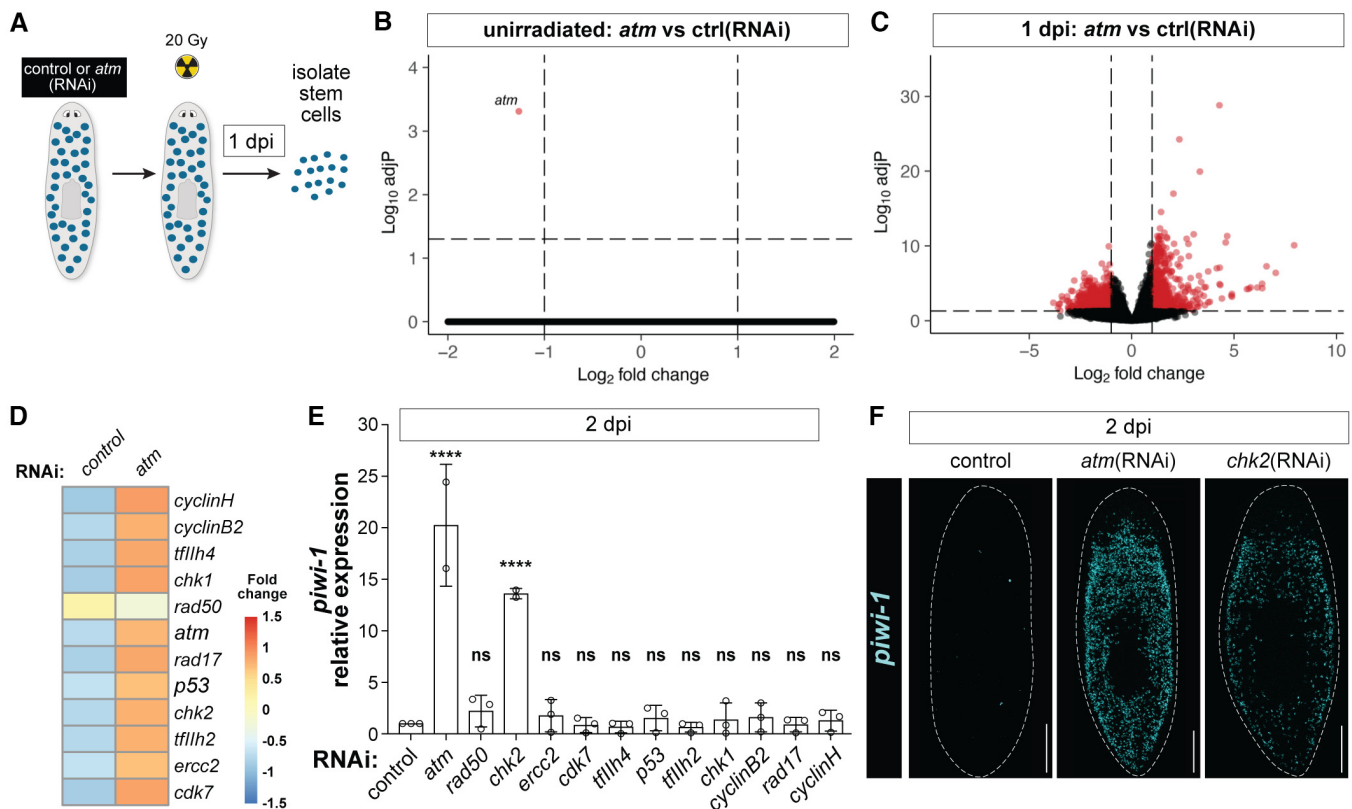


Figure 2. RNA-seq analysis of stem cells following *atm*(RNAi).

A Experimental schematic showing strategy to purify stem cells from RNAi animals prior to RNA-seq.
 B Volcano plot of differentially expressed transcripts in unirradiated RNAi animals as indicated. Red dots indicate transcripts with $|\log_2 \text{fold change}| < 1$ and adjusted P -value < 0.01 .
 C Volcano plot of differentially expressed transcripts in RNAi animals 1 day after irradiation (dpi). Red dots indicate transcripts with $|\log_2 \text{fold change}| < 1$ and adjusted P -value < 0.01 .
 D Heat map of upregulated transcripts used for RNAi in Fig 2E.
 E qRT-PCR of *piwi-1* relative to GAPDH in RNAi animals as indicated, 2dpi. Data are averages $\pm$ SD; circles represent biological replicates; statistics represent ordinary one-way ANOVA compared to controls; **** $P < 0.0001$, ns, not significant.
 F *piwi-1* fluorescent *in situ* hybridization (FISH) of RNAi animals as indicated, 2 dpi.
 Source data are available online for this figure.

controls, most *atm*(RNAi) animals had abundant EdU signal 1 day after radiation (Fig 3A and B), indicating that despite damage, DNA replication continues, suggestive of a failure to activate either the G1/S or intra-S checkpoint. This transient progression into S phase after *atm* knockdown phenocopies the radioresistant DNA synthesis observed in human cells with mutations in ATM (Painter, 1981). Second, to determine whether cells have also progressed into G2, we quantified DNA content with DAPI staining, and observed an increase in G2/M cells in *atm*(RNAi) animals (Fig 3C). However, staining for the mitotic marker phosphohistone-H3 (pH3) 2 days after radiation showed no cells in M phase (Fig 3D and E), suggesting that stem cells in *atm*(RNAi) animals accumulate in S and G2, but cannot progress into metaphase. Similar to radiated human cells (Xu *et al*, 2002), planarian ATM is likely required for immediate cell cycle arrest because of its ability to detect double-strand breaks, but is dispensable for G2/M transitions. Therefore, upon DNA damage, ATM is necessary for G1/S and intra-S checkpoints, but not the G2/M checkpoint.

Stem cells in *atm*(RNAi) animals resume cycling after radiation

Radiation exposure causes an immediate arrest in cell cycle progression (Verma *et al*, 2021). Sufficiently low doses allow a small number of stem cells to persist and gradually form colonies, or clusters, of *piwi-1*⁺ cells as they resume dividing (Wagner *et al*, 2011, 2012). To determine whether the stem cells that escape apoptosis in *atm*(RNAi) animals are capable of resuming mitosis and repopulating the animal, we monitored stem cells for 3 weeks after radiation. We detected the reappearance of phosphohistone-H3-positive cells in *atm*(RNAi) animals beginning 1 week after radiation, with their numbers increasing significantly thereafter (Fig 3D and E). In unirradiated RNAi animals, we observed similar rates of proliferation over this timecourse (Fig 3F), indicating that ATM is not required for cell cycle progression until animals are exposed to ionizing radiation.

To assess whether the rate of colony formation is altered by the increased number of stem cells that persist in *atm*(RNAi) animals

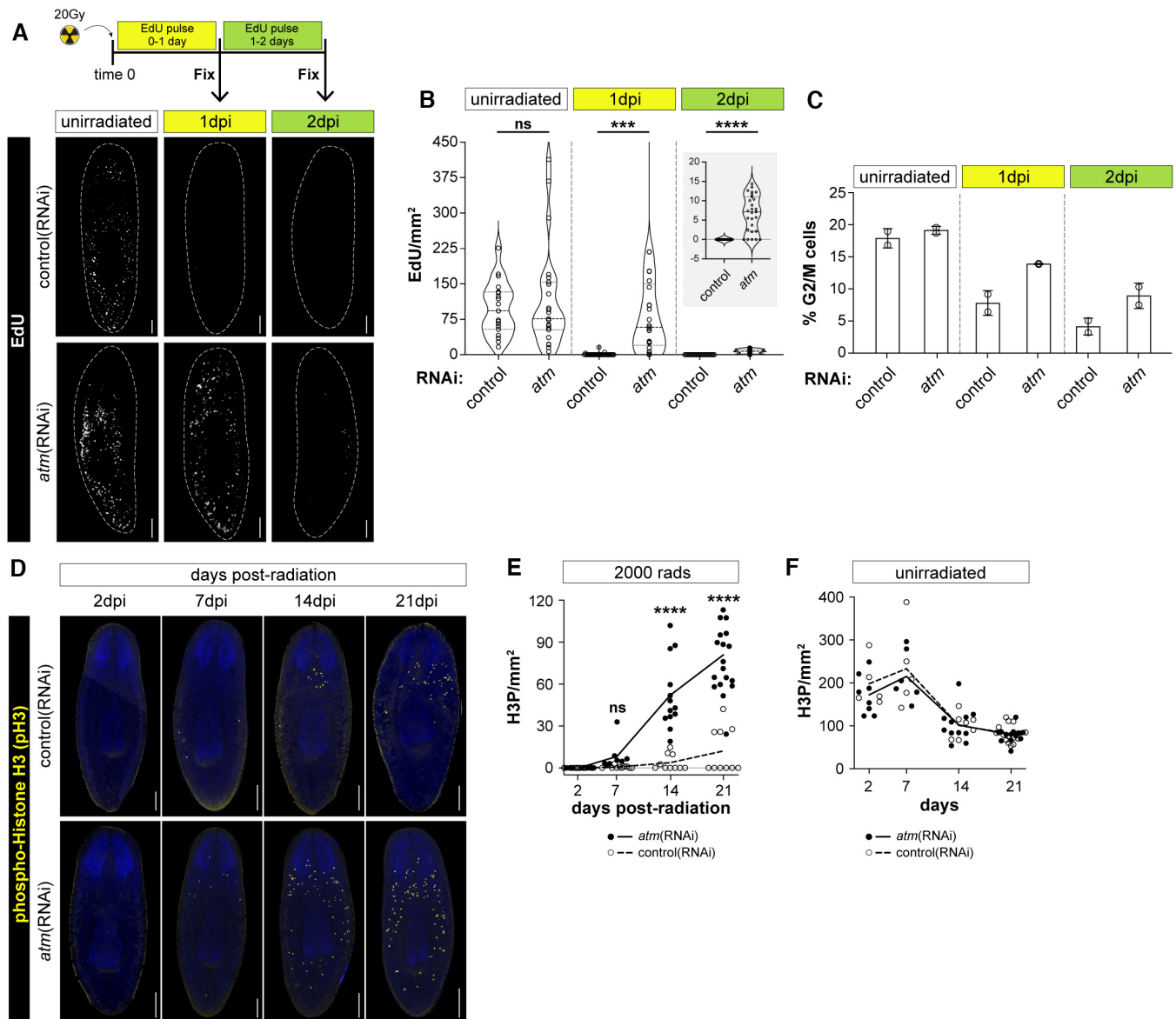


Figure 3. *atm*(RNAi) compromises G1/S, but not G2/M checkpoints, after radiation.

A Top: Strategy for EdU pulse and animal fixation. Bottom: Confocal images of EdU-stained *atm* and control RNAi animals.
B Quantification by area of EdU⁺ cells from (A). Circles represent individual animals. Inset, expanded scale showing compressed data below. Data include two independent experiments.
C Graph showing percentage of G2/M cells in RNAi animals on indicated dpi.
D Confocal images of phosphohistone H3 (pH3)-stained RNAi animals after radiation exposure. Blue, DAPI.
E, F Quantification by area of pH3 cells in radiated (E) or unirradiated (F) animals after *atm* (solid line) and control (dashed line) RNAi.

Data information: Data are averages $\pm$ SD; circles represent biological replicates in (C); in all other panels they represent quantified individual animals; (E–F) show data from a single experiment that has been repeated > 3 times; statistics represent unpaired t-test between control and *atm*(RNAi), **** p < 0.0001, *** p < 0.0004, ns, not significant. Scale bars = 250 μ m.

Source data are available online for this figure.

after radiation exposure, we quantified *piwi*⁺ cells over the course of 3 weeks. We observed a drop in the abundance of stem cells 1 week after radiation (Fig 4A and B), followed by significant repopulation thereafter, while no change occurred in unirradiated controls (Fig 4C). The initial decline of stem cells in *atm*(RNAi) animals

between 2 and 7 days after radiation (Fig 4B) implies that either ATM is not required for apoptosis during this time, or that there may be residual ATM activity eliminating stem cells with severe DNA damage. Despite this decrease in stem cell number, more stem cells persist in *atm*(RNAi) animals 7 days after radiation as

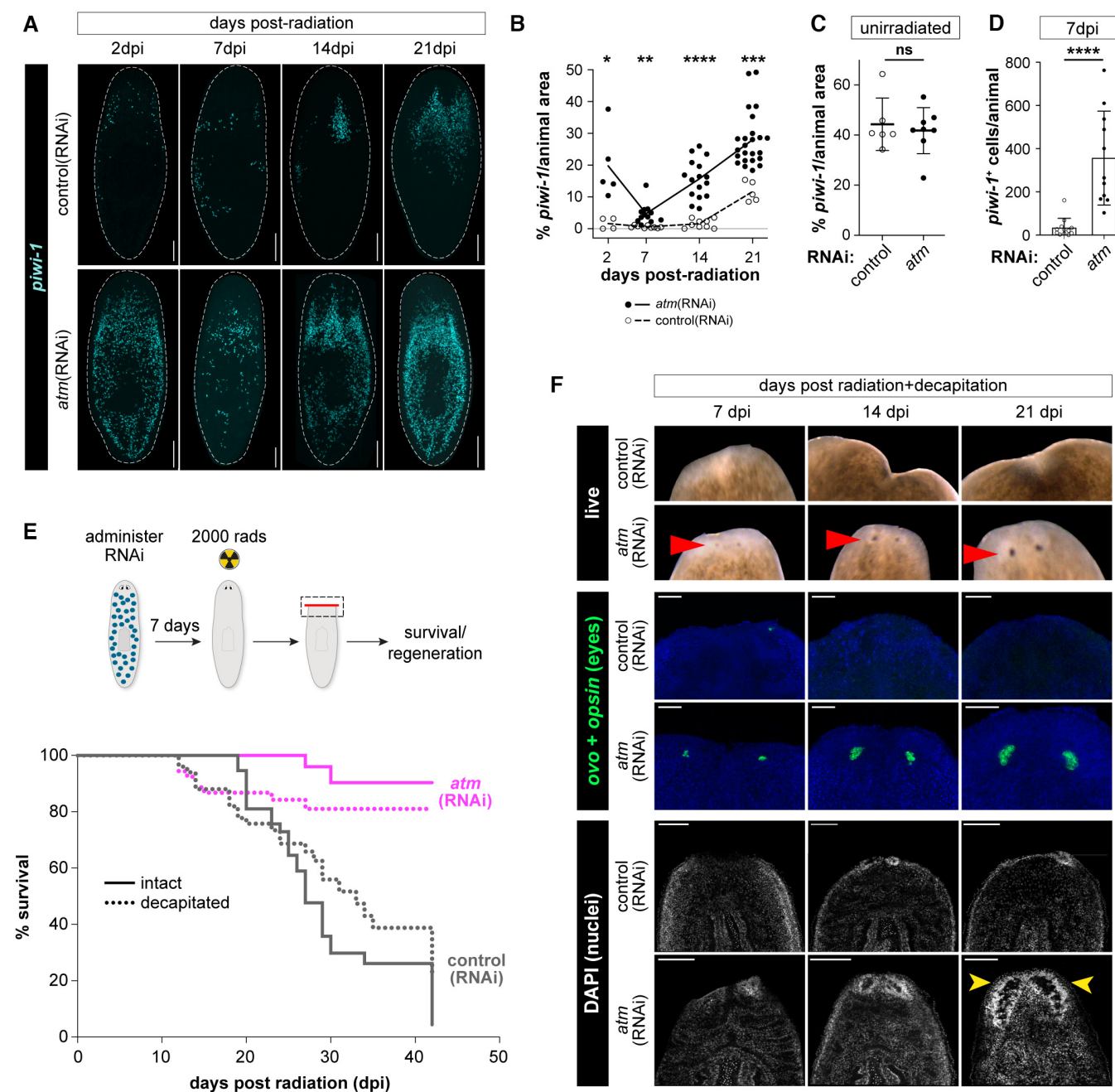


Figure 4. Stem cells repopulate and fuel long-term survival of *atm*(RNAi) animals.

A Confocal images of *piwi-1* FISH in RNAi animals after radiation exposure. Scale bars = 250 μ m.

B, C Percentage of animal area occupied by *piwi-1* FISH divided by total animal area, in radiated (**B**) or unirradiated (**C**) RNAi animals.

D Total number of *piwi-1*⁺ cells in RNAi animals, 7 dpi. Experiments in (A–D) were repeated twice.

E Schematic of experimental design. Below, Kaplan–Meier survival curves of intact (solid line) and decapitated (dashed line) animals after RNAi as indicated. $n = 45$ animals, repeated twice.

F Live images of head region (dashed box in [E]); middle, *ovo + opsin* FISH for eyes (green) counterstained with DAPI (blue); bottom, DAPI staining of the brain. Red arrowheads, eyes; yellow arrowheads, brain.

Data information: Data are averages $\pm$ SD; in (B–D), circles represent individual animals from two independent experiments; statistics represent unpaired t-test between control and *atm*(RNAi), **** $P < 0.0001$, *** $P < 0.0004$, ** $P < 0.0002$, * $P < 0.05$, ns, not significant. Scale bars = 100 μ m.

Source data are available online for this figure.

compared to controls (Fig 4D). This accelerated repopulation of stem cells is likely generated by the steady increase in proliferation in *atm*(RNAi) animals (Fig 3A and B). Together, these findings indicate that *atm*(RNAi) stem cells can ultimately pass through cell cycle checkpoints, suggesting that DNA damage has been repaired.

Stem cells in *atm*(RNAi) animals fuel regeneration and long-term animal survival

Planarians require stem cell division and differentiation for tissue renewal and regeneration. Radiation interrupts cell divisions, and normally results in animal death in about 6 weeks (Wagner et al, 2011; Li et al, 2021). To test whether the stem cells that repopulate *atm*(RNAi) animals are functional, we monitored animal survival after exposing them to a dosage of 20 Gy (Fig 4E). In control animals, only a few stem cells persist after this dose, and these cells are insufficient to support regeneration (Wagner et al, 2011; Shiroor et al, 2020). Unexpectedly, over 90% of radiated *atm*(RNAi) animals survived long-term without developing gross abnormalities (Fig 4E and F; Pearson & Sánchez Alvarado, 2010). To examine regeneration, we decapitated animals immediately after radiation and allowed them to recover. Consistent with the survival data, 80% of *atm*(RNAi) animals fully regenerated new heads, including photoreceptors and the central nervous system (Fig 4F). The recovery of regenerative capacity indicates that stem cells in *atm*(RNAi) animals are competent to restore stem cell function, including normal differentiation and patterning.

Long-term survival relies on homologous recombination-mediated DNA repair

Cells repair DSBs through activation of homologous recombination (HR) and nonhomologous end joining (NHEJ) (Ciccia & Elledge, 2010; Thompson, 2012; Santivasi & Xia, 2014). To test the dependence of surviving stem cells on these DNA repair pathways, we simultaneously knocked down components of HR or NHEJ in *atm* (RNAi) animals. We used the recombinase *rad51* or its interacting partner *brca2* as a proxy for HR (Peiris et al, 2016b; Sahu et al, 2021), and *dna-pk* as a proxy for NHEJ. Knockdown of either *rad51* or *brca2* accelerated animal death after radiation (Fig 5A). This accelerated death was unaffected by simultaneous knockdown of *atm*, suggesting that although *atm* preserves stem cells initially after radiation by preventing apoptosis, long-term survival requires homologous recombination-mediated DNA repair. By contrast, knockdown of the apical kinase *dna-pk*, which mediates NHEJ, did not substantially alter the timing of animal death after radiation as compared to controls (Fig 5B). However, animals in which *dna-pk* was knocked down along with *atm* survived long-term. Moreover, we also tested the canonical downstream effectors of NHEJ including DNA ligase IV (*ligIV*) and the nuclease *artemis*. Single knockdown of these components, similar to *dna-pk*, resulted in animal death on the same timescale as control animals. By contrast, double knockdown with *atm* led to long-term animal survival, which phenocopied what we observed with *dna-pk* (Fig 5B). Although we could not verify knockdown of NHEJ components at the transcript level, the fact that knockdown of *artemis*, *ligIV*, and *dna-pk* along with *atm* all promoted long-term animal survival supports the conclusion that NHEJ is incapable of

restoring stem cell function after radiation. Together, these results demonstrate that even when apoptosis is inhibited by *atm*(RNAi), NHEJ-mediated DNA repair is insufficient to support stem cell recovery from the deleterious effects of radiation. Rather, in stem cells that escape apoptosis, HR-mediated repair likely acts to restore stem cell function.

To determine how NHEJ and HR components contribute to the persistence and recovery of stem cells, we analyzed the impact of their knockdown at earlier times after radiation. Two days after radiation, stem cell abundance was not significantly altered by knockdown of the NHEJ apical kinase *dna-pk*, or of HR components *brca2* or *rad51* when combined with *atm* knockdown (Figs 5D and EV5A). However, the robust protection of stem cells after radiation in *atm*(RNAi) animals confounded our ability to identify precise roles for NHEJ or HR. Therefore, we titrated the dose of radiation in an effort to preserve more stem cells and provide a sensitized background to test the role of NHEJ and HR components. Single knockdown of both *brca2* and *rad51* caused a pronounced depletion of stem cells after exposure to 5 or 15 Gy as compared to controls (Fig EV5B and C), indicating that HR-dependent repair contributes to acute stem cell persistence after radiation. By contrast, knockdown of NHEJ-specific components *artemis*, *ligIV*, *ku70*, and *ku80* caused modest increases in stem cell survival and proliferation 2 days after radiation (Fig EV5D–G). This effect of NHEJ suppression in preserving stem cells lasts for 7 days after radiation in *atm*;*dna-pk* double knockdown animals (Fig 5E), while stem cells are lost in *atm*;*brca2* or *atm*;*rad51* double knockdown animals. Taken together, our data demonstrate that both the initial persistence of stem cells and long-term survival strongly rely on HR-dependent mechanisms, rather than on NHEJ.

Discussion

Radiation sensitivity has been crucial for uncovering diverse aspects of planarian stem cell biology. The ability to deplete stem cells via exposure to ionizing radiation has established these cells to be the sole drivers of regeneration and tissue repair (Reddien et al, 2005b), integral to animal survival. Planarian stem cells are sensitive to radiation-induced apoptosis in a dose-dependent manner, with exposure to 15 Gy or higher eliminating the vast majority of them (Wagner et al, 2011, 2012; Lei et al, 2016; Zeng et al, 2018; Shiroor et al, 2020). Even though a few stem cells might survive, they persist only transiently, and cannot support animal survival. These observations raise many questions. What are the factors that dictate stem cell persistence or death? Are the mechanisms of DNA damage response conserved in planarians? What facilitates stem cell recovery?

We investigated these questions by examining stem cell persistence after radiation and find that the highly conserved, apical DNA damage response kinase ATM is a primary driver of planarian stem cell apoptosis after radiation. ATM acts as an executioner after DNA damage, efficiently removing the vast majority of stem cells. In other systems, ATM is required for cell cycle progression, stem cell renewal, apoptosis, and normal development even in the absence of severe DNA damage (Barlow et al, 1996; Herzog et al, 1998; Ito et al, 2004). By contrast, planarian ATM acts strictly after ionizing radiation to enforce cell cycle checkpoints and cull damaged cells

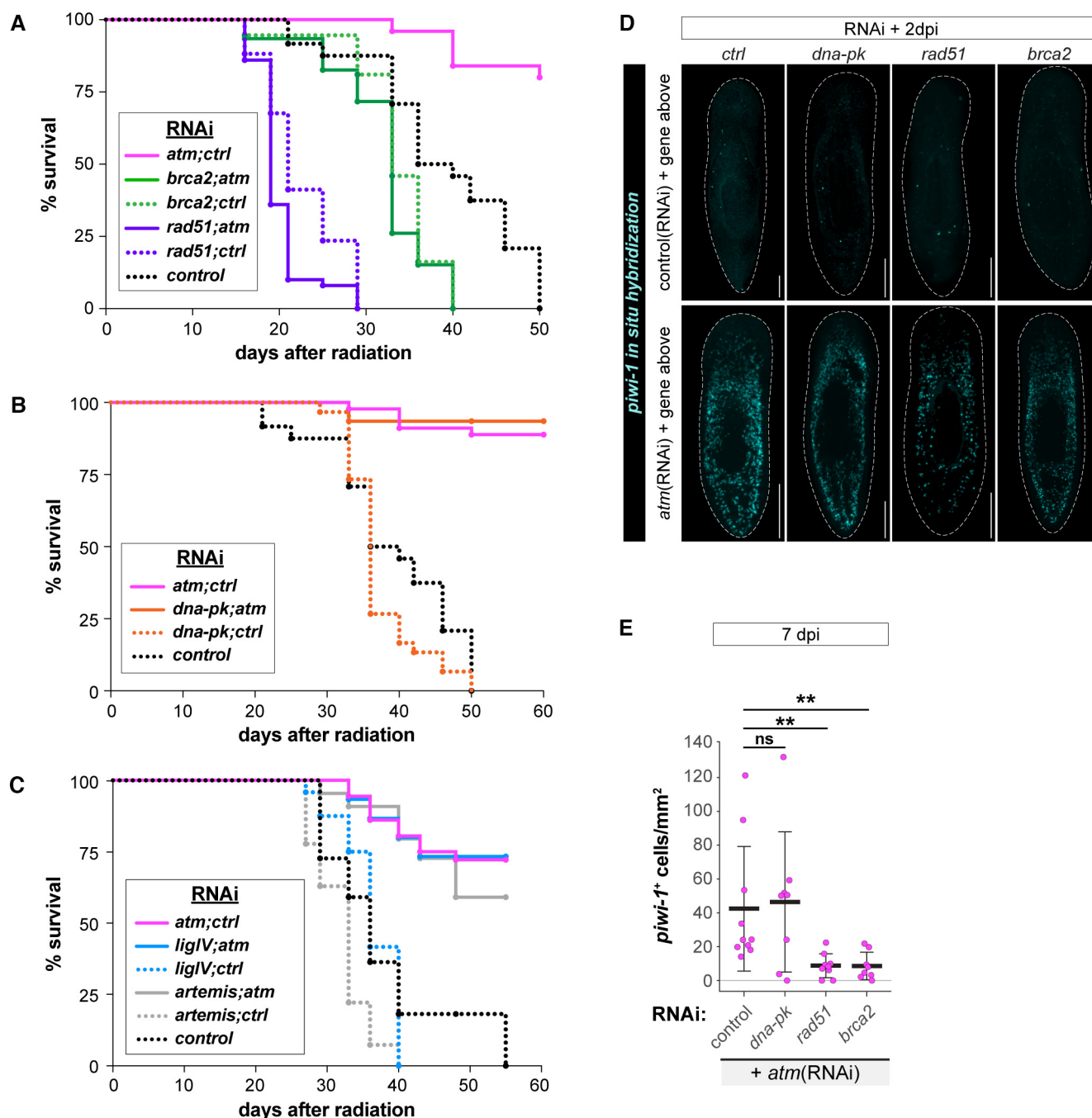


Figure 5. Animal survival after radiation relies on homologous recombination.

A–C Kaplan–Meier survival curves of intact RNAi animals after radiation, $n = 50$ animals, repeated twice. Experiments in (A) and (B) were performed simultaneously but split into two graphs for clarity; control and *atm*(RNAi) values are replicated in both panels.

D Confocal images of *piwi-1* FISH in RNAi animals after exposure to 20 Gy, 2 dpi. Scale bars = 250 μ m.

E Quantification by area of *piwi-1*⁺ cells in RNAi animals after 20 Gy exposure, 7 dpi.

Data information: Data are averages $\pm$ SD; in (E), circles represent individual animals from a single experiment; statistics represent ordinary one-way ANOVA compared to controls, $**P < 0.005$, ns, not significant. (D) and (E) include data from a single experiment.

Source data are available online for this figure.

via apoptosis. Surprisingly, when ATM is knocked down, stem cells recover full functionality, driving regeneration, and long-term animal survival through high-fidelity homologous recombination (HR)-

mediated repair. We propose that escaping acute ATM-dependent apoptosis after radiation enables faithful DNA repair and stem cell recovery (Fig 6A).

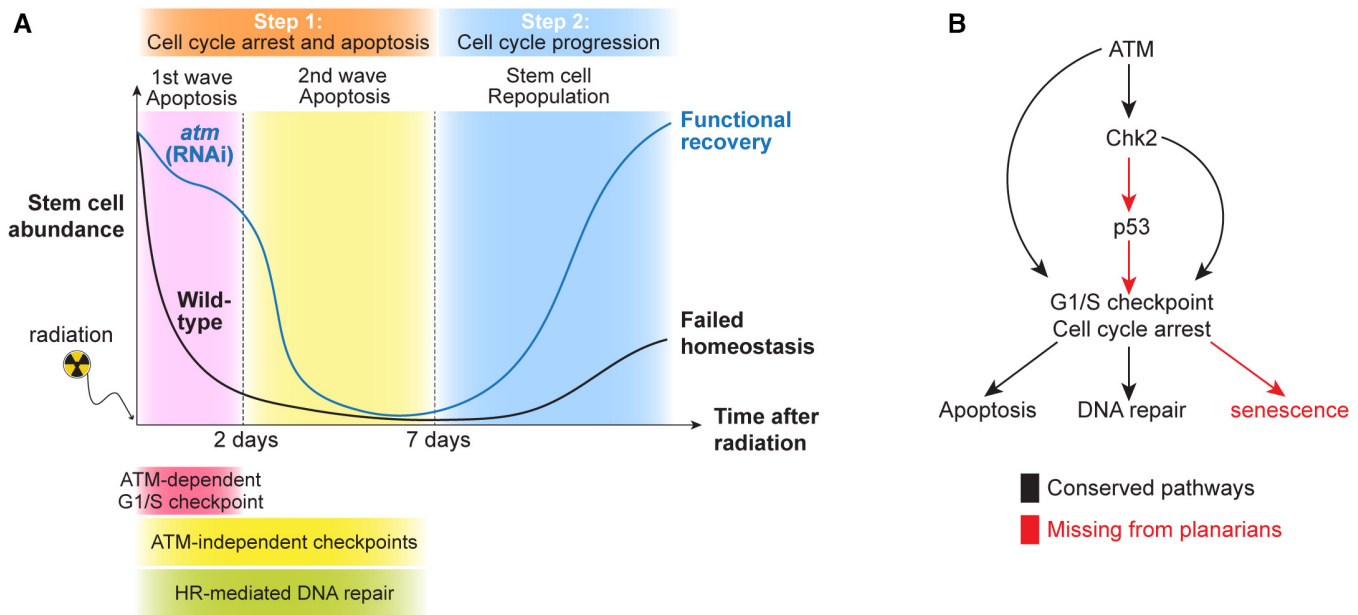


Figure 6. Mechanisms underlying ATM-dependent rescue from radiation in planarians.

A Schematic of the dynamics of stem cell abundance after radiation. x-axis represents the time after radiation; y-axis represents the abundance of stem cells; bottom boxes indicate underlying molecular mechanisms.

B Schematic of ATM pathway in planarians. Black represents the genes or pathways active in planarians; red represents the absence of the pathways.

ATM arrests cell cycle progression in response to DNA damage

In humans, ATM regulates multiple cell cycle checkpoints including G1/S, intra-S, and G2/M (Khanna *et al*, 2001). Despite the existence of conserved components of cell cycle checkpoints in the planarian genome, functional analysis of these proteins has been limited (Reddien *et al*, 2005a; Kang & Sánchez Alvarado, 2009). We show that planarian ATM primarily regulates the G1/S but not the G2/M checkpoint (Fig 3). When ATM is knocked down, stem cells continue replicating DNA, and accumulate in the S and G2 phases, but fail to progress into metaphase (Fig 3C and D). Continued DNA synthesis after radiation also occurs in ATM-deficient human cells (Painter, 1981). We propose that this checkpoint failure in *atm* (RNAi) animals generates stem cells with replicated chromosomes that could serve as a template for subsequent DNA repair (see below).

The acute burst of apoptosis induced by radiation depends on ATM (Fig 1). Despite conservation at the molecular level, mechanisms responsible for cell death in planarians have remained elusive (Pellettieri & Sánchez Alvarado, 2007; Bender *et al*, 2012). In other systems, ATM is recruited to sites of damage by the MRN complex (Blackford & Jackson, 2017). Then, it induces apoptosis by phosphorylating its downstream effector CHK2, which in turn phosphorylates p53 (Figs 6B and EV1A). Although our results confirm that the MRN complex and *chk2* components of this signaling axis are conserved (Fig 2E and F), the function of p53 in driving apoptosis in response to genotoxic stress seems to have been lost in planarians (Fig 2E; Cheng *et al*, 2018; Wendt *et al*, 2022). Determining what proteins act downstream of ATM to mediate apoptosis will be an interesting avenue for future investigation.

Despite their initial preservation after radiation in *atm* knock-down animals, stem cells continue to decrease in number for a week. This loss of stem cells may be due to a second wave of stem cell apoptosis that is likely induced by ATM-independent checkpoints that detect DNA damage and induce apoptosis as cells progress through the cell cycle. Once the cell cycle resumes (7–14 days post-irradiation), stem cells repopulate *atm*(RNAi) animals much faster than in wild-type counterparts. This accelerated repopulation may be due to the increased absolute number of stem cells that are present in *atm* knockdown animals as compared to controls (Fig 4A, B and D). Alternatively, the continued DNA replication that occurs after radiation exposure in *atm*(RNAi) animals may enable high-fidelity DNA repair that allows stem cells to successfully pass through cell cycle checkpoints.

DNA repair mechanisms in planarians

Stem cells in *atm*(RNAi) animals can ultimately repopulate and sustain animal survival after radiation, suggesting that DNA repair mechanisms remain intact in the absence of ATM. In other contexts, ATM activates both HR and NHEJ (Blackford & Jackson, 2017). Stem cell recovery depends strongly on HR (Fig 5), demonstrated by the fact that radiated animals with *rad51* or *brca2* knockdown succumb faster than controls, even when combined with *atm*. By contrast, single knockdown of NHEJ-specific components *artemis*, *ligIV*, or *dna-pk* does not alter animal lifespan, indicating that even when HR is intact, it is insufficient to drive stem cell or animal survival. Combining these NHEJ-specific knockdowns with *atm*(RNAi) (Fig 5B and C) reveals that animal survival relies on a two-step mechanism. First, stem cells escape the initial wave of ATM-dependent

apoptosis and fail to arrest in G1 or S phase after radiation. Second, homologous recombination, using a template generated during checkpoint evasion, may repair chromosomes sufficiently to progress through the cell cycle (Fig 6A). In *atm*;HR double knockdown animals where error-prone NHEJ is the sole repair mechanism available, stem cells likely trigger cell cycle checkpoints as they progress through the cell cycle, resulting in cell death. The dependence on HR is not surprising because this is the predominant mechanism used by actively proliferating cells in G2/M (Waterman *et al*, 2020). In wild-type animals, while both HR- and NHEJ-mediated repair are presumably activated by radiation, functional ATM precludes repair by triggering stem cell apoptosis before either mechanism can operate.

In planarians, knockdown of most HR and alternative NHEJ genes, with the exception of *rad51*, do not cause lethality during homeostasis, suggesting that they are either unnecessary or redundant for cell cycle regulation (Peiris *et al*, 2016b; Sahu *et al*, 2021). By exposing animals to variable radiation doses, we delineate how DNA repair controls stem cell behavior after radiation. Initially, HR is required for enabling stem cell persistence (Fig EV5B and C), but it is also essential for long-term stem cell and animal survival. The activity of NHEJ, on the other hand, may facilitate stem cell death and checkpoint evasion after radiation, because knockdown increases stem cell survival and enables proliferation (Fig EV5D and G). Together, these differences suggest that when intact, NHEJ and HR could compete for repair (Krenning *et al*, 2019), contributing to the initial persistence of stem cells after radiation.

Stem cell responses to radiation

The stochastic persistence of small numbers of cells after low doses of radiation raises the possibility that variation in cell cycle status could contribute to the ability to escape apoptosis (Shaltiel *et al*, 2015; Raz *et al*, 2021). Cell cycle status can also impact differential deployment of DNA repair. For example, stem cells that proliferate regularly (e.g. transit amplifying cells) rely on HR-mediated repair through generation of secondary repair templates during S phase. Quiescent stem cells, which spend the majority of their time in G0, therefore, primarily deploy NHEJ-mediated repair because they lack a copied sister chromatid (Mohrin *et al*, 2010; Oh *et al*, 2014). In addition, the intrinsic radiation responses of cells that either cycle regularly or are quiescent can influence outcomes (Blanpain *et al*, 2011; Buczacki *et al*, 2013). In adult mice for example, ATM mitigates radiation-induced damage propagation by accelerating differentiation of neuronal

progenitor cells and activating quiescent neural stem cells (Barazuol *et al*, 2017).

Key differences between planarian stem cells and mammalian stem cells contribute to the novelty of our study and highlight the advantage of studying radiation responses in planarians. Although the existence of quiescent stem cells in planarians has recently been shown by long-term label retention (Molinaro *et al*, 2021), the vast majority of stem cells in planarians are thought to cycle regularly (Newmark & Sánchez Alvarado, 2000). The sheer abundance of stem cells in planarians contrasts with the rarity of stem cells in most vertebrate species. For this reason, we speculate that unlike mammalian systems where maintaining the integrity of stem cells is of the utmost importance, planarian stem cells may be expendable. ATM readily culls cells with DNA damage, but because of the vast number of proliferating stem cells, losing some may not affect overall animal viability. Therefore, the dependence on HR-mediated DNA repair is consistent with other stem cells that divide regularly. In planarians, with such an abundant stem cell population that proliferates regularly, it is likely that rapid culling of damaged cells by ATM efficiently prevents the propagation of mutations.

The fact that planarian ATM seems to strictly function in the context of radiation may be due to altered protein interactions or pathway evolution. Planarian ATM lacks the N-terminal solenoid domains found in human ATM (Fig EV2A), where interactions with the MRN complex are thought to occur (Baretic & Williams, 2014; Stakyte *et al*, 2021). In addition, planarians lack several components of the DDR and cell cycle checkpoints (Fig EV1B), suggesting that while individual components of these pathways may be conserved, their collective functions, or their interactions, may have changed over the course of evolution (Grohme *et al*, 2018; Sahu *et al*, 2021). Intriguingly, our study reveals that not all effectors of the ATM pathway retain conserved function in planarians, including p53 (Fig 6B). The apparent loss of p53 function in driving apoptosis downstream of ATM provides an opportunity to explore how ATM mediates apoptosis independently of p53 in planarians.

In conclusion, our study identifies a new role for ATM in planarians influenced by the unique biology of these animals. While its function in regulating checkpoint progression and apoptosis is shared with other systems, its role in promoting stem cell exhaustion seems to have diverged. This difference may be due to the absence of significant portions of the protein (Fig EV2A) or from evolutionary divergence of the pathway (Fig 6B; Grohme *et al*, 2018). The evasion of ATM-induced apoptosis opens the door to elucidating the role of checkpoint proteins and DNA repair pathways in these fascinating stem cells.

Materials and Methods

Reagents and Tools table

Reagent/resource	Reference or source	Identifier or catalog number
Experimental models		
<i>Schmidtea mediterranea</i>	Grown in our lab	N/A
Recombinant DNA		
N/A		

Reagents and Tools table (continued)

Reagent/resource	Reference or source	Identifier or catalog number
Antibodies		
anti-DIG-AP (1:3,000)	Roche	Cat#11093274910; RRID: AB_514497
anti-DIG-POD (1:1,000)	Roche	Cat#11207733910; RRID:AB_514500
anti-FITC-POD (1:1,000)	Roche	Cat#11426346910; RRID:AB_840257
Rabbit anti-phosphohistone H3 (Ser10) (1:300)	Millipore Sigma	Cat#ZRB1226
Anti-Oregon Green HRP (1:1,000)	ThermoFisher	Cat#A21253
Goat anti-rabbit-HRP (1:1,000)	ThermoFisher	Cat#31460; RRID:AB_228341
Oligonucleotides and other sequence-based reagents		
<i>smedwi-1</i>	Thermo Fisher	AI89MBJ
GAPDH	Thermo Fisher	AI6RPY3
Gene-specific primers		Table EV2
Chemicals, enzymes and other reagents		
Annexin V-APC	ThermoFisher	Cat#A35110
Propidium Iodide	Sigma	Cat#P4170
F-ara-EdU	Sigma	Cat#T511293
Oregon Green 488 azide	ThermoFisher	Cat#O10180
Liberase™	Roche	Cat#5401135001
Hoechst 33342	ThermoFisher	Cat#H3570
DRAQ5	ThermoFisher	Cat#65-0880-96
Calcein-AM	ThermoFisher	Cat#C3100MP
Software		
Fiji/ImageJ	Schindelin et al (2012)	https://imagej.net/Fiji/Downloads ; RRID:SCR_001935
PRISM	GraphPad Software	www.graphpad.com ; RRID:SCR_002798
FlowJo™	Becton, Dickinson and Company	www.flowjo.com ; RRID:SCR_008520
Other		
FACS Symphony Analyzer	Becton Dickinson	
Attune NxT Analyzer	ThermoFisher	
MA900 Cell Sorter	Sony	
NEBNext UltraII Directional Library Prep Kit for Illumina	New England Biolabs	
NextSeq500	Illumina	

Methods and Protocols

Planarian care

Asexual planarians from the *Schmidtea mediterranea* clonal line CIW4 were maintained in a recirculating system containing Montjuïc salts (Arnold et al, 2016; Merryman et al, 2018) with constant UV sanitization of water. Experimental animals were transferred to a static culture of planaria water supplemented with 50 µg/ml gentamicin sulfate.

Irradiation

A J.L. Shepherd & Associates Mark I-68 Irradiator was used to radiate planarians at the doses indicated in the figures. Animals were rinsed immediately after exposure and transferred into fresh planarian water on the following day. Radiated animals maintained longer than 2 days were rinsed on alternate days.

RNA interference

RNAi was carried out as previously described, either using *in vitro* synthesized RNA (Rouhana et al, 2013) or by feeding animals with double stranded RNA-containing bacteria (Adler & Sánchez Alvarado, 2018). For *in vitro* synthesized RNAi, double stranded RNA (dsRNA) was generated using PCR products of genes as a template and mixed with a 4:1 liver:water paste containing 4 µg of dsRNA per 10 µl of liver. Animals were fed every other day, for a total of 6 feeds. For bacterial RNAi, cDNA for genes of interest were cloned into either pJC53.2 or T4P vector and transformed into HT115 competent cells. Cultures were grown in 2XYT broth at 37°C, shaking at 200–250 rpm until reaching an OD₆₀₀ of 0.6, when IPTG was added to a final concentration of 1 mM, and shaken for 2 hours. Bacterial cultures were then spun down and resuspended in a 9:1 liver:water paste. For double RNAi, equal volumes of two bacterial liquid cultures were mixed before spinning

down. Bacterial RNAi was administered for a total of 4 feeds every other day. Animals were used for experiments 5–7 days after the last RNAi feed. All experiments use *unc-22*(RNAi) as control. See Table EV2 for primer sequences used to amplify genes.

qRT-PCR

For each biological replicate, 10 animals were homogenized in Trizol (Thermo Fisher 15596018) in Lysing Matrix D Tubes (MP Biomedicals 116913100) followed by homogenization in a Bead Bug homogenizer (Benchmark). RNA was extracted using a standard Trizol extraction protocol. cDNA was synthesized with Superscript VILO (Life Technologies 11754250). PCR mixes were made with TaqMan Gene Expression Master Mix (Life Technologies) and run using custom primers for *smcdwi-1* (AI89MBJ) and GAPDH (AI6RPY3) (ThermoFisher) on an Applied Biosystems Viia7 Real Time PCR System. For each biological replicate, three technical replicates were performed and C_t values were averaged. Data were analyzed using C_t methods.

Fixations

Animals were fixed and labeled as previously described (Pearson *et al.*, 2009). Briefly, animals were processed in 7.5% N-acetylcysteine in PBS for 7.5 min and fixed in 4% paraformaldehyde for 30 min at room temperature. Worms were then rinsed twice with PBSTx (PBS + 0.3% Triton X-100), which was replaced with a prewarmed reduction solution (PBS containing 1% NP-40, 50 mM DTT, and 0.5% SDS) and incubated at 37°C for 10 min. After rinsing twice with PBSTx, animals were dehydrated in serial solutions of 50:50 methanol:PBSTx and 100% methanol, and stored at −20°C.

Whole-mount *in situ* hybridizations

Colorimetric *in situ* hybridizations were performed as described (Pearson *et al.*, 2009) and fluorescent *in situ* hybridizations (King & Newmark, 2013) with some modifications. Briefly, fixed animals were rehydrated, bleached (5% formamide, 1.2% H₂O₂ in 0.5× SSC), and treated with proteinase K (4 µg/ml in PBSTx, Thermo Fisher 25530049) for 10 min followed by fixation in 4% formaldehyde (10 min). After 2 h in pre-hybe, probes were added, and incubated overnight at 56°C. The next day, samples were washed 2× in wash hybe (5 min), 1:1 wash hybe:2× SSC-0.1% Tween 20 (10 min), and 2× SSC-0.1% Tween 20 (30 min), 0.2× SSC-0.1% Tween 20 (30 min) at 56°C followed by 3 × 10 min MABT washes (for colorimetric *in situ*) or PBSTx washes (fluorescent *in situ*) at room temperature. Animals were then placed in blocking solution (0.5% Roche Western Blocking Reagent and 5% inactivated horse serum diluted in MABT or PBSTx) for 2 h at room temperature and incubated with an appropriate antibody overnight at 4°C: 1:3,000 anti-DIG-AP (Roche 11093274910), 1:1,000 anti-DIG-POD (Roche 11207733910), or 1:1,000 anti-FITC-POD (Roche 11426346910) diluted in blocking solution. Subsequent washes and AP or tyramide development were performed as previously described. After development, animals were soaked in ScaleA2 (4 M urea, 20% glycerol, 0.1% Triton X-100, 2.5% DABCO) and mounted in Aqua-Polymount (Polysciences Inc. 18606). Some *in situ* hybridizations were carried out in a CEM InSituPro Hybridization robot up to the development stage, using the same protocol. Animals were imaged either on a Leica M165F with a DFC7000T, a Zeiss 710 confocal microscope or a Keyence BZ-X800 microscope. For all *in situ* hybridizations, representative images are shown from a population of $n \geq 10$ animals.

Phosphohistone H3 labeling

Animals were stained with phosphohistone H3 following fluorescent *in situ* hybridization. After inactivation of peroxidase with 4% H₂O₂ in PBSTx for 1 h at RT, animals were rinsed in PBSTx (6× washes). Then, animals were incubated in anti-phosphohistone H3 (Ser10) antibody (Millipore Sigma, ZRB1226) 1:300 in blocking solution for 2 days at 4°C. After washing off the primary with PBSTx, animals were incubated in a goat anti-rabbit-HRP secondary antibody (Thermo Fisher 31460) 1:1,000 in PBSTx overnight at 4°C. Antibody was washed off with PBSTx and development was carried out as follows. Animals were preincubated in rhodamine tyramide (1:5,000 in PBSTx) for 10 min, followed by development with 0.005% H₂O₂ in PBSTx for 15 min at room temperature. After development, samples were rinsed in PBSTx, then counterstained with DAPI (1:5,000 in PBSTx, Thermo Scientific) before soaking in ScaleA2 and mounting in Aqua-Polymount. Animals were imaged on a Zeiss 710 confocal microscope or a Keyence BZ-X800 microscope.

F-ara-EdU staining

Animals were soaked for 24 h in 0.5 mg/ml F-ara-EdU (Sigma T511293) diluted in planaria water containing 3% DMSO. Soaking began either immediately after radiation or 24 h later. Animals were fixed immediately after 24 h in F-ara-EdU.

F-ara-EdU detection

F-ara-EdU was detected as in (Bohr *et al.*, 2021). Fixed animals were rehydrated and bleached overnight in 6% H₂O₂ in PBSTx. Bleached animals were treated with proteinase K (10 µg/ml proteinase K + 0.1% SDS in PBSTx) for 15 min, and fixed in 4% formaldehyde in PBSTx for 10 min. F-ara-EdU was developed by soaking animals in development solution containing PBS + 1 mM CuSO₄ and 100 µM Oregon Green 488 azide (Thermo Fisher O10180) with freshly made 100 mM ascorbic acid added immediately before administering. Animals were incubated in this solution for 30 min in the dark. After rinsing with PBSTx, animals were postfixed in 4% formaldehyde and rinsed 2× in PBSTx. Animals were then placed in K block (5% inactivated horse serum, 0.45% fish gelatin, 0.3% Triton-X, and 0.05% Tween-20 diluted in PBS) at room temperature for 4 h. This was followed by incubation with 1:1,000 anti-Oregon Green-HRP (Thermo Fisher A21253) in K block overnight. The following day, after 6× PBSTx rinses, antibody was developed with FAM tyramide (1:2,000 in borate buffer (King & Newmark, 2013)) for 10 min, followed by development with 0.005% H₂O₂ in borate buffer for 15 min at room temperature.

Flow cytometry and Annexin V staining

Flow cytometry of Hoechst-stained cells was conducted as previously described (Reddien *et al.*, 2005b; Hayashi *et al.*, 2006) with minor modifications. Thirty animals per group were rinsed in CMFB (CMF containing 0.5% BSA) and dissected into pieces. Fragments were dissociated in 1:100 Liberase™ (2.5 mg/ml, Roche 5401135001) in CMFB at 30°C, agitating at 300 rpm for 30 min on an Eppendorf ThermoMixer™. Fragments were triturated with a pipette periodically to aid dissociation. Dissociated cells were then diluted with equal volume of CMFB and centrifuged for 5 min at 500 g. Pelleted cells were diluted in 1 ml of CMFB and passed through a 30 µm cell strainer (BD 340627). Cell density was assessed with an automated cell counter (Bio-Rad TC20™).

2.8×10^6 cells/group were stained with 5 $\mu\text{g/ml}$ Hoechst 33342 (ThermoFisher H3570) diluted in CMFB for 70 min in the dark with gentle rotation. Cells were spun at 500 g for 5 min, then resuspended in Annexin V staining buffer (2 μl Annexin V-APC [ThermoFisher A35110] diluted in 100 μl freshly made $1\times$ Annexin V buffer from a $10\times$ stock solution [0.1 M HEPES pH 7.4, 1.4 M NaCl, and 25 mM CaCl_2]) and incubated for 15 min at RT. Then, 400 μl of $1\times$ Annexin V buffer with 1 $\mu\text{g/ml}$ Propidium Iodide (Sigma P4170) was added to each tube. Cells were analyzed on a BD FACS Symphony Analyzer and data were processed in FlowJo (TreeStar, Ashland, OR).

Flow cytometry with DAPI staining

Thirty animals per condition were dissociated in 1:100 Liberase in CMFB as above. After dissociation and filtration, pelleted cells were fixed by gently resuspending in 4% paraformaldehyde in CMFB for 15 min with gentle agitation. Cells were then rinsed twice in PBS-1%BSA and permeabilized with 0.2% Tween-20 in 1X PBS for 15 min with gentle rocking, then rinsed twice in PBS-1%BSA. Cells were then incubated in 400 μl of DAPI staining solution made by diluting a 1 mg/ml DAPI solution 1:1,000 in 1X PBS-0.1% Triton-X, and then gently rocking at room temperature for 30 min in the dark. Samples were run on an Attune NxT analyzer and data were processed using the ModFit LT program.

RNA-seq library preparation and analysis

RNA-seq libraries were generated from stem cells isolated from either *unc-22*(RNAi) or *atm*(RNAi) animals 1 day after radiation. Data were generated from three biological replicates with 5–10 animals. Stem cells were isolated as previously described (Peiris *et al*, 2016a). Briefly, animals were dissociated as described above, cells were stained with Draq5 (ThermoFisher 65-0880-96) and Calcein-AM (ThermoFisher C3100MP), then sorted on a Sony MA900 Cell Sorter. RNA was isolated from a total of 100,000 stem cells per replicate. RNA-seq libraries were constructed using NEBNext[™] Ultra II Directional RNA Library Prep Kit for Illumina®, and run on a NextSeq500 sequencer. Sequencing reads were aligned using STAR aligner against schMed4-2018 genome assembly, and genes were annotated using the v6 Dresden transcriptome (Grohme *et al*, 2018; Rozanski *et al*, 2019). Differential gene expression analysis was carried out using DESeq2. Using the DESeq2 default parameters, the Wald test was used to compare between groups (unirradiated, *atm*(RNAi) vs. *control*(RNAi); or 1dpi, *atm*(RNAi) vs. *control*(RNAi)) and Benjamini–Hochberg for multitesting correction (Love *et al*, 2014). For identifying differentially expressed genes, $|\log_2\text{FC}| > 1$ and adjusted p-value < 0.01 were used as the cutoff. The upregulated genes in the comparison between *atm*(RNAi) versus *control*(RNAi) at 1dpi are listed in Dataset EV1, and the downregulated genes are listed in Dataset EV2. For GO analysis, topGO was used for identifying enriched GO terms among upregulated or downregulated genes (Alexa & Rahnenführer, 2007).

Single-cell RNA-seq analysis

Single-cell RNA-seq analysis utilized published datasets generated from whole animals (Fincher *et al*, 2018). The analysis was conducted using the function “DotPlot” from Seurat (4.0.4) in R (4.0.5) (Hao *et al*, 2021). Following the original cell annotation from the publication, we divided the cells into two categories: those

annotated as “Neoblast”, and the rest of the cells, which were annotated here as “Differentiated cells”. Dot size represents the percentage of cells in each category that expressed at least 1 normalized count. The color scale represents the average expression of each annotation (neoblast or differentiated cells), calculated across all cells included in the analysis.

Image quantification

All image quantification was carried out in Fiji (Schindelin *et al*, 2012). For quantification of fluorescent confocal images of *piwi-1*, phosphohistone-H3, and EdU, maximum projections of confocal images were generated. For EdU and pH3, images were thresholded and counted using the Analyze tool in Fiji. DAPI signal was used to threshold images and measure total area of the animal. Quantification of the number of *piwi-1* cells from colorimetric and fluorescent images was done manually using the CellCounter Plugin. To calculate the percentage of animal area occupied by fluorescent signal for *piwi-1*, we determined the area occupied by *piwi-1* by thresholding, and divided this by the total area of the animal, calculated based on the DAPI signal. No blinding was used in the quantification.

Statistical analysis and reproducibility

Statistical analysis was performed using PRISM-Graphpad version 9. Statistical details of all experiments are noted in each figure legend. Data represent ≥ 2 independent experiments.

Data availability

All data are available upon request. RNA-seq data have been deposited into NCBI Accession number GSE190454 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE190454>).

Expanded View for this article is available [online](#).

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Author contributions

Divya A Shiroor: Conceptualization; data curation; formal analysis; validation; investigation; visualization; methodology; writing – original draft; writing – review and editing. **Kuang-Tse Wang:** Conceptualization; data curation; formal analysis; validation; investigation; visualization; methodology; writing – original draft; writing – review and editing. **Bhargav D Sanketi:** Investigation; writing – review and editing. **Justin K Tapper:** Investigation; writing – review and editing. **Carolyn E Adler:** Conceptualization; resources; supervision; funding acquisition; writing – original draft; project administration; writing – review and editing.

Disclosure and competing interests statement

The authors declare that they have no conflict of interest.

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Expanded View Figures

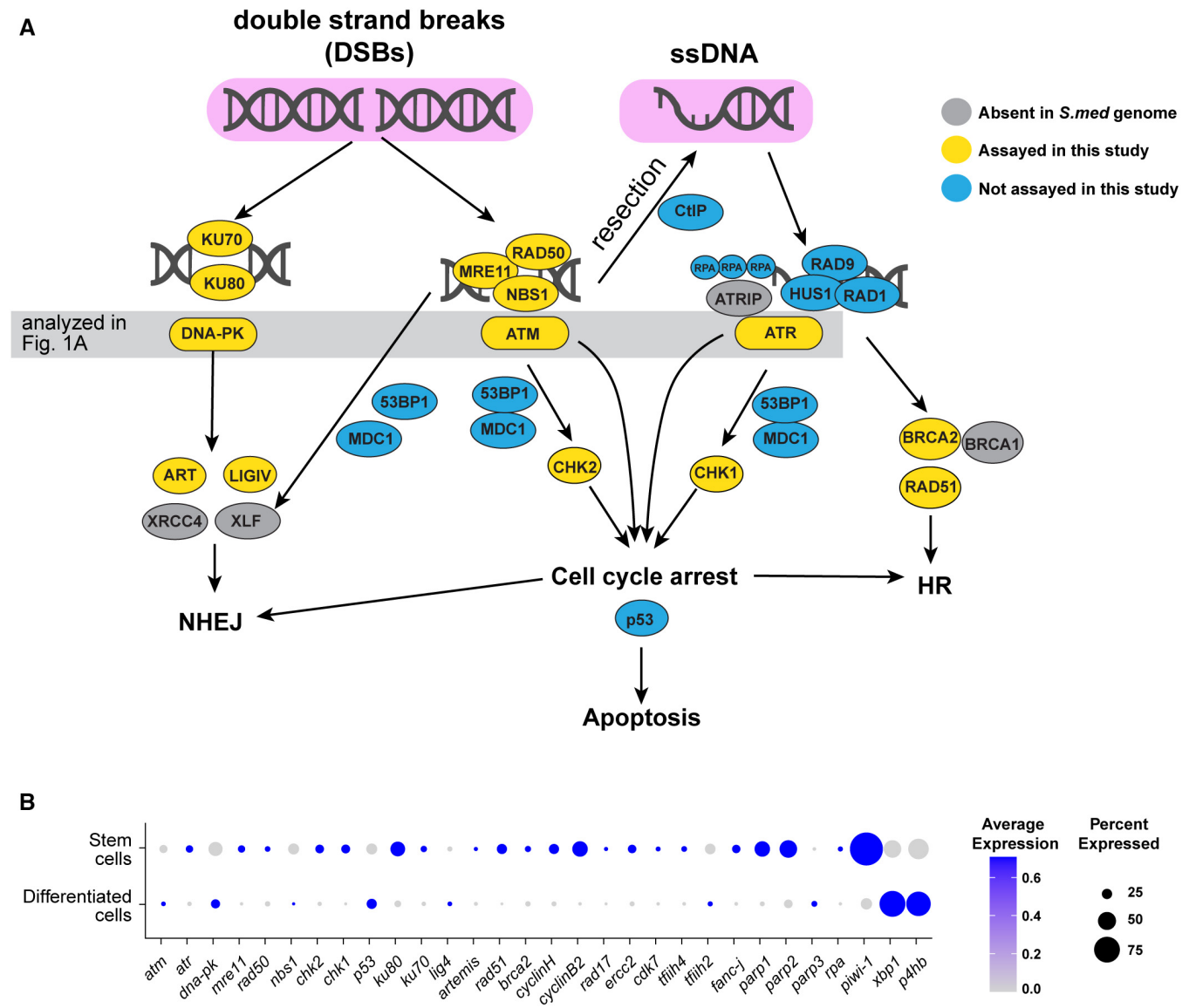


Figure EV1. Schematic of DNA damage response pathways.

A Schematic of DNA damage response pathway in humans. Homologs of proteins highlighted in yellow were tested in this study. Those in gray are absent in the *Schmidtea mediterranea* genome (Grohne et al, 2018; Sahu et al, 2021), and those in blue were not examined here. The interactions and relationships among the proteins are unverified in planarians. The *S. mediterranea* p53 homolog is not involved in DNA-damage induced apoptosis (Wendt et al, 2022).

B Dot plot showing expression levels of DNA damage response genes in stem cells (top) and differentiated cells (bottom) in single-cell RNA-seq data (Fincher et al, 2018). Cells were grouped into two categories (stem cells vs. differentiated cells); in each population, the average expression and percent of cells expressing a given transcript were calculated. *piwi-1* expression is shown as a control for stem cells, and *xbp1* and *p4hb* are included as references for differentiated cells (Raz et al, 2021).

Figure EV2. ATM knockdown acts only after radiation.

- A Schematic of planarian and human ATM showing domain conservation. Blue and purple boxes show nonoverlapping RNAi constructs tested.
- B *piwi-1* WISH of RNAi animals as indicated, 2 dpi. Scale bars = 250 μ m.
- C qRT-PCR of *piwi-1* relative to GAPDH in RNAi animals. Statistics represent ordinary one-way ANOVA compared to controls, *** $P < 0.0004$.
- D *piwi-1* WISH of unirradiated RNAi animals. Scale bars = 250 μ m.
- E qRT-PCR of *piwi-1* relative to GAPDH in unirradiated RNAi animals. Statistics represent unpaired *t*-test; ns = not significant.
- F Source data for Fig 1D. FACS plots of Hoechst-stained X1 cells from unirradiated control and *atm*(RNAi) animals. Black polygon = X1 cells.
- G Source data for Fig 1F. Histograms of X1s from (F) stained with Annexin V. Brackets and red shaded area = Annexin V mean fluorescent intensity staining $> 10^3$.
- Data information: In (C) and (E), data are averages $\pm$ SD; circles represent biological replicates.

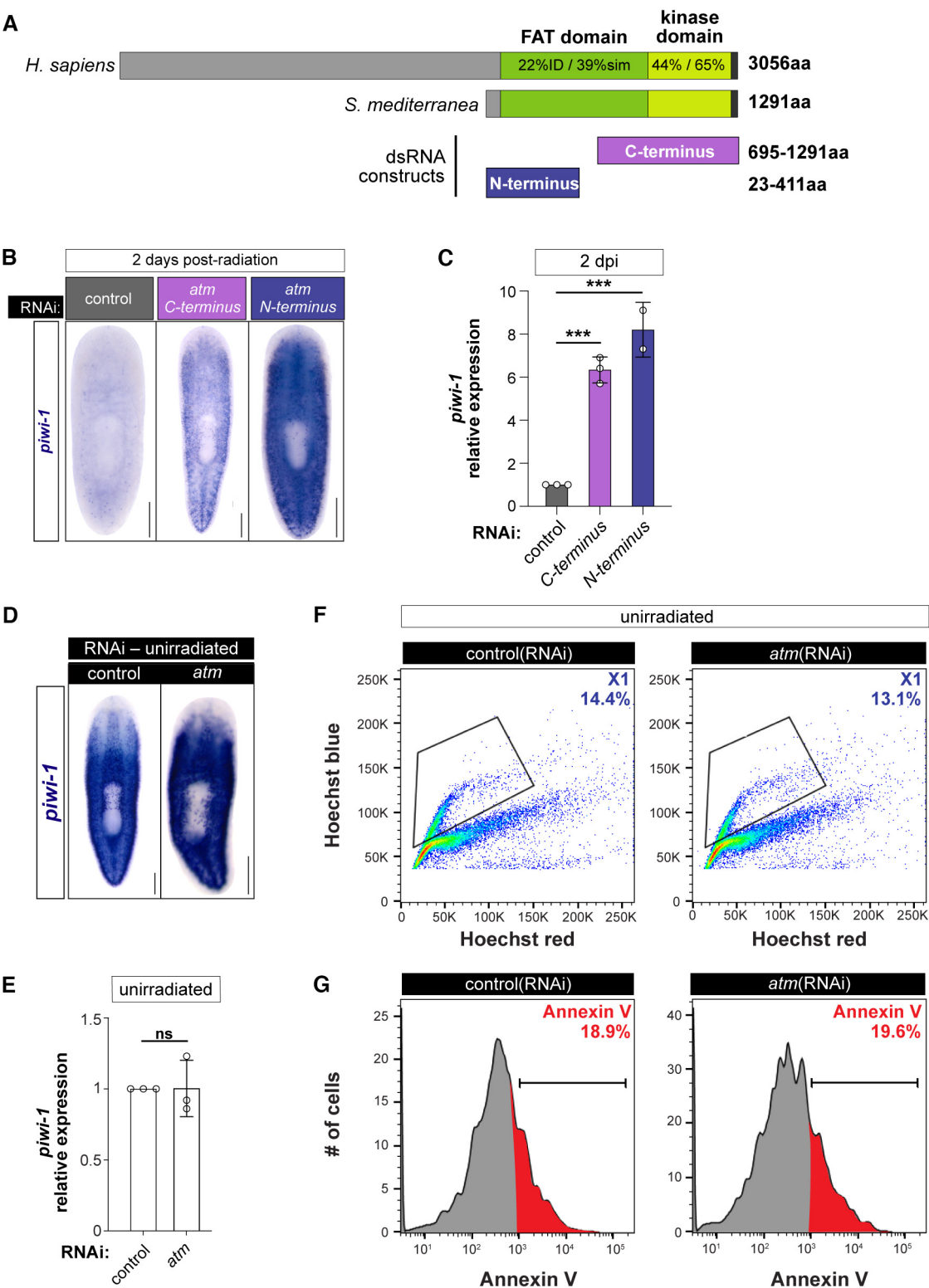


Figure EV2.

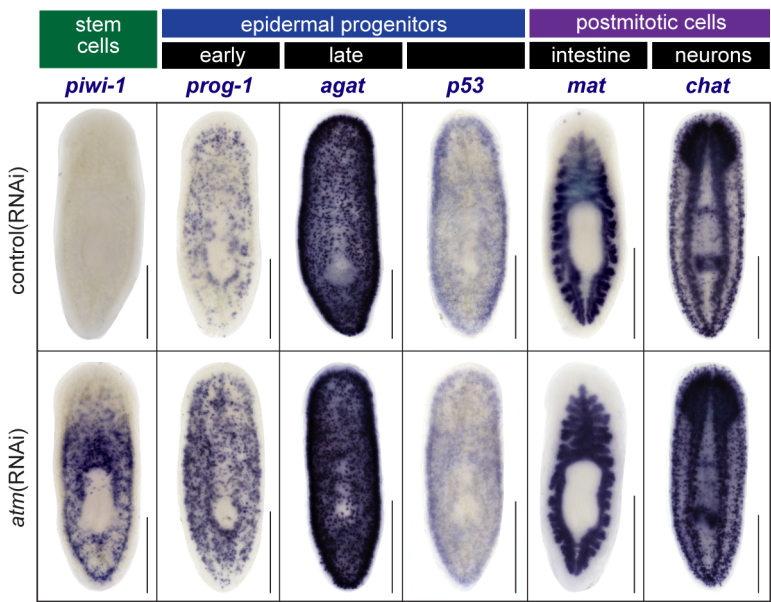


Figure EV3. Knockdown of *atm* does not alter abundance of mature and postmitotic cells.

In situ hybridizations of *piwi-1* (stem cell marker), *prog-1* (early progeny marker), *agat* (late progeny marker), *p53* (late progeny marker), *mat* (gut marker), and *chat* (neuron marker) in RNAi animals 2 days after exposure to 20 Gy. Scale bars = 250 μm.

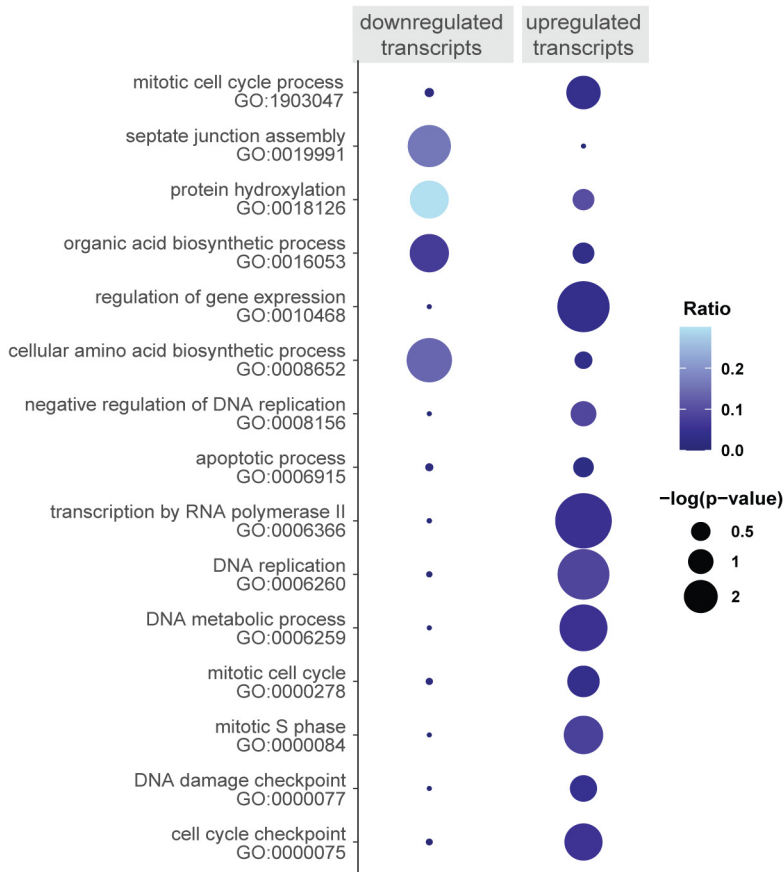


Figure EV4. Gene ontology (GO) analysis of differentially expressed transcripts in *atm*(RNAi) stem cells after radiation.

Enrichment of each GO term across downregulated and upregulated transcripts in *atm*(RNAi) stem cells, extracted from animals 1 day after radiation.

Figure EV5. Knockdown of HR, but not NHEJ, exaggerates stem cell apoptosis after radiation.

- A Percentage of animal area occupied by *piwi-1* FISH divided by total animal area, in radiated RNAi animals as indicated.
- B Confocal images of *piwi-1* FISH (cyan) after RNAi of HR genes 2 days after exposure to either 5 or 15 Gy.
- C Quantification by area of *piwi-1*⁺ cells in RNAi animals (from panel B) after 1.5 Gy exposure, 2 dpi.
- D Confocal images of *piwi-1* FISH (cyan) after RNAi of NHEJ genes 2 days after exposure to either 5 or 15 Gy.
- E Quantification by area of *piwi-1*⁺ cells in RNAi animals (from panel B) after 15 Gy exposure, 2 dpi.
- F Confocal images of animals stained with pH3 (yellow) and DAPI (DNA, blue) after RNAi of NHEJ genes, 2 days after exposure to 5 Gy.
- G Quantification by area of pH3⁺ mitotic nuclei in RNAi animals (from panel D) after exposure to 5 Gy.

Data information: Data are averages $\pm$ SD; circles represent biological replicates; statistics represent ordinary one-way ANOVA compared to *atm*(RNAi) in (A); controls in (C), (E) and (G); * $P < 0.05$, *** $P < 0.0005$, **** $P < 0.0001$, ns, not significant. Scale bars = 250 μ m.

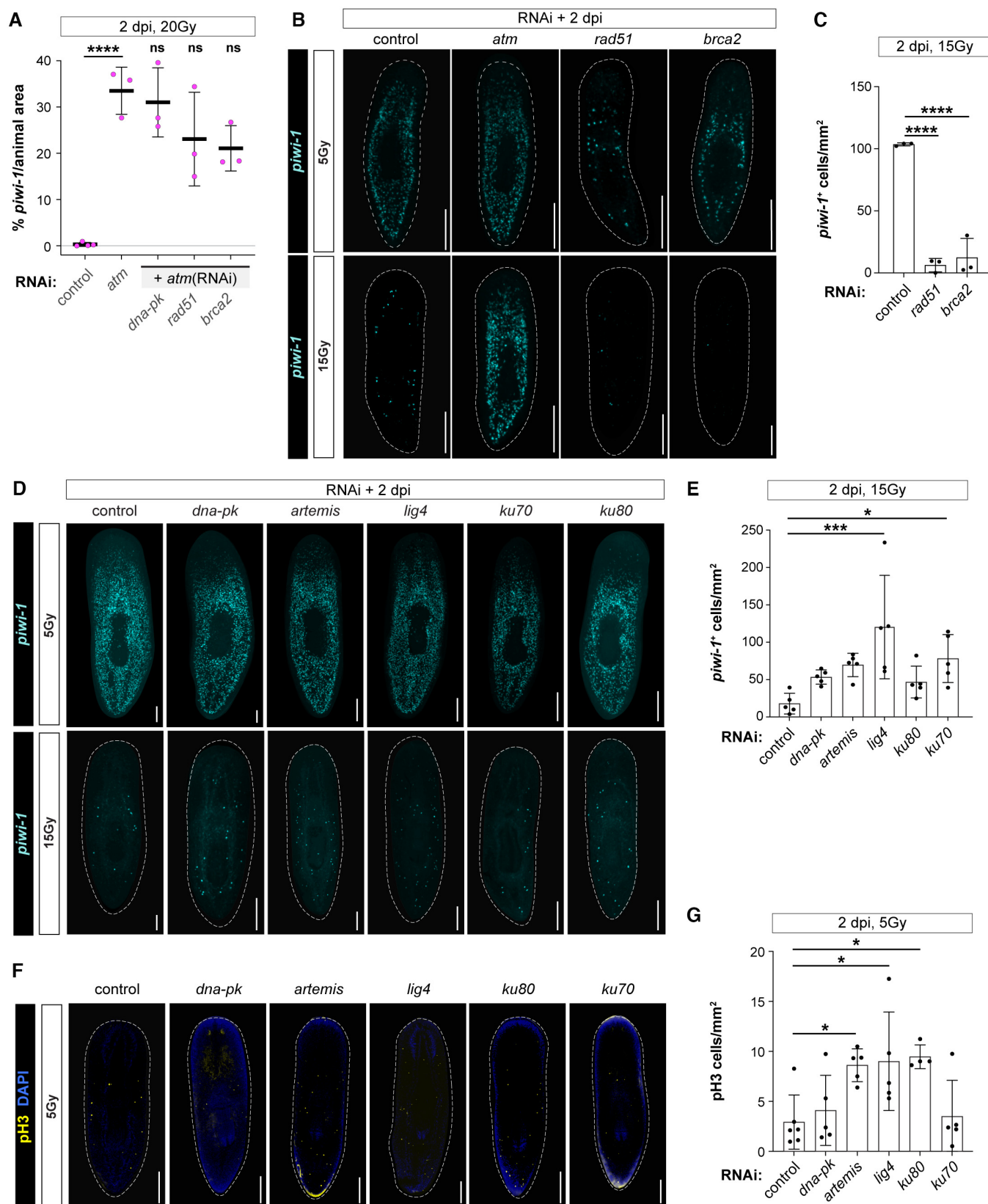


Figure EV5.