Expanded View Figures

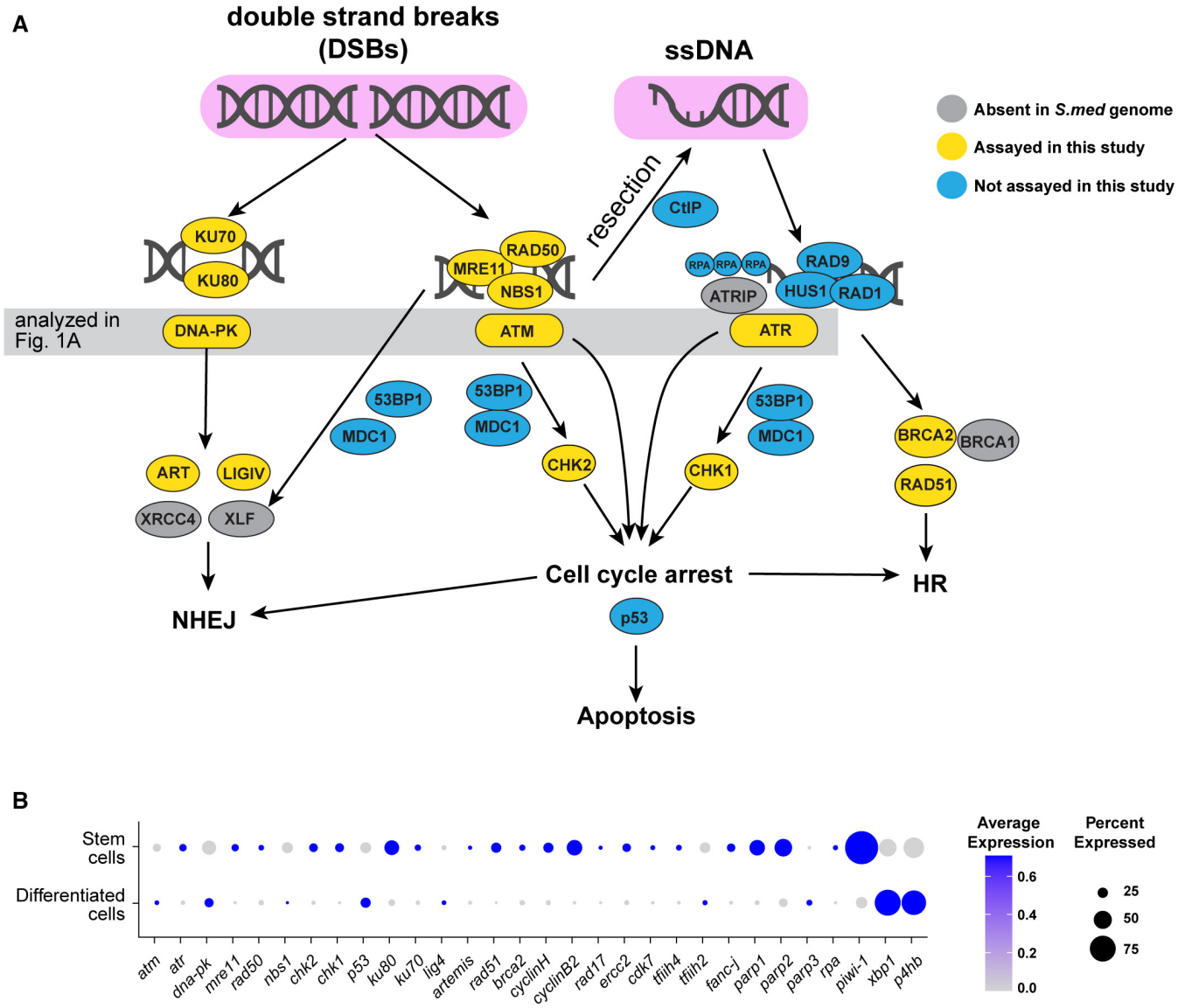

Figure EV1. Schematic of DNA damage response pathways.

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 (Grohme *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 μm.
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 μm.
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 ± SD; circles represent biological replicates.

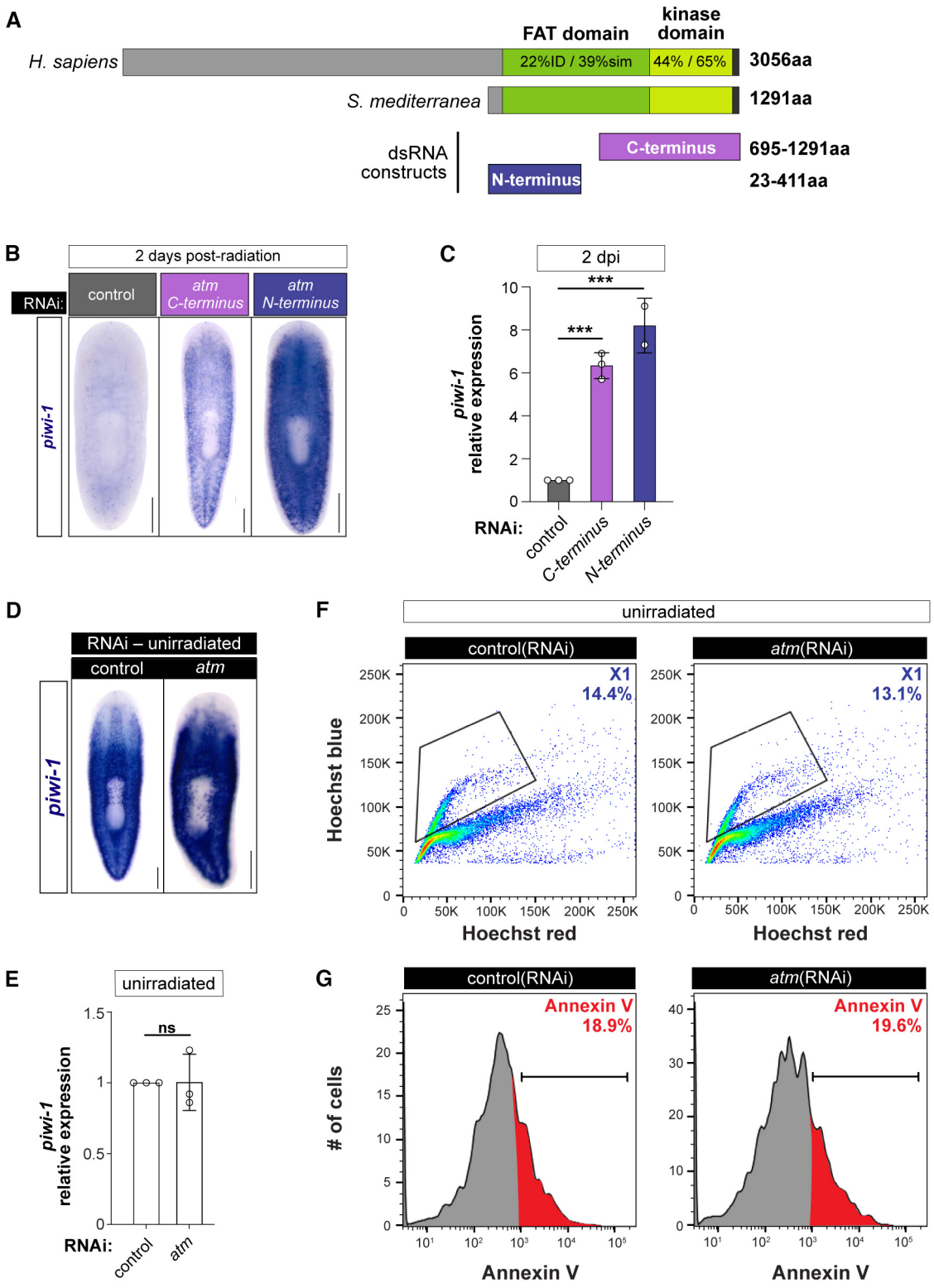

Figure EV2.

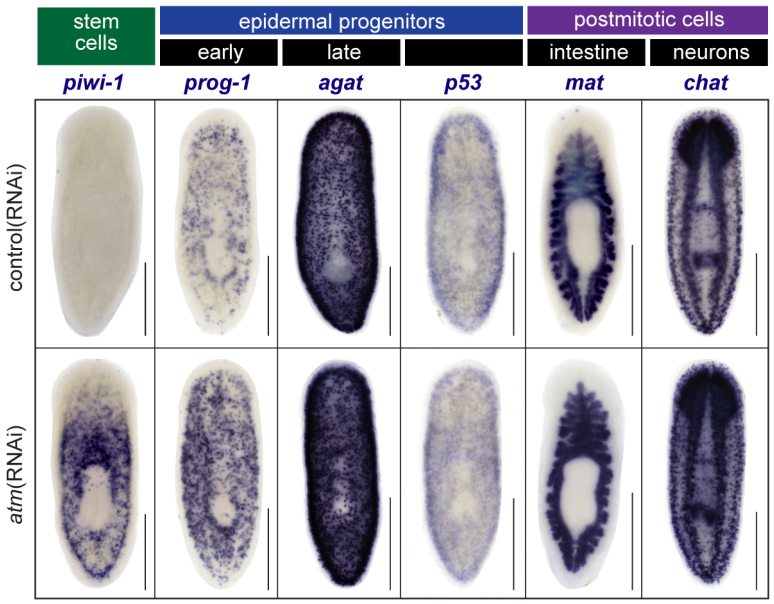

Figure EV3. Knockdown of *atm* does not alter abundance of mature and postmitotic cells.

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.

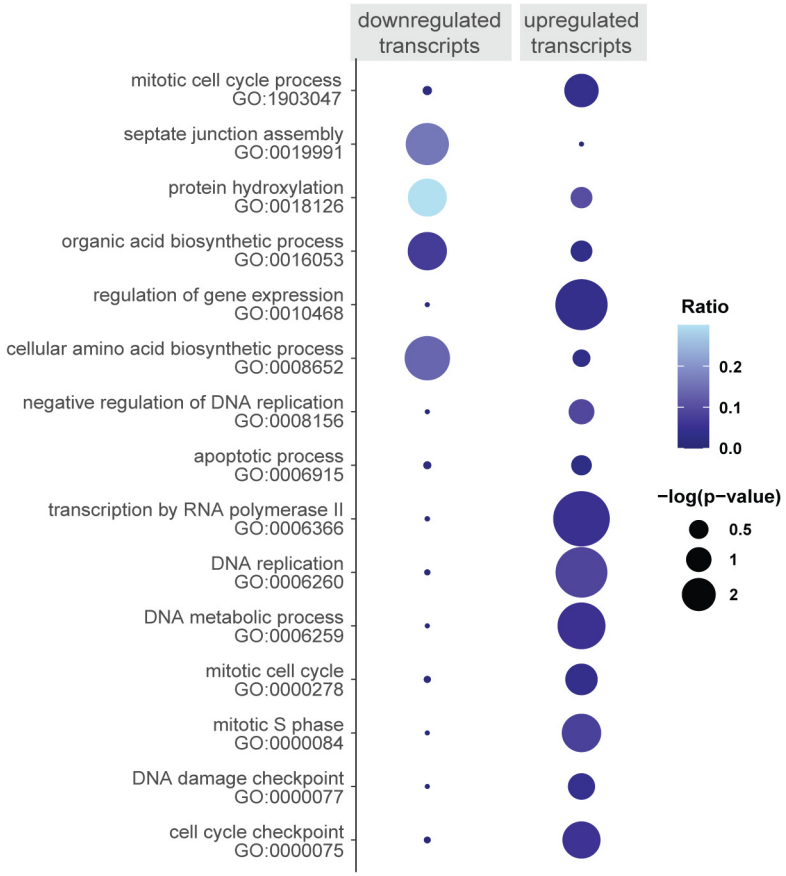

Figure EV4. Gene ontology (GO) analysis of differentially expressed transcripts in *atm*(RNAi) stem cells after radiation.

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 ± 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 μm.

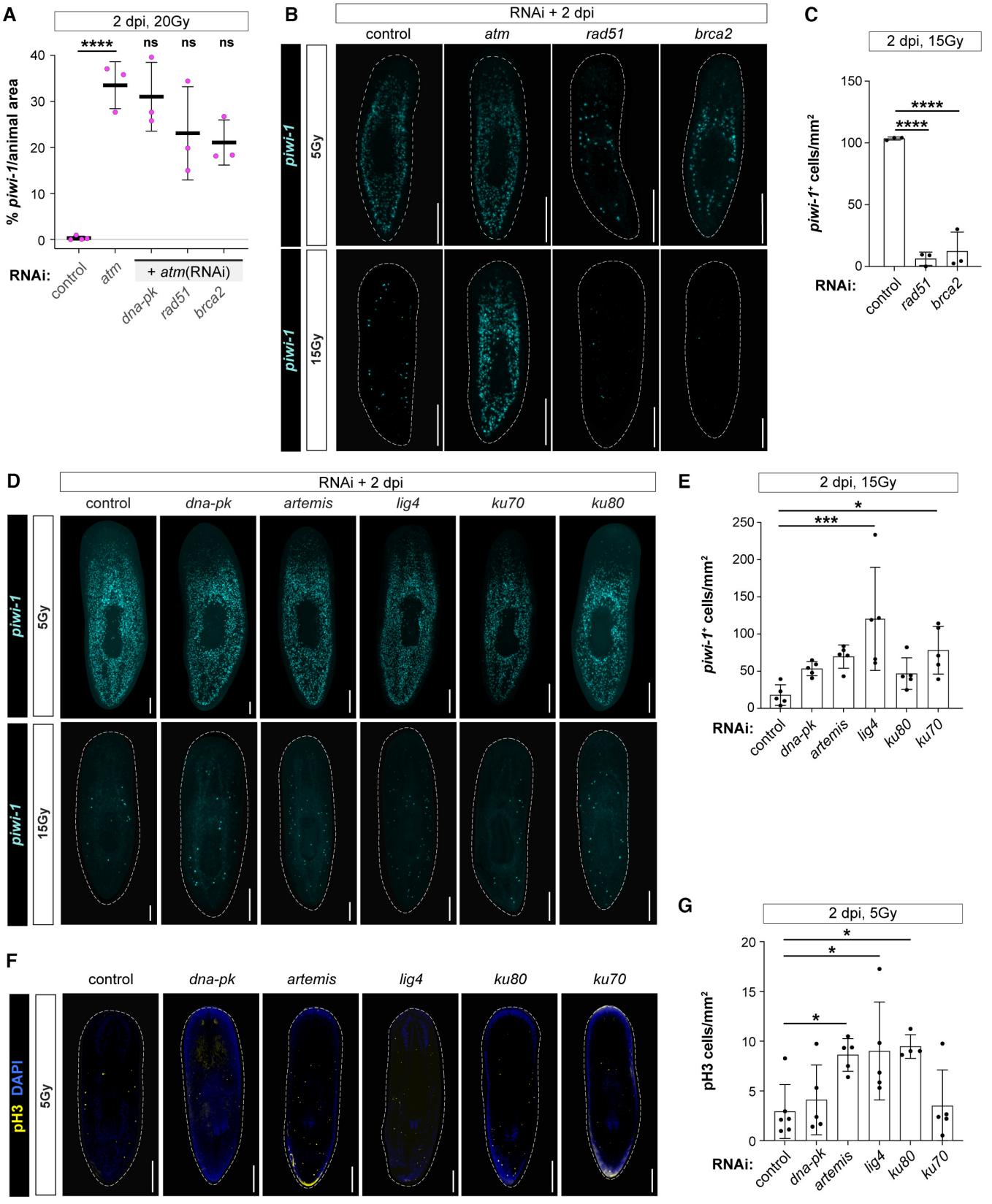
A
2 dpi, 20Gy
****
ns
ns
ns
% piwi-1/animal area
40
30
20
10
0
RNAi:
control
atm
+ atm(RNAi)
dna-pk
rad51
brca2
B
RNAi + 2 dpi
control
atm
rad51
brca2
piwi-1
5Gy
piwi-1
15Gy
C
2 dpi, 15Gy
piwi-1+ cells/mm2
150
100
50
0
****
****
RNAi:
control
rad51
brca2
D
RNAi + 2 dpi
control
dna-pk
artemis
lig4
ku70
ku80
piwi-1
5Gy
piwi-1
15Gy
E
2 dpi, 15Gy
piwi-1+ cells/mm2
250
200
150
100
50
0
*
***
RNAi:
control
dna-pk
artemis
lig4
ku80
ku70
F
control
dna-pk
artemis
lig4
ku80
ku70
pH3 DAPI
5Gy
G
2 dpi, 5Gy
pH3 cells/mm2
20
15
10
5
0
*
*
*
RNAi:
control
dna-pk
artemis
lig4
ku80
ku70

Figure EV5.