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Convergent evolution of a genotoxic stress response in a parasite-specific p53 homolog

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P53 is a widely studied tumor suppressor that plays important roles in cell-cycle regulation, cell death, and DNA damage repair. P53 is found throughout metazoans, even in invertebrates that do not develop malignancies. The prevailing theory for why these invertebrates possess a tumor suppressor is that P53 originally evolved to protect the germline of early metazoans from genotoxic stress such as ultraviolet radiation. This theory is largely based upon functional data from only three invertebrates, omitting important groups of animals including flatworms. Previous studies in the freshwater planarian flatworm *Schmidtea mediterranea* suggested that flatworm P53 plays an important role in stem cell maintenance and skin production, but these studies did not directly test for any tumor suppressor functions. To better understand the function of P53 homologs across diverse flatworms, we examined the function of two different P53 homologs in the parasitic flatworm *Schistosoma mansoni*. The first P53 homolog (*p53-1*) is orthologous to *S. mediterranea* P53(*Smed-p53*) and human TP53 and regulates flatworm stem cell maintenance and skin production. The second P53 homolog (*p53-2*) is a parasite-specific paralog that is conserved across parasitic flatworms and is required for the normal response to genotoxic stress in *S. mansoni*. We then found that *Smed-p53* does not seem to play any role in the planarian response to genotoxic stress. The existence of this parasite-specific paralog that bears a tumor suppressor–like function in parasitic flatworms implies that the ability to respond to genotoxic stress in parasitic flatworms may have arisen from convergent evolution.

p53 | parasitology | flatworms | schistosomes

Significance

P53 is an important tumor suppressor that is found throughout metazoans, including invertebrates that do not develop malignancies. The prevailing theory for why these invertebrates possess a tumor suppressor is that P53 originally evolved to protect the germline of early metazoans from genotoxic stress such as ultraviolet radiation. Here, we examine the function of two P53 homologs in the parasitic flatworm *Schistosoma mansoni*. The first is orthologous to canonical P53 and regulates flatworm stem cell maintenance and differentiation. The second P53 gene is a parasite-specific paralog that is required for the normal response to genotoxic stress. The existence of this parasite-specific paralog implies that the ability to respond to genotoxic stress in parasitic flatworms may have arisen from convergent evolution.

First described as a "guardian of the genome" in 1992 (1), P53 has long been studied in the context of vertebrate cancer in which its function and regulation are relatively well understood (2). The P53 family of proteins, however, is widely conserved across metazoans, including invertebrates that do not seem to develop malignancies (3, 4). Studies in invertebrate model organisms have suggested that invertebrate P53 homologs generally respond to genotoxic stress by inducing cell death, much like vertebrate P53. In the absence of malignancies, this behavior is hypothesized to be instrumental in eliminating germ cells that have acquired mutations, supporting the idea that the ancestral function of P53 is to defend the integrity of the genome.

One problem with this inference of ancestral function, however, is that only a handful of invertebrate P53 homologs have been functionally studied to date. Although P53-like molecules have been shown to act as "genome guardians" (i.e., respond to genotoxic stress by inducing cell death) in invertebrates, including *Caenorhabditis elegans* (5), *Drosophila melanogaster* (6), and *Nematostella vectensis* (7), examination of Platyhelminthes (flatworms) has been limited to the planarian flatworm *Schmidtea mediterranea* in which the role of P53 in response to genotoxic stress was not thoroughly investigated (8, 9). Knockdown of the planarian *S. mediterranea* p53 homolog (*Smed-p53*) resulted in both an increase in stem cell proliferation (consistent with a tumor suppressor–like function) and an increase in animal sensitivity to radiation (inconsistent with a tumor suppressor–like function) (8). The best-characterized function of the planarian P53 homolog (*Smed-p53*) is the regulation of the stem cell–mediated production of epidermal cells. *Smed-p53* RNA interference (RNAi) results in loss of the ability to produce epidermal progenitor cells (10) and loss of expression of the flatworm-specific transcription factor *zfp-1* (9), a known regulator of skin production in free-living and parasitic flatworms (11, 12) that is required for normal skin production in both types of worms. Because planarian epidermal cells are required for animal survival and represent a significant portion of the cells generated by stem cells, it has been difficult to tease apart the role *Smed-p53* plays in regulating genotoxic stress.

Flatworm skin production is fascinating from both an evolutionary and a medical perspective. This is because the clade Neodermata (literally "new skin"), the group of

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Platyhelminthes that contains virtually all parasitic flatworms, is united by the presence of a skin-like tissue known as the tegument. Unlike in free-living flatworms that possess a simple multicellular epidermis, the tegument is a syncytial tissue that covers the entire surface of the organism, acting as an important interface between the parasite and the host (13). The tegument is known to play roles critical for survival inside the harsh environment of a host. In schistosomes, the tegument is involved in evading the hosts' immune system, preventing hemostasis, and acquiring nutrients (14–17). Even more remarkable, throughout the course of evolution, tapeworms lost their gut in favor of absorbing nutrients through their tegument (18). That the tegument appears along with the adaptation to a parasitic lifestyle in flatworms suggests that the tissue may have been a key driving force that enabled these flatworms to become some of nature's most prolific parasites (19). Furthermore, because the tegument plays critical roles in processes essential for parasite survival, better understanding of how the tegument is made and maintained could lead to the development of new methods of treatment and prevention to combat these deadly parasites, which are responsible for hundreds of thousands of deaths, billions of dollars in damage, and unquantifiable morbidity, primarily in the developing world (20).

Schistosomes are the most medically important parasitic flatworm, killing more than 200,000 people every year and infecting more than 200 million, with morbidity comparable to that of leishmaniasis, trypanosomiasis, and even malaria (21). Recent studies of the schistosome tegument and planarian epidermis demonstrated that despite having vastly different tissue organization, schistosomes and planarians have several commonalities in terms of skin production (9, 11, 12). Both schistosomes and planarians possess somatic stem cells termed neoblasts that constantly give rise to progenitor cells which migrate through the worm's parenchyma until they eventually become part of the organisms' tegument or epidermis, respectively. Aside from these anatomical similarities, both animals also rely on the flatworm-specific transcription factor *zfp-1* to produce their skin. The presence of these similarities, despite millions of years of evolutionary distance, unique tissue structures, and vastly different natural histories, suggests that other factors that regulate epidermis production in planarians may be functionally conserved in schistosomes.

To that end, we decided to investigate homologs of *Smed-p53* in schistosomes. In doing so, we found two *Smed-p53* homologs in the schistosomes genome, which we refer to as *p53-1* and *p53-2*. *p53-1* is orthologous to *Smed-p53* (as well as other canonical P53 molecules such as human *TP53*) and seems to be functionally analogous to *Smed-p53*. *p53-2*, however, is a paralog of *Smed-p53* that seems to have arisen from a gene duplication event that occurred in the Neodermata and functions more like a genome guardian by inducing cell death after genotoxic stress. Closer examination of *Smed-p53* suggests that the function of *p53-2* is unique to schistosomes and represents convergent evolution of a genotoxic stress response function in a P53 homolog, which raises questions about the ancestral function of this important gene.

Results

The *S. mansoni* genome contains two genes with the p53 tumor suppressor family InterPro ID (IPR002117) (22), Smp_139530 (henceforth *p53-1*), and Smp_136160 (henceforth *p53-2*). Both genes contain a p53 DNA binding domain (Pfam PF00870) but no apparent P53 transactivation domain (Pfam PF08563), P53 tetramerization domain (Pfam PF07710), or sterile alpha motif (Pfam 00536) (Fig. 1*A*). Gene models from WormBase Parasite Version WBPS16 (23, 24) identify 86 orthologs of *p53-1*, including *Homo sapiens TP53/TP63/TP73*, *D. melanogaster P53*, and *Smed-p53*, suggesting that it represents the canonical P53 (Fig. 1*B* and *SI Appendix*, Figs. S1 and S2 and Dataset S1). Examination of *p53-2*, however, identifies only 17 orthologs, all of which are present only in trematodes (flukes). Closer examination of other flatworms reveals that at least one apparent ortholog (identified by using reciprocal BLAST [Basic Local Alignment Search Tool]) of *p53-2* is also present in 15 of 16 cestode (tapeworm) genomes examined but not in any of the 2 monogenean or 19 free-living flatworm genomes examined (Dataset S2). Together, these data suggest that a gene duplication event occurred in the common ancestor of Neodermata that resulted in *p53-2* representing a parasite-specific P53 paralog (Fig. 1*C*). These data must be cautiously interpreted, however, because of the limited number and quality of flatworm genomes and transcriptomes available. All *p53-1*/*p53-2* orthologs and genome/transcriptome databases referred to are listed in Datasets S1 and S3, respectively.

Epidermal production in planarians mirrors tegument development in schistosomes in many ways, including how both processes are regulated by the same molecules (12). *Smed-p53* is a known regulator of epidermal production in planarians (9, 11), so we hypothesized that one or both schistosome P53 homologs may also regulate tegument production. We began by examining the expression patterns of *p53-1* and *p53-2*. *Smed-p53* is expressed in planarian neoblasts and epidermal progenitors (8), so a functionally conserved molecule would likely be expressed in analogous cells. Whole-mount in situ hybridization (WISH) of schistosome *p53* homologs (Fig. 2*A*) revealed a punctate pattern of gene expression for *p53-1*, which is similar to other genes expressed in schistosome neoblasts and tegument progenitor cells (12, 25, 26). However, *p53-2* had a more diffuse expression pattern in addition to apparent enrichment in the parasite's gut and reproductive organs. Double fluorescence in situ hybridization (FISH) experiments demonstrated that *p53-1* is indeed expressed in schistosome neoblasts and tegument progenitor cells (Fig. 2*B* and *SI Appendix*, Fig. S2*A*). *p53-2* is also expressed in many of these cells (Fig. 2*B* and *SI Appendix*, Fig. S2*B*) in addition to many other cell types, including gut and reproductive cells (*SI Appendix*, Fig. S2*B*). To explore expression data across the schistosome lifecycle, we also accessed schisto.xyz (27) and found that *p53-1* expression is highest in adult male worms, sporocysts, and eggs (*SI Appendix*, Fig. S2*C*) whereas *p53-2* expression is highest in gonads, eggs, and adult female worms (*SI Appendix*, Fig. S2*D*). Finally, we examined the expression patterns of *p53-1* and *p53-2* using a recently published single-cell RNA sequencing (RNA-seq) atlas (26, 28) (Fig. 2*C*) and found that the single-cell RNA-seq data generally agree with our WISH and FISH results. Together, all of these data show that *p53-1* has an expression pattern very similar to that of *Smed-p53*, whereas *p53-2* expression is unlike *p53-1* or *Smed-p53*.

Next, we tested whether either one of the schistosome P53 homologs was functionally related to *Smed-p53* (i.e., a regulator of the skin lineage). *Smed-p53* RNAi eliminates epidermis-producing neoblasts, depletes epidermal progenitors, and ultimately results in impaired production of epidermis (9). Therefore, we examined the expression of *tsp-2* (a tegument progenitor marker analogous to epidermal progenitors) and *eled* (a gut-producing neoblast marker) as well as 5-ethynyl-2′-deoxyuridine (EdU; a thymidine analog that labels proliferative cells) after RNAi analysis of each P53 homolog (*SI Appendix*, Fig. S3*A*). We found that *p53-1*

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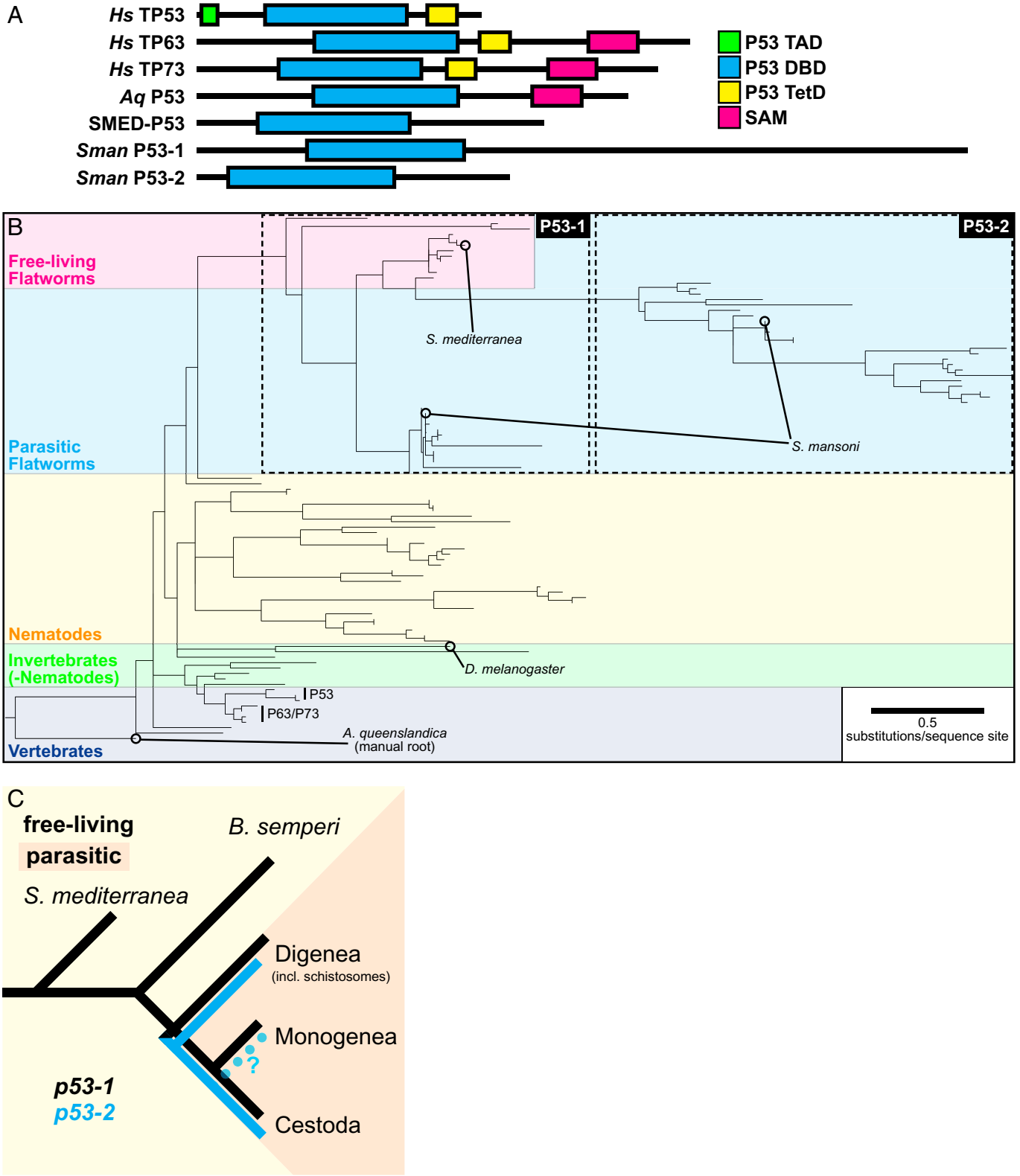

Fig. 1. Phylogenetic analysis of p53-1 and p53-2 orthologs. (*A*) Schematic of domain structure of human TP53/TP63/TP73, *Amphimedon queenslandica* (*Aq*) P53, *S. mediterranea* Smed-p53, and *S. mansoni* p53-1/p53-2. TAD, transactivation domain; DBD, DNA binding domain; TetD, tetramerization domain; SAM, sterile alpha motif. (*B*) Phylogenetic trees of p53-1 and p53-2 orthologs color coded by remarkable clades. Flatworm p53-1 and p53-2 orthologs are indicated with dashed lines. (*C*) Cartoon depicting model of *p53-1* and *p53-2* evolution. Dotted line with question mark indicates the possible loss of *p53-2* orthologs in Monogenea.

RNAi results in complete loss of *tsp-2* cells and all *eled*-negative neoblasts but no significant change in the number of *eled*-positive neoblasts (Fig. 3 *A*–*C* and *SI Appendix*, Fig. S3 *B* and *C*). This was accompanied by a decrease in the expression of *nanos2*, a marker of schistosome neoblasts, suggesting that the loss of EdU-positive cells represents a depletion of neoblasts rather than a cessation of the cell cycle. Loss of tegument progenitor cells and retention of gut-producing neoblasts should result in a loss

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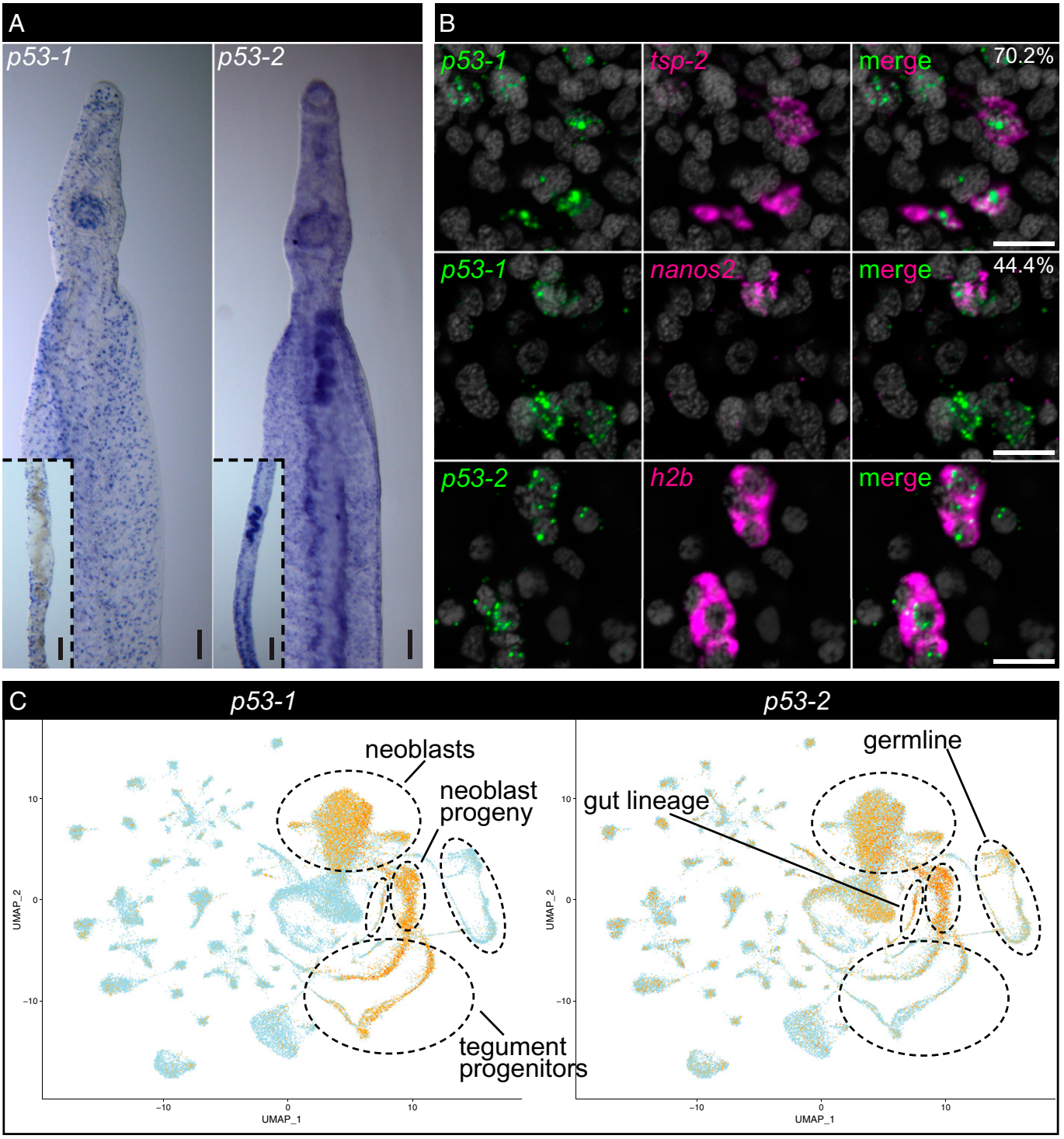

Fig. 2. Expression pattern of schistosome p53 homologs. (*A*) Colorimetric WISH showing expression patterns of *p53-1* and *p53-2*. (*B*) Double FISH experiment showing expression of *p53-1* relative to the tegument progenitor marker *tsp-2* and the neoblast marker *nanos2* as well as the expression of *p53-2* relative to the proliferative cell marker *h2b*. (*C*) Uniform manifold approximation plots showing expression patterns of *p53-1* and *p53-2* in adult schistosomes. Red indicates high expression, orange indicates medium expression, and blue indicates low or no expression. Important cell populations are indicated. Percentage of *p53-1*–positive cells that are also marker positive is indicated in the upper right of appropriate panels in *B*. Counts were performed in three separate animals with more than 130 cells per gene comparison. (Scale bars, 100 μm [*A*], 10 μm [*B*]).

of tegument production with no change in gut production, so we next examined tegument and gut production using EdU pulse-chase experiments. Consistent with loss of *tsp-2*–positive tegument progenitor cells but preservation of *eled*-positive gut-producing neoblasts, *p53-1* RNAi resulted in complete loss of tegument production (Fig. 3*B* and *SI Appendix*, Fig. S3*F*) but did not cause any observable changes in gut production (Fig. 3*C* and *SI Appendix*, Fig. S3*G*). However, it is possible that there were subtle gut phenotypes akin to what is observed in planaria after *Smed-p53* RNAi (9) that we failed to detect in our experiments. *p53-2* RNAi did not seem to have any effect on tegument production and even led to a modest increase in gut production (Fig. 3 and *SI Appendix*, Fig. S3 *F*–*G*). Together, these data support a model in which *p53-1* is a

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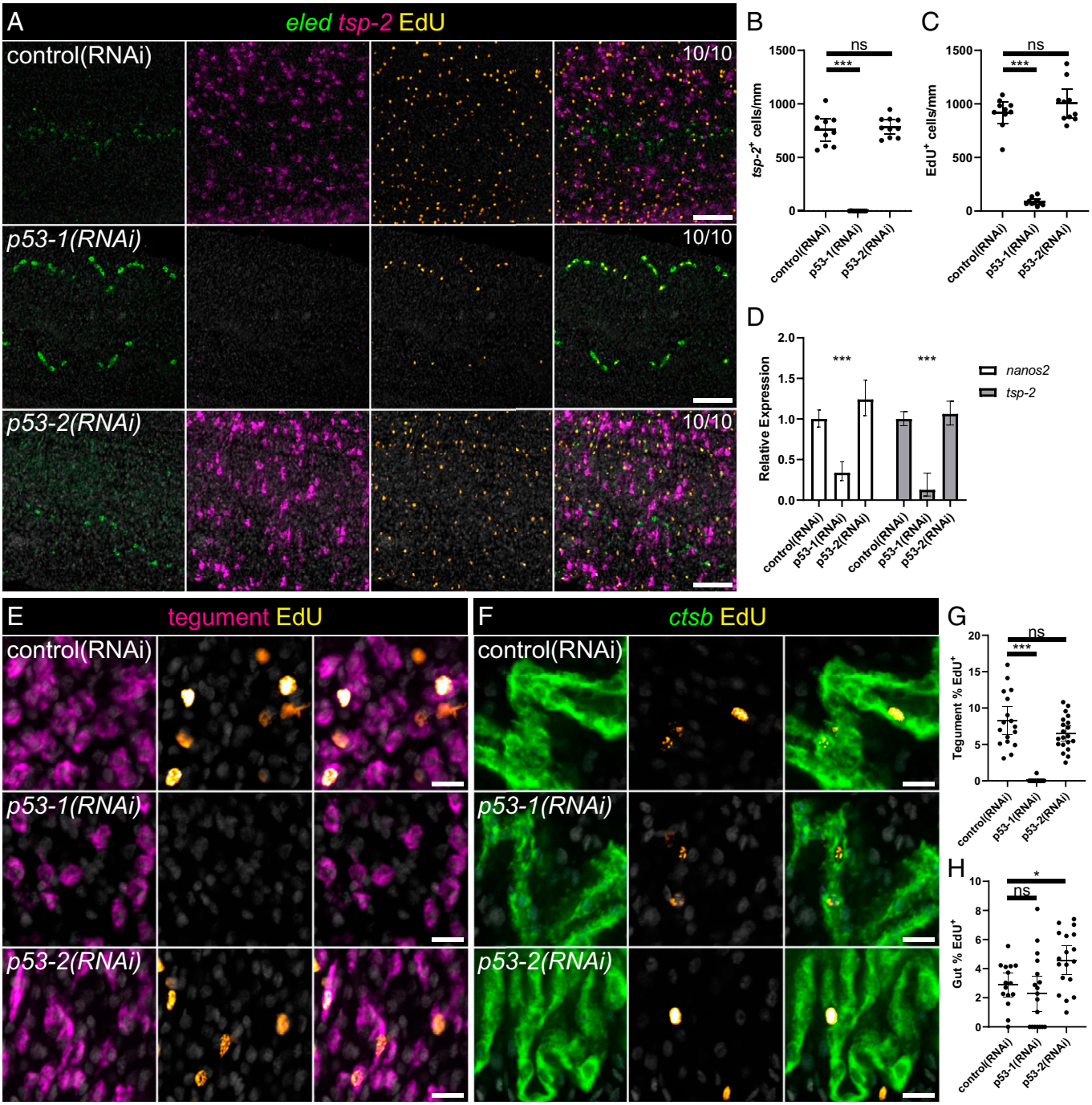

Fig. 3. *p53-1* RNAi perturbs neoblast maintenance and differentiation. (*A*) Double FISH of the gut neoblast marker *eled* and the tegument progenitor marker *tsp-2* after *p53-1* and *p53-2* RNAi. Neoblasts are labeled with the thymidine analog EdU. (*B* and *C*) Quantification of *tsp-2*–positive cells and EdU-positive cells, respectively, after *p53-1* and *p53-2* RNAi (corresponds to 3*A*). (*D*) qPCR detection of expression of the neoblast marker *nanos2* and the tegument progenitor marker *tsp-2*. (*E*) FISH of a mixture of genes that mark the tegument during *p53-1* and *p53-2* RNAi. Neoblast progeny are labeled with EdU via a pulse-chase experiment. (*F*) FISH of the gut marker *ctsb* after *p53-1* and *p53-2* RNAi. Neoblast progeny are labeled with EdU via a pulse-chase experiment. (*G* and *H*) Quantification of data from *E* and *F*, respectively. Fraction indicates number of worms that are similar to representative image. Data are from 10 animals per condition from one biological replicate (*A*) or >12 animals per condition from two biological replicates (*E* and *F*). *$P < 0.05$; ***$P < 0.001$; ns, not significant. Error bars indicate the 95% confidence interval. (Scale bars, 50 μm.)

functional homolog of *Smed-p53*, whereas *p53-2* seems to have a different role.

The prevailing theory regarding the function of invertebrate P53 homologs is that they respond to DNA damage by eliminating affected cells (4). Given that *p53-2* had no apparent role in tegument production but is still a P53 homolog, we next tested whether *p53-2* has any role in the parasite's response to genotoxic stress. A sublethal dose (20 Gy) of radiation is sufficient to deplete the vast majority of proliferative cells in adult parasites (Fig. 4*A*). *p53-1* RNAi offers no protection from this effect, but *p53-2* RNAi limited the impact of radiation on EdU-positive proliferative cells (Fig. 4*A*). This protection from radiation extended beyond simply preserving EdU-positive proliferative cells. The EdU-positive neoblasts remaining after radiation still expressed some markers of specialization (*eled*) and gave rise to *tsp-2*–positive tegument progenitors (*SI Appendix*,

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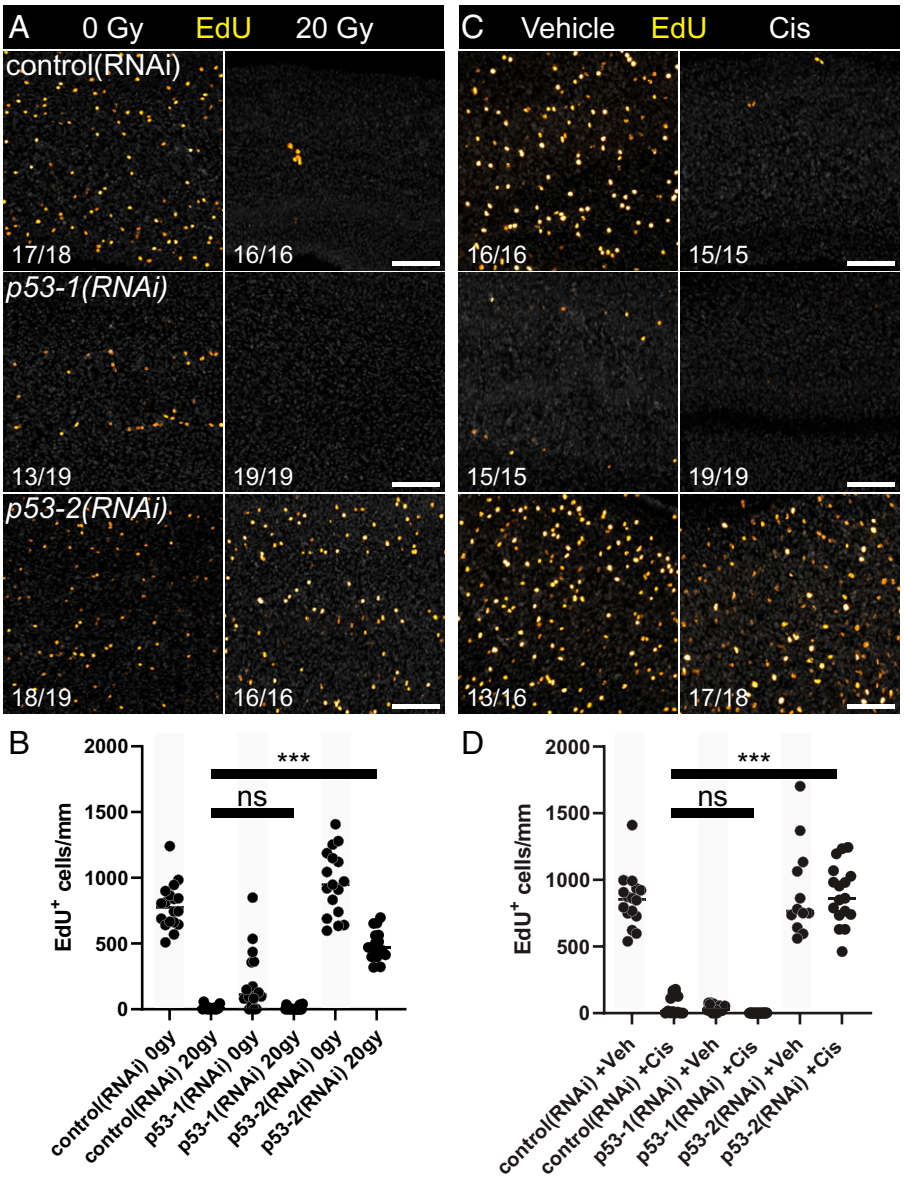

Fig. 4. *p53-2* RNAi abrogates normal response to genotoxic stress. (*A*) EdU-labeled neoblasts following radiation after *p53-1* or *p53-2* RNAi. (*B*) Quantification of data from *A*. (*C*) EdU-labeled neoblasts following cisplatin treatment after *p53-1* or *p53-2* RNAi. Cis, cisplatin. (*D*) Quantification of data from *C*. Data are from >12 animals per condition from two biological replicates. Fraction indicates number of worms that are similar to representative image. Veh, vehicle. ***$P < 0.001$; ns, not significant. Horizontal bars indicate the conditions that are being compared with respect to statistical tests. (Scale bars, 50 μm.)

Fig. S4), suggesting that they are at least partially functional. *p53-2* RNAi also protected the parasite's neoblasts from the effects of chemical genotoxic stress in the form of the DNA cross-linking agent cisplatin (29) (Fig. 4*B* and *SI Appendix*, Fig. S5). These data support the existence of a genotoxic stress response function for *p53-2* that is not present in *p53-1*.

The ancestral function of P53 in regulating the response to genotoxic stress may have been lost or may have diverged between planarians and schistosomes. In planarians, radiation exposure rapidly induces body-wide apoptosis (30) and loss of *Smed-p53* expression (8, 9), but the precise role of p53 in initiating apoptosis after DNA damage remains unresolved. To directly test whether *Smed-p53* is required for stem cell death in response to DNA damage, we exposed *Smed-p53* (RNAi) animals (*SI Appendix*, Fig. S6*A*) to radiation and analyzed stem cell abundance and apoptosis. Stem cells were depleted in animals 1 or 2 d after radiation exposure, suggesting that radiation-induced apoptosis still occurred even after knockdown of *Smed-p53* (*SI Appendix*, Fig. S6 *B* and *C*). To determine whether stem cells were lost as a result of apoptosis, we stained purified neoblasts with the apoptosis marker annexin-V to quantify apoptosis within stem cells (31). Unirradiated *Smed-p53(RNAi)* animals showed no change in overall numbers of neoblasts or rates of apoptosis (Fig. 5). Radiation exposure caused a depletion of neoblasts and a corresponding increase in apoptosis equivalent to that in control animals, indicating that *Smed-p53* is not required for apoptosis in response to genotoxic stress in planarians. Together, these results suggest that the genotoxic stress response function of *p53-2* is unique to schistosomes and the function of the *p53-1* ortholog in both schistosomes and planarians is to regulate neoblast survival and differentiation.

Discussion

The evolution of the P53 family of proteins is both complex and fascinating. Based on data from the nematode *C. elegans*, insect *D. melanogaster*, and sea anemone *N. vectensis*, the ancestral role of P53 was likely to defend the germline from

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genotoxic stress (5–7). This stress could be in the form of exogenous radiation or endogenous transposable elements (32). This is an attractive hypothesis because it explains why invertebrates that do not seem to be susceptible to malignancies would possess a tumor suppressor (4).

Our studies in flatworms, however, suggest that the ancestral flatworm P53 (i.e., flatworm *p53-1* orthologs) functions in stem cell maintenance and differentiation as opposed to regulating the response to genotoxic stress. The expression pattern and RNAi phenotype of *p53-1* and *Smed-p53* are virtually identical, suggesting that *p53-1* orthologs may be functionally conserved across Platyhelminthes. Future investigation of *p53-1* orthologs (as well as *zfp-1* homologs) in other flatworm models will help clarify the extent of the evolutionary conservation of the regulation skin production and neoblast differentiation. Conversely, *p53-2* seems to be a more recent invention, emerging concurrently with Neodermata. Although further studies of *p53-2* orthologs in other flatworms are required, it is very tempting to speculate that their genotoxic stress response function represents convergent evolution of this proposed ancient function of the P53 family of proteins. This raises two major questions: Is the ancestral function of P53 actually stem cell regulation (e.g., controlling epidermal differentiation) rather than the genotoxic stress response? Or did the genotoxic stress response function spontaneously appear in an early metazoan ancestor and simply persist because of its usefulness? In addition, if *p53-2* is in fact a parasite-specific homolog that responds to genotoxic stress, why do parasites but not free-living flatworms need such a gene?

Ultimately, it is not possible to definitively answer fundamental questions regarding evolutionary history, but we come closer to understanding things such as ancestral function with every new model organism that we functionally study. Given that our understanding of the ancestral function of P53 is based on only a handful of model organisms, it is perhaps not surprising that these data challenge the existing notion that the ancestral function is to respond to genotoxic stress. We should note that these data do not refute previous theories of ancestral function; there are several possible explanations for our data. The most obvious would be that we failed to identify any *p53-2* orthologs in free-living flatworms because we have not examined enough high-quality genomes. We surveyed the genomes of 35 parasitic flatworms in which we identified at least 1 *p53-2* ortholog in 31 of these genomes, as opposed to our examination of 19 free-living flatworm genomes/transcriptomes in which we failed to identify any *p53-2* orthologs. Even taking genome/transcriptome quality into account, it is very unlikely that we would have failed to identify *p53-2* orthologs in 19 separate genomes if they indeed existed in free-living flatworms. It is also possible that *S. mediterranea* is the outlier whose *p53-1* ortholog does not function in response to DNA damage, whereas *p53-1* orthologs in other free-living flatworms could behave like *S. mansoni p53-2*. Another potential explanation for the appearance of a genotoxic stress response function in *p53-2* is that the said function existed in ancestral p53 homologs but was lost early on in flatworm evolution and then spontaneously reappeared in Neodermata. While this is not the most parsimonious explanation of our data, it is possible that P53 family

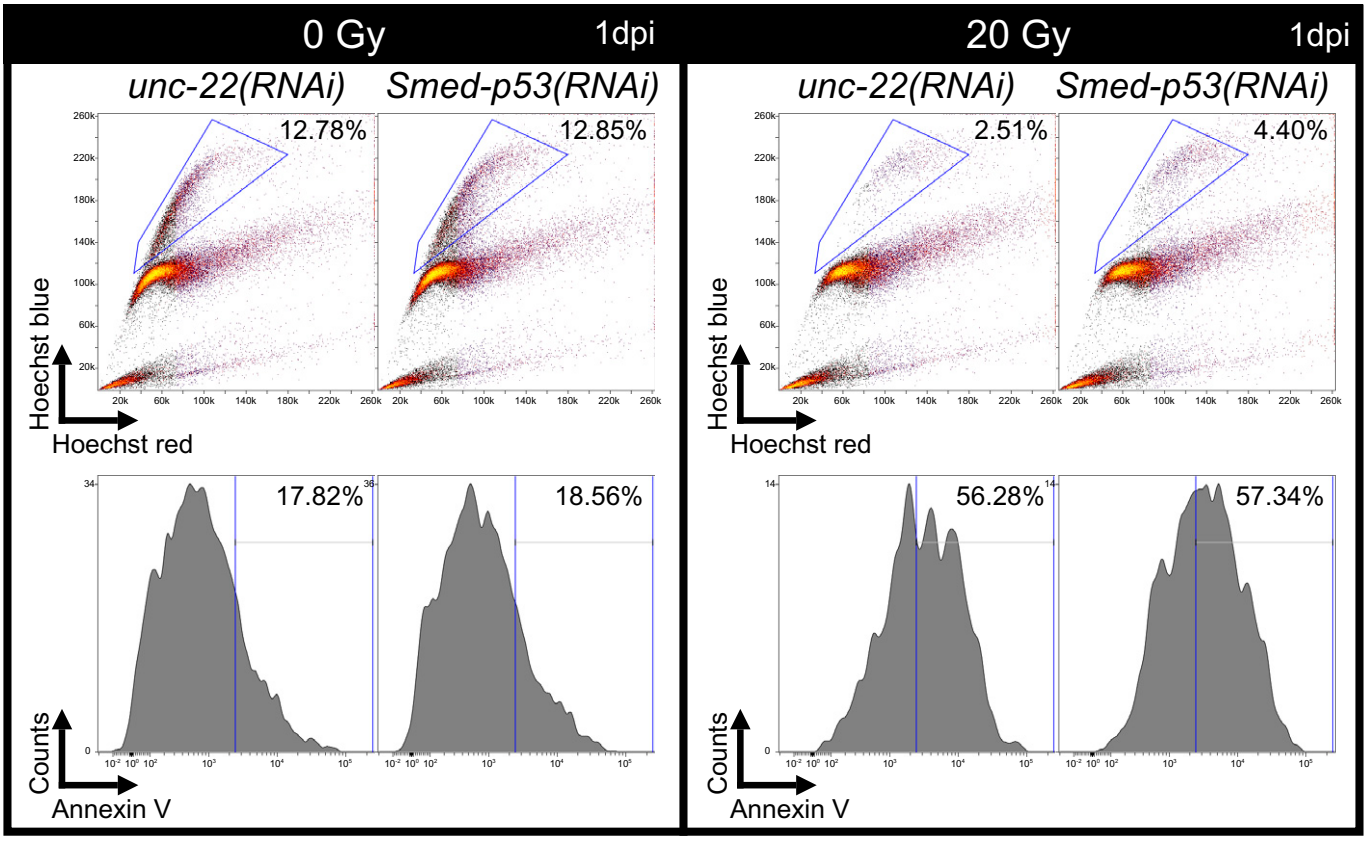

Fig. 5. *Smed-p53* RNAi does not protect planarian neoblasts from genotoxic stress. FACS plots of *Smed-p53* RNAi annexin V flow cytometry experiment. Percentage of neoblasts remaining following irradiation (*Upper*) and percentage of apoptotic neoblasts (*Lower*) are indicated in the 2D flow plot and flow histogram, respectively. Graphs show representative data from one of two biological replicates. Each biological replicate used 30 RNAi-treated animals per condition.

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proteins simply have a propensity for acquiring the ability to respond to genotoxic stress because of the nature of the DNA sequences that they bind. Indeed, if the ancestral function of P53 was in fact the regulation of stem cell maintenance and differentiation, then the ability to respond to genotoxic stress independently evolved at least twice (once in a basal ancestral metazoan and once again in parasitic flatworms).

The second question that arises from these studies is simpler but much harder to answer: Why do parasites need a P53 homolog that responds to genotoxic stress? Unlike free-living flatworms, parasitic flatworms actually spend much of their life living inside a host where they are presumably protected from environmental sources of genotoxic stress such as ultraviolet radiation. One factor to consider is the complex lifecycles that are characteristic of parasitic flatworms. Most alternate between at least one vertebrate and one invertebrate host, meaning that they are not always protected from environmental genotoxic stress (i.e., ultraviolet radiation). Thus, *p53-2* might respond to experimental genotoxic stress in adult schistosomes, but physiologically, it may be important only in other developmental stages that were not examined in this study. Another possible explanation pertains to endogenous threats to genome stability: transposable elements. *p53-2* orthologs could be involved in the suppression of transposable elements as has been demonstrated for P53 proteins in flies, zebrafish, and humans (32, 33). This hypothesis is especially attractive because many parasitic flatworm genomes studied to date seem to have lost components of the transposon-suppressing piwi-interacting RNA (piRNA) pathway (34, 35), which raises questions regarding how they protect their genome from transposable elements. Further investigation of transposon activity after *p53-2* RNAi may yield interesting results.

As more flatworms become available for study in the laboratory, we will be able to better answer these questions specifically and possibly answer broader evolutionary questions as well. Despite being arguably the most widely studied gene in history, there is still a great deal that we do not know about the evolutionary origins of P53. Further studies of P53 homologs in flatworms could clarify the origins of this important gene while also teaching us more about the basic biology of parasitic flatworms, which will help in the development of novel therapeutics against these deadly pathogens.

Materials and Methods

Phylogenetic Analysis. Orthologs of schistosome P53 homologs were identified by using Wormbase Parasite version WBPS16 (23, 24). Examination of *p53-2* (Smp_136160) orthologs initially revealed only trematode orthologs of *p53-2*. A reciprocal BLAST search of the *Hymenolepis diminuta* genome identified the *p53-2* ortholog WMSIL1_LOCUS11874. Wormbase Parasite then identified 15 orthologs of WMSIL1_LOCUS11874 in 14 unique cestode genomes, each of which was confirmed to be a *p53-2* ortholog via a reciprocal BLAST search. PlanMine v3.0 (https://planmine.mpibpc.mpg.de/planmine/begin.do) was also used to search 19 additional free-living flatworm genomes for *p53-2* orthologs. The domain structure of P53 homologs was obtained by using SMART v9.0 (http://smart.embl-heidelberg.de/) (36, 37).

Phylogenetic trees were generated from p53 protein sequences by first using the FastME/OneClick Workflow function at NGPhylogeny.fr (38) to perform multiple sequence alignment with Multiple Alignment using Fast Fourier Transform (MAFFT) (auto flavor, gap extend penalty = 0.123, gap opening penalty = 1.53) and alignment trimming with Block Mapping and Gathering with Entropy (BMGE) (estimated matrix BLOSUM = 62, sliding window size = 3, maximum entropy threshold = 0.5, gap rate cutoff = 0.05, minimum block size = 5). BMGE trimmed sequences were next filtered for identical sequences (i.e., p53 homologs from closely related animals) and were then analyzed using Randomized Axelerated Maximum Likelihood (RAxML) version 8.2.12 (39) via raxmlGUI version 2.0.6 (40) with the following parameters: -f a -x 256425 -p 256425 -N 1000 -m PROTGAMMAPMB -k -O. The best model (PROTGAMMAPMB) was determined by using ModelTest-NG version 0.1.7 (41). The best maximum-likelihood tree was visualized by using FigTree version 1.4.4 and was rooted to *Amphimedon queenslandica*, a representative of Porifera, the sister phylum to Eumetazoa.

All *p53-1* and *p53-2* orthologs are listed in Dataset S1, organisms examined and the genome/transcriptome databases referred to are listed in Dataset S3, the unaligned sequences submitted to NGPhylogeny are provided in Dataset S4, the BMGE trimmed sequences are listed in Dataset S5, and the input sequences for RAxML analysis (with duplicate sequences removed) are listed in Dataset S6.

Accession of Data from schisto.xyz. Schisto.xyz plots (27) in *SI Appendix*, Fig. S2 were obtained from v7test.schisto.xyz by querying Smp_139530 (p53-1) and Smp_136160 (p53-2). Data are from multiple RNA-seq experiments, details of which are available at https://v7test.schisto.xyz/dataused/.

Parasite Acquisition and Culture. Adult *S. mansoni* (NMRI strain, 6–7 wk after infection) were obtained from infected female mice by hepatic portal vein perfusion with 37 °C Dulbecco modified Eagle medium (DMEM; Sigma-Aldrich, St. Louis, MO) plus 10% serum (either fetal calf serum or horse serum) and heparin. Parasites were cultured as previously described (25). Unless otherwise noted, all experiments were performed with male parasites to maximize the amount of somatic tissue present and to avoid additional experimental modifications that have to be undertaken for successful in vitro culture of female parasites (42). Experiments with and care of vertebrate animals were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center (approval APN: 2017–102092).

Planarian Husbandry. Asexual *S. mediterranea* (strain CIW-4) stocks were maintained in 1× Montjuïc water and were fed homogenized beef liver as previously described (43). Worms were kept at 20 °C in a shaded environment with a 12-h light–dark cycle.

Radiation.

Schistosome radiation. Schistosomes were exposed to a radiation dose of 2,000 rad using a Precision X-Ray X-Rad320 system (Madison, CT). All animals were rinsed and placed in fresh Basch media after being irradiated.

Planarian radiation. Planarians were exposed to a radiation dose of 2,000 rad by using a J.L. Shepherd & Associates Mark I-68 Irradiator (San Fernando, CA). All animals were rinsed immediately after being irradiated, and planarian water was replaced.

Fluorescence-Activated Cell Sorting (FACS). Hoechst-stained cells were analyzed by using annexin V flow cytometry as previously described (31, 44, 45) with some modifications. Thirty animals per group were dissected in calcium, magnesium-free phosphate buffer (CMFB) (calcium, magnesium-free [CMF] containing 0.5% bovine serum albumin [BSA]) and dissociated using 1:50 Liberase (2.5 mg/mL, Roche 5401135001) in CMFB at 30 °C with agitation at 300 rpm for 30 min on an Eppendorf ThermoMixer. Samples were triturated every 5 min with a pipette to aid dissociation. Dissociated cells were then diluted with equal volumes of CMFB and pelleted by centrifugation (500 × *g*, 5 min, room temperature [RT]). Pellets were resuspended in 1 mL of CMFB and passed through a 30-mm cell strainer (BD 340627). Strained cells were counted on an automated cell counter (Bio-Rad TC20), and 2.83×10^6 cells per group were stained with 5 μg/mL Hoechst 33342 (Thermo Fisher Scientific H3570) in CMFB for 70 min in the dark with gentle agitation. Cells were subsequently pelleted as before, and Hoechst solution was replaced with annexin V staining buffer (2 μL annexin V allophycocyanin [APC; Thermo Fisher Scientific A35110] diluted in 100 μL freshly made 1× annexin V buffer from a 10× stock solution [0.1M *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), pH 7.4, 1.4 M NaCl, and 25 mM $CaCl_2$]). After staining for 15 min at RT, 400 μL of 1× annexin V buffer with 1 μg/mL propidium iodide (Sigma P4170) was added to each tube. Cells were analyzed on a BD FACSymphony Analyzer. Data were analyzed in FlowJo (TreeStar, Ashland, OR).

Labeling and Imaging. Schistosome colorimetric and FISH analyses were performed as previously described (12, 25, 46) with the following modification. To improve signal-to-noise ratio for colorimetric in situ hybridization, all probes

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were used at 10 ng/mL in hybridization buffer. In vitro EdU labeling and detection were performed as previously described (46). All fluorescently labeled parasites were counterstained with DAPI (1 μg/mL), cleared in 80% glycerol, and mounted on slides with Vectashield (Vector Laboratories).

Confocal imaging of fluorescently labeled samples was performed on a Nikon A1 Laser Scanning Confocal Microscope. Unless otherwise mentioned, all fluorescence images represent maximum intensity projection plots. To perform cell counts, cells were manually counted in maximum intensity projection plots derived from confocal stacks. To normalize counts, we collected confocal stacks and normalized the number of cells counted to the length of the parasite in the imaged region. Brightfield images were acquired on a Zeiss AxioZoom V16 microscope equipped with a transmitted light base and a Zeiss AxioCam 105 color camera.

RNAi. Planarian RNAi: For *Smed-p53* RNAi FACS experiments, RNAi was carried out as previously described (47). Briefly, double-stranded RNA (dsRNA) was synthesized in vitro by using PCR products for *Smed-p53* and control gene *unc-22* as templates. Synthesized dsRNA was then mixed with a 4:1 liver:water paste containing 4 μg of dsRNA per 10 μL of liver. Animals were fed every 2 d for a total of three feeds. Radiation was carried out 4 d after the last feed, and animals were processed for annexin V FACS 24 h later.

Schistosome RNAi: All RNAi experiments used freshly perfused male parasites at 6 to 7 wk after infection (separated from females). dsRNA treatments were all carried out at 30 μg/mL in Basch Media 169. dsRNA was generated by in vitro transcription and was replaced once per day for 3 d and then every 3 d until the end of the experiment. EdU pulses were performed at 5 μM for 4 h before either fixation or chase as previously described.

We used a nonspecific dsRNA containing two bacterial genes (48) as a negative control for RNAi experiments. Complementary DNAs (cDNAs) used for RNAi, and in situ hybridization analyses were cloned as previously described (48); oligonucleotide primer sequences are listed in Dataset S7.

Statistical Analysis. Statistical analysis was carried out using one-way ANOVA tests (qPCR experiments), two-way ANOVA with Tukey's multiple comparisons tests (radiation and cisplatin experiments), or Brown-Forsythe and Welch ANOVA with Dunnett's T3 multiple comparisons tests (all other experiments). All statistical tests were performed using GraphPad Prism v9.3.1. The *P* value for symbols denoting significance are provided in the figure legends.

Data Availability. All study data are included in the article and/or supporting information.

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