Supporting Online Material

Materials and Methods

Planaria Culture and Irradiation

Schmidtea mediterranea asexual strain ClW4 was maintained as described (*1*). Animals were starved in the presence of Gentamicin (Gibco) for ten days prior to irradiation experiments. Individual irradiation experiments used size-matched animals with identical feeding and culturing histories. Irradiation was delivered to animals at 79-82 rads/min using dual Gammacell-40 ^{137}Cesium sources (i.e., sources positioned both above and below the specimen). For survival experiments, irradiated animals were maintained in 6 cm Petri dishes (10 worms/dish) in the dark; water and dishes were changed every 3-4 days.

In situ Hybridization and Tissue Sectioning

Whole-mount *in situ* hybridizations (ISH) and fluorescent *in situ* hybridizations (FISH) were performed and RNA probes prepared as described (*2*). Tyramide-conjugated fluorophores were generated from AMCA, Fluorescein, Rhodamine (Pierce), and Cy5 (GE Healthcare) NHS esters as previously reported (*3*). For double/triple labeling, HRP-inactivation was performed between labelings in 4% formaldehyde, 45 min. Tissue sectioning was performed as previously described (*4*).

BrdU labeling and Immunofluorescence

Animals were fed or injected with 5 mg/mL BrdU (Fluka) as previously described (*5*). Specimens were fixed in 4% formaldehyde as described (*2*) and antibody labelings performed as reported (*4*) using rat anti-BrdU (1:100, Oxford Biotech), rabbit anti-H3P (1:100, Millipore), or rabbit anti-SMEDWI-1 (*6*) (1:2000).

Microscopy

Microscopy images were captured with an AxioCam HRm on a Zeiss Stereo Lumar V12 or an Axio Imager Z1 using Zeiss Axiovision software. Double-positive cells were scored in optical sections obtained with an Apotome (Zeiss). Additional images were collected on a Zeiss LSM 700 confocal microscope using Zen software.

Phylogenetic Analysis

Putative members of *gata4/5/6* and *hnf4* gene families were identified in the *Schmidtea mediterranea* genome. Peptide sequences were aligned with well-known members of these families using ClustalW with default settings (*8*). Alignments were trimmed using GBlocks (*9*). Neighbor-joining trees were generated using ClustalW using default settings and 1,000 bootstrap replicates. Maximum likelihood analyses using 100 bootstrap replicates were run on each alignment using PhyML (*10*) with WAG model of amino acid substitution, four substitution rate categories, and the proportion of invariable sites estimated from the dataset. Maximum likelihood trees are shown in Supplemental Figures 7 and 9. Maximum likelihood bootstrap values greater or equal to 50 (50%) and neighbor-joining bootstrap values greater than 500 (50%) are indicated in bold and italics, respectively.

X1(FS) Cell Collection

Animals were starved for at least seven days prior to harvesting. For control cells, animals were macerated in 1.0 mg/ml collagenase (Sigma) and 0.3 mM N-acetyl-L-cysteine (Sigma) for 1 hour and labeled in 0.4mg/ml Hoechst 33342 (Invitrogen) for 45 minutes. For transplant cells, animals were macerated in 1.0 mg/ml collagenase and 0.3 mM N-acetyl-L-cysteine for 20 minutes. The X1 population from Hoechst-labeled control cells was used to define the forward scatter/side scatter gate. Cells were sorted with a Dako Cytomation MoFlo sorter.

Single Cell Transplantation

Animals to receive transplants were starved in the presence of Gentamicin for at least seven days prior to onset of experiments. Three days prior to transplantation, irradiation was delivered to animals at 79-82 rads/min for 76 minutes. Cells collected by flow cytometry were loaded at low density onto glass cover slips treated with 2% dimethyldichlorosilane (Sigma) in chloroform. Individual cells were selected based on morphology with 10x magnification and loaded by mouth pipetting into the tip of pulled borosilicate glass microcapillaries (Sutter) treated with 0.1% polyvinylpyrrolidone (Sigma). Loaded cells were injected into the post-pharyngeal midline of cold-immobilized animals at 1.5-2.5 psi (Eppendorf FemtoJet). For survival experiments, transplant recipients were maintained in 6 cm Petri dishes (3 worms/dish) in the dark; water and dishes were changed every 3 days.

SNP Discovery

Short (36 bp) sequencing reads from both asexual Clone 4 and sexual S2F1L3F2 strain expressed sequences were obtained by mRNA-Seq (Illumina). Reads were mapped to an assembly of planarian expressed sequences and Single Nucleotide Polymorphisms (SNPs) were identified with MAQ using default settings (*7*). Only SNPs based on at least 10X read depth for both strains were considered. Candidate loci were selected based on presence of multiple homozygous SNPs. SNP-containing loci were validated by PCR and Sanger sequencing (see SNP sequencing below).

Genomic DNA Isolation

Animals used for genotyping were subjected to two rounds of regeneration and starved for at least five days. DNA was isolated from intact regenerated animals using Easy-DNA kit (Invitrogen).

PCR-RFLP Analysis

Restriction Fragment Length Polymorphism (RFLP) loci were amplified from genomic DNA samples (Finnzymes Phusion Polymerase). PCR product was purified and digested with restriction enzymes (HpaI or ScaI-HF, New England Biolabs) for two hours. Digested DNA was purified by phenol-chloroform extraction and run on a 1.4% agarose gel.

SNP Sequencing

SNP loci were amplified from genomic DNA. PCR products were gel-purified, A-tailed (Roche Taq Polymerase), and ligated into pGEM-T Easy vector (Promega). 94 bacterial colonies from each locus for each strain were Sanger sequenced with M13F primer. For genotyping, reads were counted if at least four SNPs corresponding to a single haplotype were present.

RFLP loci sequences

> RFLP 00310 (S2F1L3F2)

TCGGATACAGTAAATCACCTGATACTATTGCTACGGGCTATTCTGGTGAT
GCTCCCCCGTCTATAACTGCTGCACAATTACAACTGAGTCCTGGTCAAGC
GGACACGGGATACGTATCATTGACGTGGAATATACTGACCCAGTCCGACA
TTGCCACGAATGTGAACGGATTTTTCCGTGGATATCGAATTGAATGGTGC
TTGGCAAACCTGATTGATGCGGAATGTGATGCATCAACTCAATATCAGGT
AAATAGGTAATTAGACGTTTTATGTTTAATATTATAAGGATGTGATTCTC
GCAACACAAAACCTCCCGGTTCTTTATGGAAATAAGCGCCGTAAAAGATC
AGTACAAGATGATGATGAAGACACACAGACGGATGAAGGAAGATTTAAAT
ATGATACTAAATATCGCCAGGTCATACCAGACAACCCAGCAACGTCAGTT
AATTTTCAAGTCTTAAGTCGTAGAAAACGAGCTGCATTAAAAAATCCTGA
TGATTGGAATTATGGAAAAAATATCACTGTAAAATTGACGATGATTCCAG
GCAATACTTGGATCAAGGTTTGGCTGAGAGTTTTGAAT

> RFLP 00310 (CIW4)

TCGGATACAGTAAATCACCTGATACTATTGCTACGGGCTATTCTGGTGAT
GCTCCCCCGTCTATAACTGCTGCACAATTACAACTAGGTCCTGGTCAAGC
GGACACGGGCTACGTATCATTGACGTGGAATATACTGACCCAGTCCGACA
TTGCCACGAATGTTAACGGATTTTTCCGTGGATATCGAATTGAATGGTGC

TTGGCAAACCTGATTGATGCAGAATGTGATGCATCAACTCAATATCAGGT
AAATAGGTAATTAGACGTTTTATGTTTAATATTATAAGGATGTGATTCTC
GCAACACAAAACCTCCCGGTTCTTTATGGAAATAAGCGCCGTAAAAGATC
AGTACAAGATGATGATGAAGACACACAGACGGATGAAGGAAGATTTAAAT
ATGATACTAAATATCGCCAGGTCATACCAGACAACCCAGCAACGTCAGTT
AATTTTCAAGTCTTAAGTCGTAGAAAACGAGCTGCATTAAAAAATCCTGA
TGATTGGAATTATGGAAAAAATATCACTGTAAAATTGACGATGATTCCAG
GCAATACTTGGATCAAGGTTTGGCTGAGAGTTTTGAAT

> RFLP 00463 (S2F1L3F2)
ATCGGATCACCTATCAATATTTGCCTCCGGCTGCATTCAACATTGAACTC
GTTCCGCAATCTTCATCAGCCAATAACAGCAGCAAAACATCTTCGGATTG
CCACAGGAATTCAGATGGCAGCCGGAAATTGAGATCGCATACTCTCCCAG
GCGACAAAATCGCTCCTGTTGTCATTGGCAATGCGCCCGCTCAACAGTCG
GCCTCCACAGCAGATTCGCCTATCATGGCAACGAGAAACCTTCGCGGATG
GATTGTAATACTCAAGGAGTACTTTGGATTCGTGGAAACGGCCGATCACA
ACGCGCTATACAAGTTCAGCCCGTTCACAATCAAGAAGAGCAAATTGGGA
GTGGAATTGAAGGTTGGCTCGGCGATTGAATTTCTGGCGGTCCCGAGCTC
TGGCAGTCGGCCTCGTCGCATCATTGAGCAGTTCCTGAAGGTCCTCACCG
AGCCGTTATCCAATGAG

> RFLP 00463 (CIW4)
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GTTCCGCAATCTTCATCAGCCAATAACAGCAGCAAAACATCTTCGGATTG
CCACAGGAATTCAGATGGCAGCCGGAAATTGAGATCGCATACTCTCCCAG
GCGACAAAATCGCTCCTGTTGTCATTGGCAATGCGCCCGCTCAACAGTCG
GCCTCCACAGCAGATTCGCCTATCATGGCAACGAGAAACCTTCGCGGATG
GATTGTAATACTCAAGGAGTATTTTGGATTCGTGGAAACGGCCGATCACA
ACGCGCTATACAAGTTCAGCCCGTTCACAATCAAGAAGAGCAAATTGGGA
GTGGAATTGAAGGTTGGCTCGGCGATTGAATTTCTGGCGGTCCCGAGCTC
TGGCAGTCGGCCTCGTCGCATCATTGAGCAGTTCCTGAAGGTCCTCACCG
AGCCGTTATCCAATGAG

Sequences of SNP loci

> SNP 00163 (S2F1L3F2)
CCCAGTGAAAAACCCAAACATGGAAATGAAGATTGTTTCAATACATTTTT
CAGTGAGACTGGAAATGGAAAATATGTTCCTCGGGCTCTTTTTGTCGATT
TGGAACCAAGTGTAATTGGTAAGTTTTAGAATTTTGTGTTTTATATTTTT
AATGCTCTCCTGCTTTAGGTGAAGTGAGAAATGGGGCTTATAGACAACTG
TTCCATCCGGAACAACTTATTAGTGGTAAAGAAGATGCAGCTAATAATTA
CGCAAGAGGACATTATACAGTGGGTAAAGAATTGATCGATCAAGTTTTAG
ATAGAATTAGAAAGGTTGCTGATAATTGTACCGGTTTGCAAGGGTTTCTA
ATGTTTCATTCATTTGGTGGTGGAACTGGTTCCGGGTTTACTTCTCTGTT
AATGGAACGGTTAAGTGTTGATTATGGTAAAAAATCCAAGTTAGAGTTTG

CTGTTTATCCTGCTCCACAAATCG

> SNP 00163 (CIW4)
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TGGAACCAAGTGTAATTGGTAAGTTTTAGATTTTTGTGTTTTCTATTTTT
AATGCTCTCCTGCTTTAGGTGAAGTGAGAAATGGGGCTTATAGACAACTG
TTCCATCCGGAACAACTTATTAGTGGTAAAGAAGATGCAGCTAATAATTA
CGCAAGAGGACATTATACAGTTGGGAAAGAATTGATCGATCAAGTTTTAG
ATAGGATAAGAAAGGTTGCTGATAATTGTACCGGTTTGCAAGGGTTTCTA
ATGTTTCATTCATTTGGTGGTGGAACTGGTTCCGGGTTTACTTCTCTGTT
AATGGAACGGTTAAGTGTTGATTATGGTAAAAAATCCAAGTTAGAGTTTG
CTGTTTATCCTGCTCCACAAATCG

> SNP 00463 (S2F1L3F2)
GACGATATTGCCGGATTGATTGAGCCGGATTTATCGGTAAAGGGTGATGA
GGACATGCTGGTAGCGTTCAAGGCCAAGGAATGGCTACCAAGCGATGAGA
AACTGACAAAGTTTGCGGTAGTTTCCTACACGCCGTTGCAAGTGTCCAGG
AACGTCGGAGGCCACCAGAAGCTGACGACAACCGCGCTGCGCATCATGCC
ATCAAAGGAGAGGGACAAAATGACCCACCTGAAGCTGTATCCGGCCGGCC
AGTTACGGGGAGTTGTCAATACTGCAGTTCACAATCCCGATGATCTCGGC
ATCATTTTCTGTCAGTTTCCCAATTCATCTACCAAACGAACGGTCGCATT
TACCAGTGAGGATCTTGTCAACTGCAAGCCCAAGGT

> SNP 00463 (CIW4)
GACGATATTGCCGGATTGATTGAGCCGGATTTATCGGTAAAGGGCGATGA
GGACATGCTGGTAGCGTTCAAGGCCAAGGAATGGTTGCCAAGCGATGAGA
AACTGACAAAGTTTGCGGTAGTTTCCTACACGCCGTTGCAAGTGTCCAGG
AACGTCGGAGGCCATCAGAAGCTGACGACAACCGCGCTGCGCATCATGCC
ATCAAAGGAGAGGGACAAAATGACCCACCTGAAGCTGTATCCGGCCGGCC
AGTTGCGGGGAGTTGTCAATACTGCAGTTCACAATCCCGATGATCTCGGC
ATCATTTTCTGTCAGTTTCCCAATTCATCTACCAAACGGACGGTCGCATT
TACCAGTGAGGATCTTGTCAACTGCAAGCCCAAGGT

> SNP 02716 (S2F1L3F2)
GCTTTCGTCTATATGTTAGAGCGTTTCATCAATACTTCACTGACTTCGGT
AAATAAATGGTGTGCTATAACATTTTTTGTTTACCTATTTACATAGGATC
AAGTACAAAATCATTTGTTAGATCTTTGCCAAATAATTTCTTCACCGAAA
AAAGAAAAAATTATCGATTTCTTGAAAAAAGAAGGACCTAAATTGATTCC
CCGAATACTTAAAAAAGACTGTCCAATAAAGATTTGTCAAATGGAAAACT
TTTGTCACAAAATCGAAGTTATATTCGAAAATGATGATAAGGTTCCAAGT
AATTTAATTTACTCTAAGGGATTTTCGAATATATCTTCTAATTAGATCCA
TCAAAATATTGTTCGATCTGCCAATCGG

> SNP 02716 (CIW4)

GCTTTCGTCTATATGTTAGAGCGTTTCATCAATACTTCACTGACTTCAGT
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GTTGTCACAAAATCGAAGTTATATTCAAAAATGATGATAAGGTTCCAAGT
AATTTAATTTACTCTAAGGGATTTTCGAATATATCTTCTACTTAGATCCA
TCAAAATATTGTTCGATCTGCCAATCGG

Primers for PCR-RFLP analysis.

RFLP_00310_F1	5’-TCGGATACAGTAAATCACCTGATAC-3’
RFLP_00310_R2	5’-ATTCAAAACTCTCAGCCAAACC-3’
RFLP_00463_F1	5’-ATCGGATCACCTATCAATATTTGC-3’
RFLP_00463_R1	5’-GATAACGGCTCGGTGAGGAC-3’

Primers for SNP loci sequencing.

SNP_00163_F1	5’-GTCTTGAACATGGTATTCAACAAGA-3’
SNP_00163_F2	5’-CCCAGTGAAAAACCCAAACA-3’
SNP_00163_R1	5’-ATGGTTCAACAACCGCTGTA-3’
SNP_00163_R2	5’-CGATTTGTGGAGCAGGATAAA-3’
SNP_00463_F1	5’-CTCGACATATCGGAGTTGTGAA-3’
SNP_00463_F2	5’-GACGATATTGCCGGATTGA-3’
SNP_00463_R1	5’-CAACTAACTGACAGGCAGCAAC-3’
SNP_00463_R2	5’-ACCTTGGGCTTGCAGTTG-3’
SNP_02716_F1	5’-TCACGATGGAAACCAAAAAG-3’
SNP_02716_F2	5’-GCTTTCGTCTATATGTTAGAGCGTTTC-3’
SNP_02716_R1	5’-TTTTCTAAGGCTACCCAGCTGAT-3’
SNP_02716_R2	5’-ACCGATTGGCAGATCGAA-3’

Figure S1

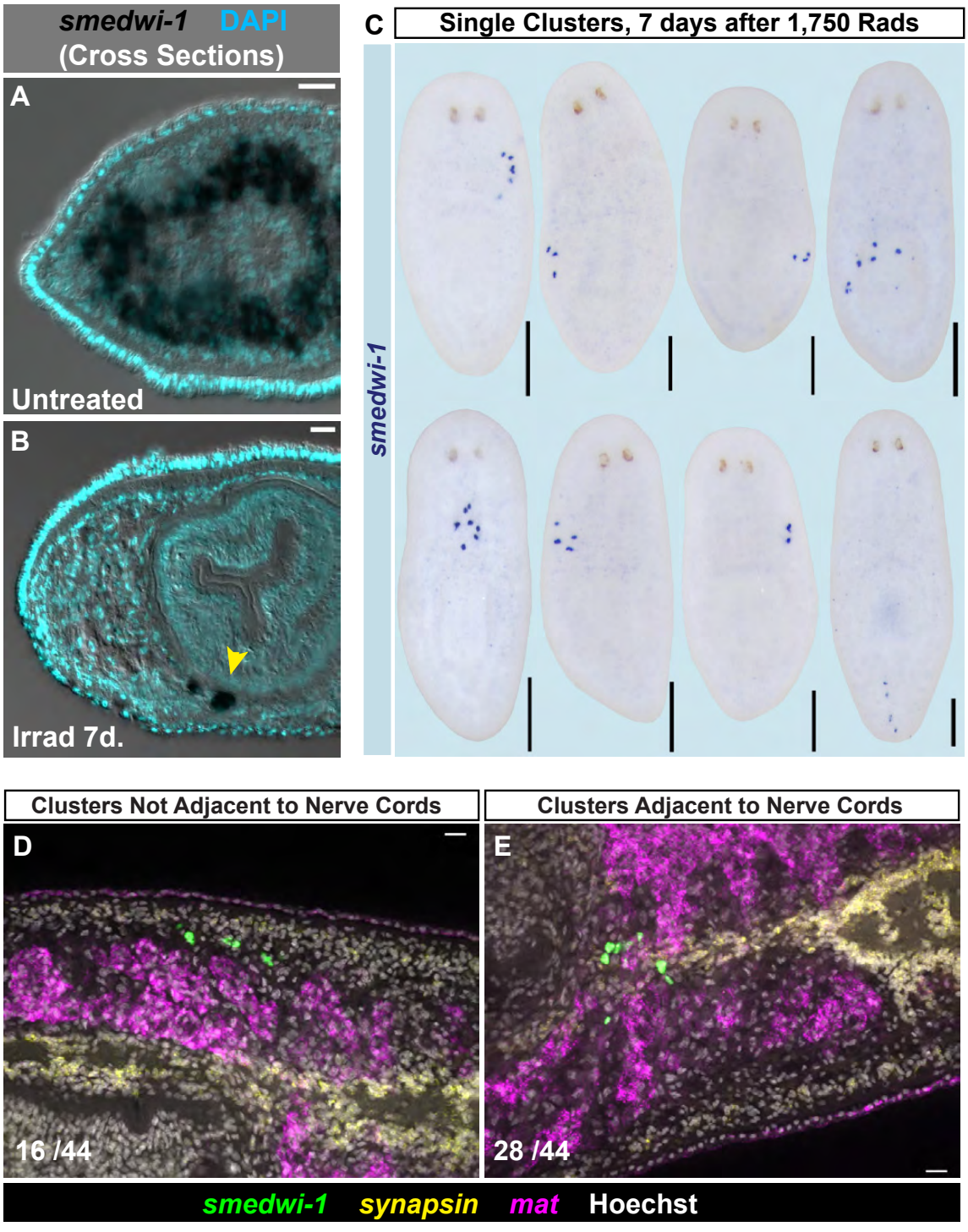

smedwi-1 DAPI
(Cross Sections)
A
Untreated
B
Irrad 7d.
C
Single Clusters, 7 days after 1,750 Rads
smedwi-1
Clusters Not Adjacent to Nerve Cords
Clusters Adjacent to Nerve Cords
D
16 /44
E
28 /44
smedwi-1 synapsin mat Hoechst

Figure S1. *smedwi-1*$^+$ clusters originate in ventral positions throughout the body

(A-B) Transverse tissue sections shown, dorsal up. Control worms display *smedwi-1*$^+$ cells distributed in parenchyma throughout the dorsal-ventral axis (A). Representative image of a *smedwi-1*$^+$ cluster (arrowhead) seven days post-1,750 rad treatment (B). **(C)** Representative images of animals displaying single *smedwi-1*$^+$ clusters seven days post-1,750 rad treatment. Whole animals, anterior up. Individual clusters were scattered throughout body, but consistently displayed compact, isolated, colony-like morphology. **(D-E)** Triple FISH for *synapsin* (central nervous system), *mat* (intestine), and *smedwi-1* seven days after 1,750 rads. Nuclei are labeled with Hoechst. Shown are projections through Apotome optical sections. Individual *smedwi-1*$^+$ clusters were distributed throughout the ventral regions and were not invariantly associated with specific organs or anatomical features. Some clusters had cells adjacent (within ~1 cell diameter) to the ventral nerve cords (28/44); some clusters were located large distances from the nerve cords (16/44). Scale bars 20μm (A-B, D-E), 200μm (C).

Figure S2

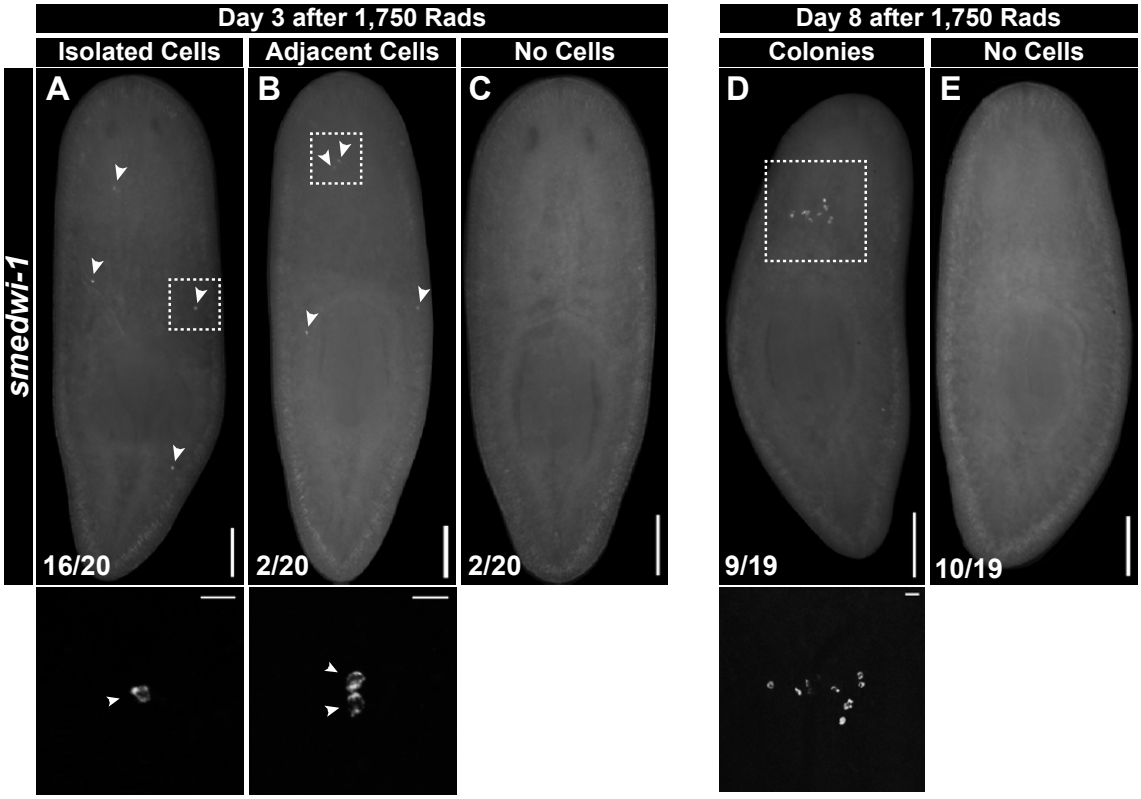

Day 3 after 1,750 Rads
Isolated Cells
Adjacent Cells
No Cells
Day 8 after 1,750 Rads
Colonies
No Cells
smedwi-1
A
B
C
D
E
16/20
2/20
2/20
9/19
10/19

Figure S2. Following irradiation, clusters are preceded by isolated *smedwi-1*$^+$ cells

(A-E) Representative images of *smedwi-1* (FISH) after 1,750 rad exposure. Whole animal, anterior up. Magnified regions are indicated by boxes. Arrowheads denote individual *smedwi-1*$^+$ cells. Three days after irradiation, the majority of animals (16/20) displayed isolated *smedwi-1*$^+$ cells (A). Animals with >1 *smedwi-1*$^+$ cell in close proximity (within 50μm) were rare (2/20) (B). Remaining animals at this condition (2/20) displayed no *smedwi-1*$^+$ cells (C). Animals fixed 8 days post-irradiation displayed either small *smedwi-1*$^+$ clusters (9/19) (D) or were devoid of *smedwi-1*$^+$ cells (E). Based on these proportions, the possibility that all 8-day clusters arose from multiple cells can be excluded ($P = 0.0138$, 2-tailed Fisher's Exact Test). Scale bars 200μm (a-e), 20μm (zoomed boxes).

Figure S3

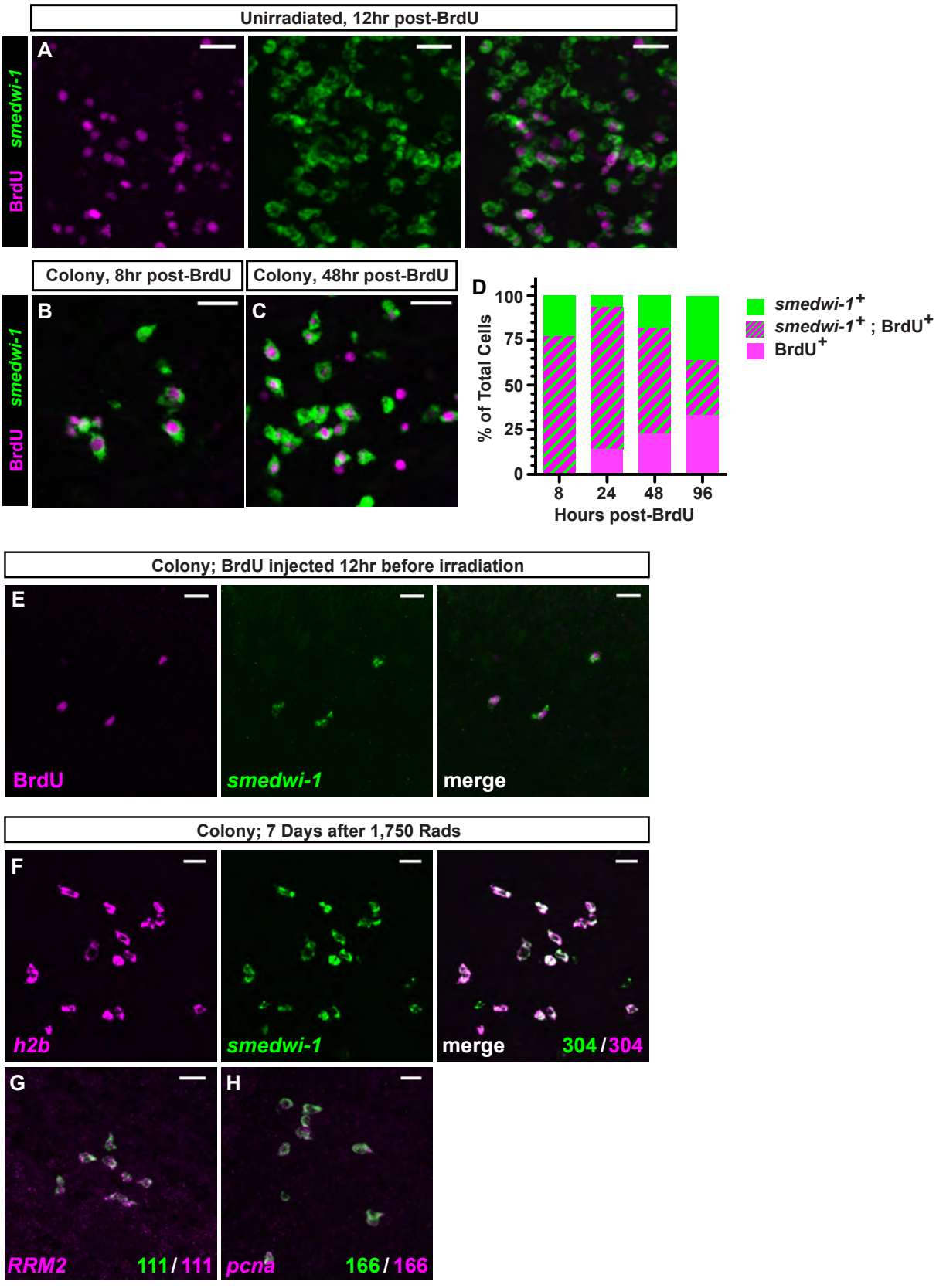

Unirradiated, 12hr post-BrdU
A
BrdU
smedwi-1
Colony, 8hr post-BrdU
Colony, 48hr post-BrdU
B
C
D
% of Total Cells
100
75
50
25
0
8
24
48
96
Hours post-BrdU
smedwi-1+
smedwi-1+ ; BrdU+
BrdU+
Colony; BrdU injected 12hr before irradiation
E
BrdU
smedwi-1
merge
Colony; 7 Days after 1,750 Rads
F
h2b
smedwi-1
merge
304/304
G
RRM2
111/111
H
pcna
166/166

Figure S3. BrdU incorporation and proliferation of *smedwi-1*$^+$ colony cells

(A) Immunofluorescence (BrdU) and FISH (*smedwi-1*). All dividing cells (571/571) detected in unirradiated, intact worms by a 12-hour BrdU pulse expressed *smedwi-1*$^+$ (see also, Guo *et al*, 2006). Thus, *smedwi-1*$^+$ cells are the sole source for BrdU-labeled cells in these animals. **(B-D)** BrdU incorporation and dilution kinetics in expanding clusters of *smedwi-1*$^+$ cells. Animals were injected with BrdU 7 days after 1,750 rads and fixed at various timepoints. Representative immunofluorescence (BrdU) and FISH (*smedwi-1*) images are shown (B-C). Percentage of *smedwi-1*$^+$, BrdU$^+$, and *smedwi-1*$^+$; BrdU$^+$ (double-positive) cells are indicated for each timepoint ($n \geq 154$ cells analyzed/ timepoint) (D). BrdU is rapidly incorporated into a majority of *smedwi-1*$^+$ cells within the first 8 hours following labeling. Following a brief 48 hour chase period, the first significant signs of BrdU dilution from the continuously dividing *smedwi-1*$^+$ population are evident. At 24 hours post-BrdU, 123/133 (92.5%) *smedwi-1*$^+$ cells were BrdU$^+$, compared with 91/119 (76.5%) at 48 hours post injection. Fisher's Exact Test indicates that this difference is significant (P=0.0006, 2-tailed). Unincorporated BrdU, therefore, is unlikely to remain within injected animals for longer than 48 hours post-injection (see also, Eisenhoffer *et al*, 2008). **(E)** Animals were irradiated (1,750 rads) 12 hours after BrdU injection and fixed 5-6 days later. Shown is a representative cluster following immunofluorescence (IF) detection of BrdU$^+$ cells and FISH (*smedwi-1*). Several colonies of 3-12 *smedwi-1*$^+$ cells were identified (n=9), and nearly all of these cells (47/48) contained BrdU$^+$ nuclei (BrdU signal is anticipated to ultimately be absent from some cells after many cell divisions). **(F-H)** Double FISH seven days after 1,750-rad irradiation. All proliferating cells expressing *Smed-h2b* (**F,** 304/304), *Smed-RRM2* (**G,** 111/111), or *Smed-pcna* (**H,** 166/166) were *smedwi-1*$^+$-colony cells. Scale bars 200μm (A-E), 20μm (zoomed images, F-H).

Figure S4

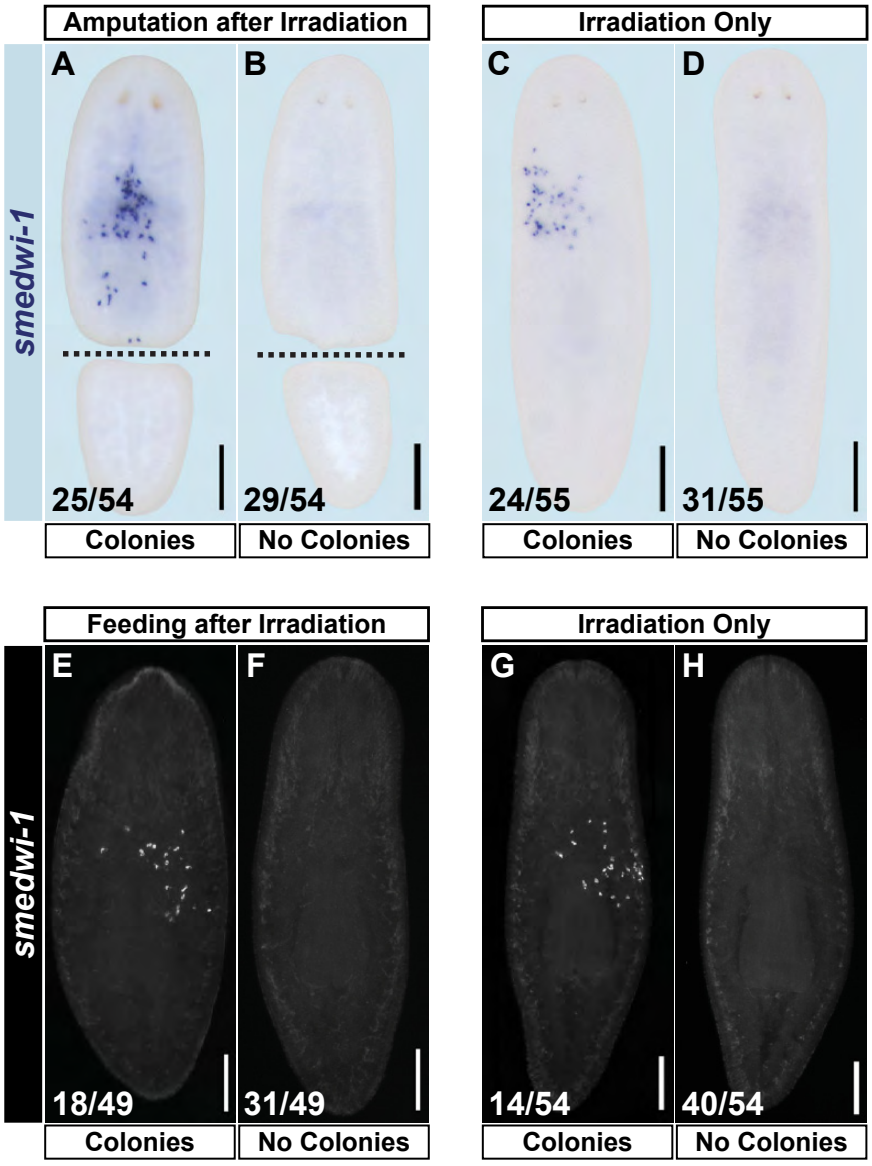
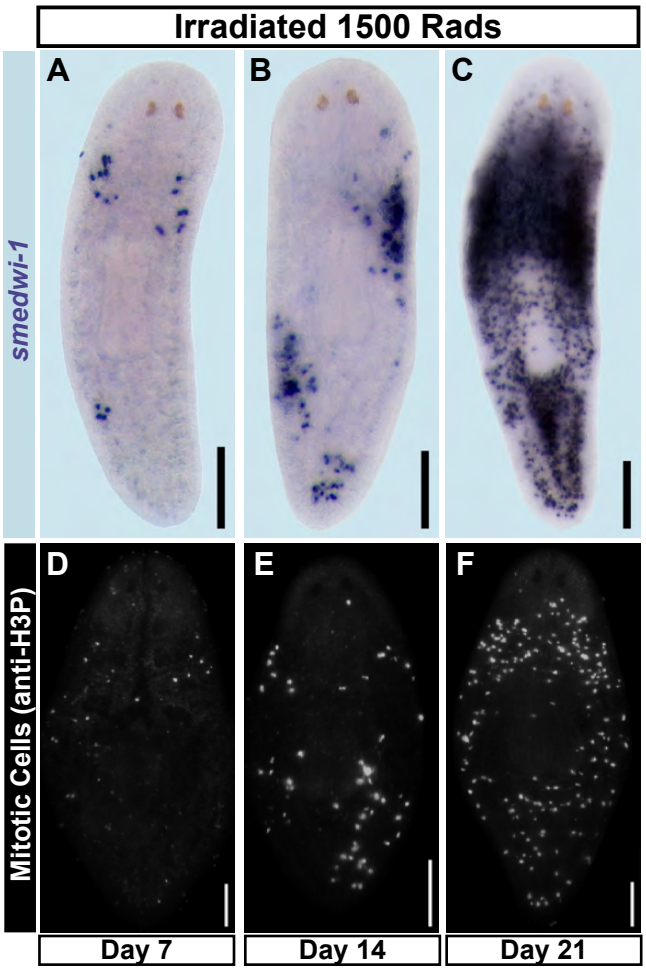

Amputation after Irradiation
Irradiation Only
smedwi-1
A
B
C
D
25/54
29/54
24/55
31/55
Colonies
No Colonies
Colonies
No Colonies
Feeding after Irradiation
Irradiation Only
smedwi-1
E
F
G
H
18/49
31/49
14/54
40/54
Colonies
No Colonies
Colonies
No Colonies

Figure S4. Amputation and feeding fail to stimulate new colony formation following irradiation

(A-D) Animals were exposed to 1,750 rads and fixed 12 days later for *smedwi-1* ISH. Shown are whole animals (anterior up) amputated into fragments five days after irradiation (A-B), or left intact (C-D). Dotted lines indicate amputation plane. Amputated fragments from individual worms were cultured, fixed, and stained independently. The proportion of amputated worms displaying colonies (25/54) was not significantly altered from that of intact worms (24/55), indicating that amputation did not stimulate formation of additional colonies (P=0.8483, Fisher's Exact Test, 2-tailed). **(E-H)** Animals were exposed to 1,750 rads and fixed on day 12 for *smedwi-1* FISH. Animals were either fed four days after irradiation (E-F), or left untreated (G-H). The proportion of fed worms displaying colonies (18/49) was not significantly different from that of control worms (14/54), indicating that feeding failed to stimulate formation of additional colonies (P=0.2885, Fisher's Exact Test, 2-tailed). Scale bars, 200 μm.

Figure S5


Irradiated 1500 Rads
A
B
C
smedwi-1
D
E
F
Mitotic Cells (anti-H3P)
Day 7
Day 14
Day 21

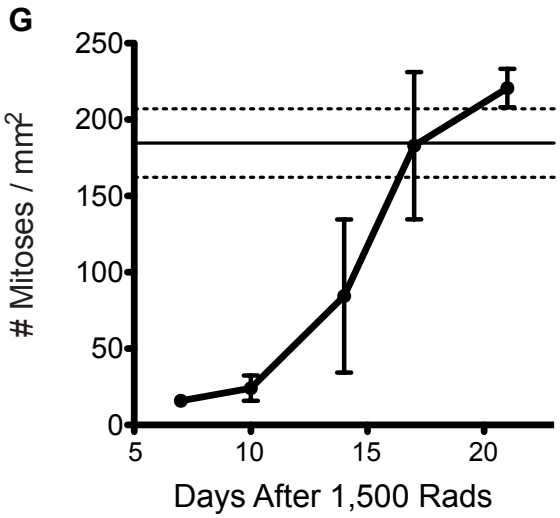

G
250
200
150
100
50
0
Mitoses / mm2
5
10
15
20
Days After 1,500 Rads

Figure S5. *smedwi-1*$^+$ cells and mitotic activity are restored to normal levels following irradiation with 1,500 rads

(A-F) Whole-mount *smedwi-1* ISH (A-C) and IF detection of Histone-H3 (phospho-serine-10)$^+$ mitotic cells (D-F). Representative images demonstrate that the number and body-wide distribution of both *smedwi-1*$^+$ cells and mitotic activity are gradually restored following irradiation at 1,500 rads. **(G)** A timecourse illustrates that mitotic numbers (normalized by worm area) are almost completely depleted 7 days after irradiation. Successive timepoints display increased levels of mitotic activity. Furthermore, the increase in mitotic activity slowed between days 17 and 21, suggesting that colony expansion is a regulated (rather than neoplastic) process. Shown are means and standard deviations for each data point. Mean and standard deviation mitotic levels for untreated worms (184.6 ± 22.4 mitoses / mm^2 tissue) are represented by solid and dotted lines, respectively. Scale bars, 200μm.

Figure S6

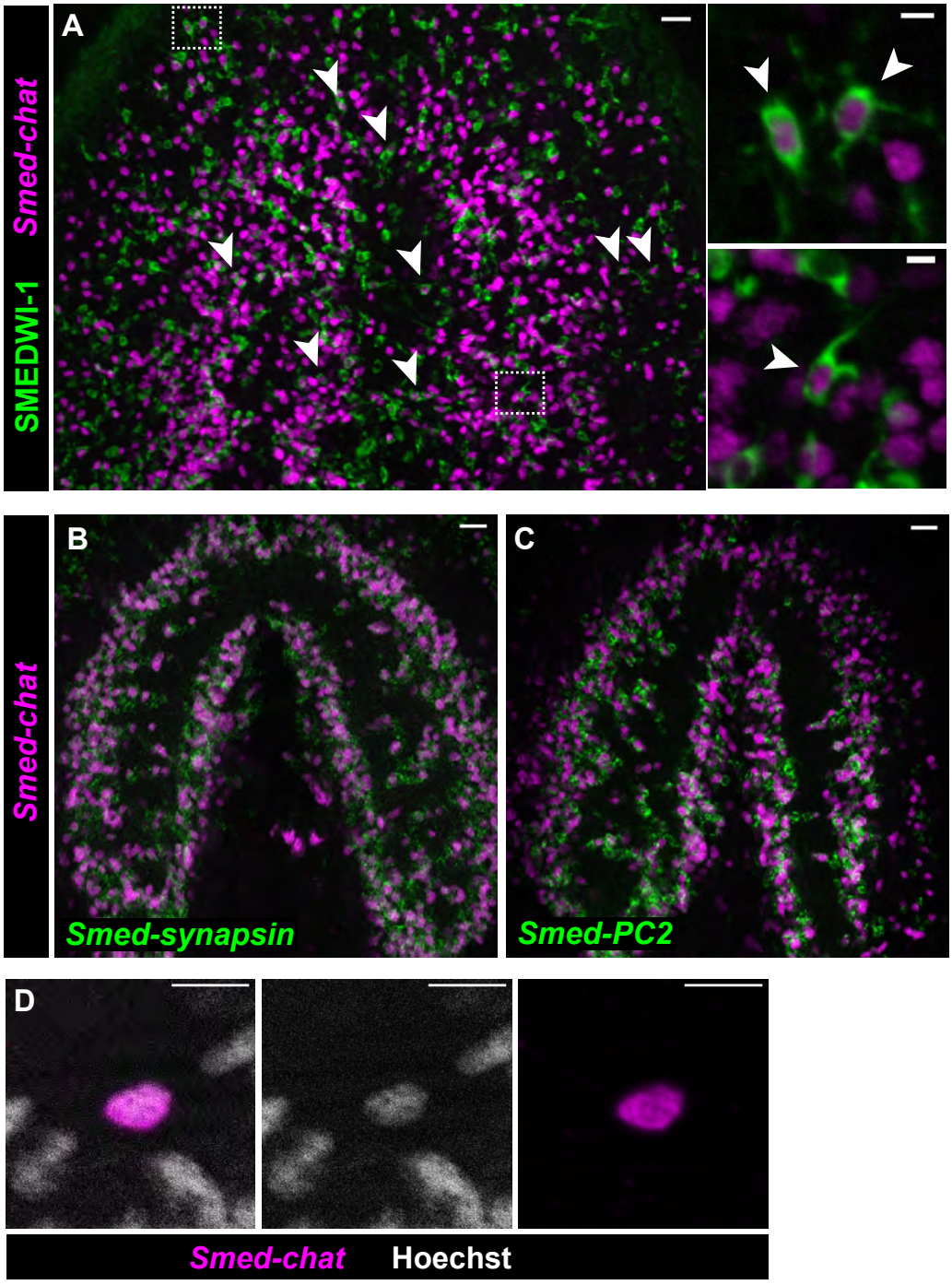

A
SMEDWI-1
Smed-chat
B
Smed-chat
Smed-synapsin
C
Smed-PC2
D
Smed-chat
Hoechst

Figure S6. A subset of SMEDWI-1$^+$ cells undergo neuronal differentiation

Double-labeling analysis of cephalic ganglia in intact, unirradiated planarians. Anterior, up. **(A)** IF (SMEDWI-1) and FISH (*Smed-chat)* identifies SMEDWI-1$^+$ cells co-expressing *Smed-chat*, a marker of differentiated neurons (double-positive cells are indicated by arrowheads). Zoomed regions are indicated by boxes. These cells were enriched in brain regions and often adopted a non-neoblast cell morphology that included long axon-like cytoplasmic processes, suggesting differentiation into neurons. **(B-C)** Double FISH in intact, unirradiated planarians indicates that *Smed-chat*$^+$ cells exist in the planarian CNS and co-express *Smed-synapsin* (b) and *Smed-proprotein-convertase-2* (*Smed-PC2*) (C), two independent markers of the planarian nervous system. **(D)** A representative FISH image shows that *Smed-chat* mRNA is localized within or in close proximity to the nucleus. Scale bars, 20μm (A-C), 5μm (zoomed images, D).

Figure S7

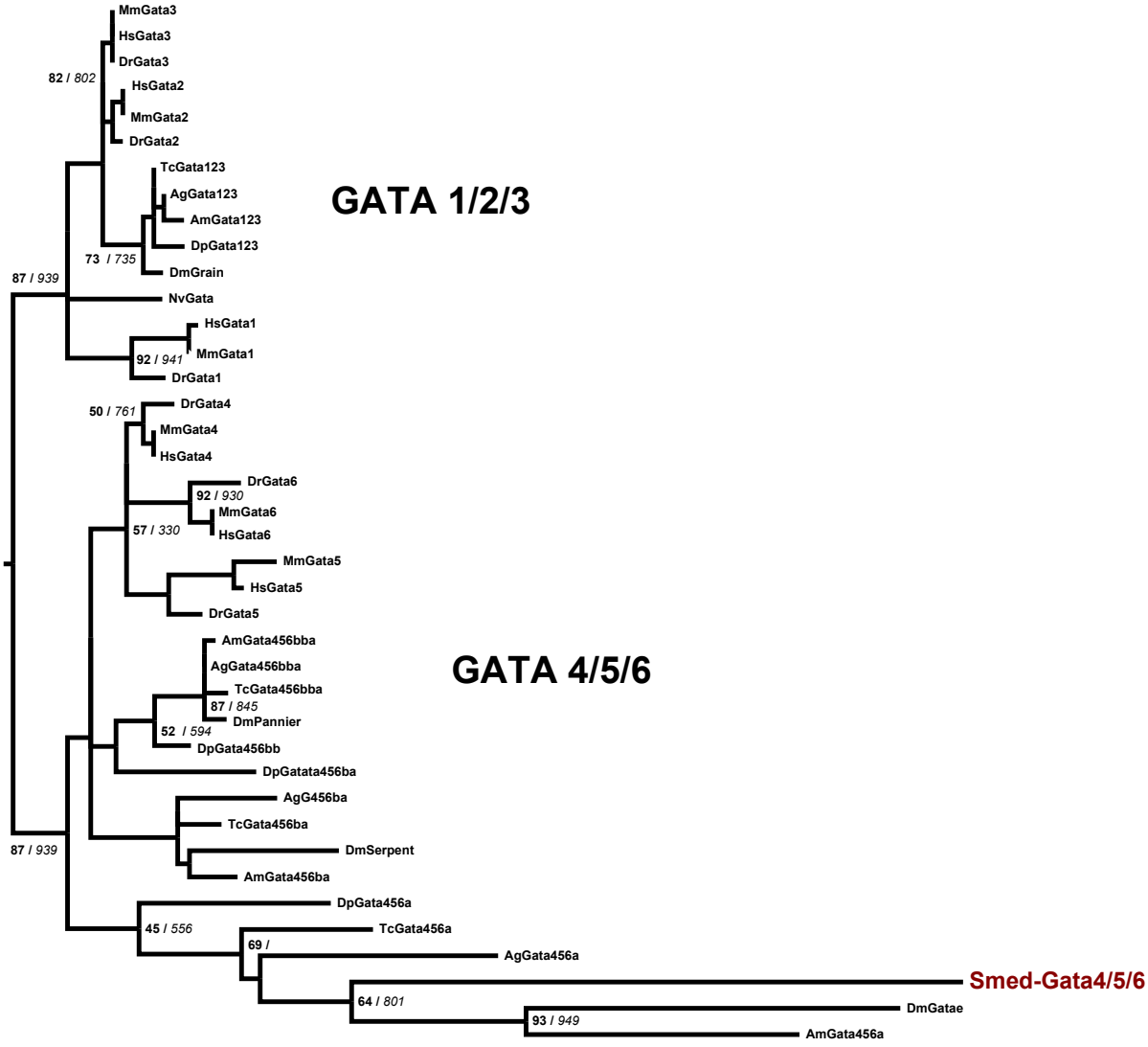

MmGata3
HsGata3
DrGata3
82 / 802
HsGata2
MmGata2
DrGata2
TcGata123
AgGata123
AmGata123
DpGata123
73 / 735
DmGrain
87 / 939
NvGata
HsGata1
92 / 941
MmGata1
DrGata1
50 / 761
DrGata4
MmGata4
HsGata4
DrGata6
92 / 930
MmGata6
HsGata6
57 / 330
MmGata5
HsGata5
DrGata5
AmGata456bba
AgGata456bba
TcGata456bba
87 / 845
DmPannier
52 / 594
DpGata456bb
DpGatata456ba
AgG456ba
TcGata456ba
DmSerpent
AmGata456ba
87 / 939
DpGata456a
45 / 556
TcGata456a
69 /
AgGata456a
Smed-Gata4/5/6
64 / 801
DmGatae
93 / 949
AmGata456a
GATA 1/2/3
GATA 4/5/6

Figure S7. Phylogenetic analysis of the *Smed-gata4/5/6* gene

Maximum likelihood and neighbor-joining analysis provide strong support for the *Schmidtea mediterranea* gene *Smed-gata4/5/6* falling within the GATA4/5/6 clade with known protostome members of this family. Genes used in this analysis are well-established representatives of the GATA1/2/3 or GATA4/5/6 gene families (*11*). Accession numbers for genes listed in this tree can be found in ref (*11*). Mm, *Mus musculus*; Hs, *Homo sapiens*; Dr, *Danio rerio*; Tc, *Tribolium castaneum*; Ag, *Anopheles gambiae*; Am, *Apis mellifera*; Dp, *Daphnia pulex*; Dm, *Drosophila melanogaster*; Nv, *Nematostella vectensis*; Smed, *Schmidtea mediterranea.*

Figure S8

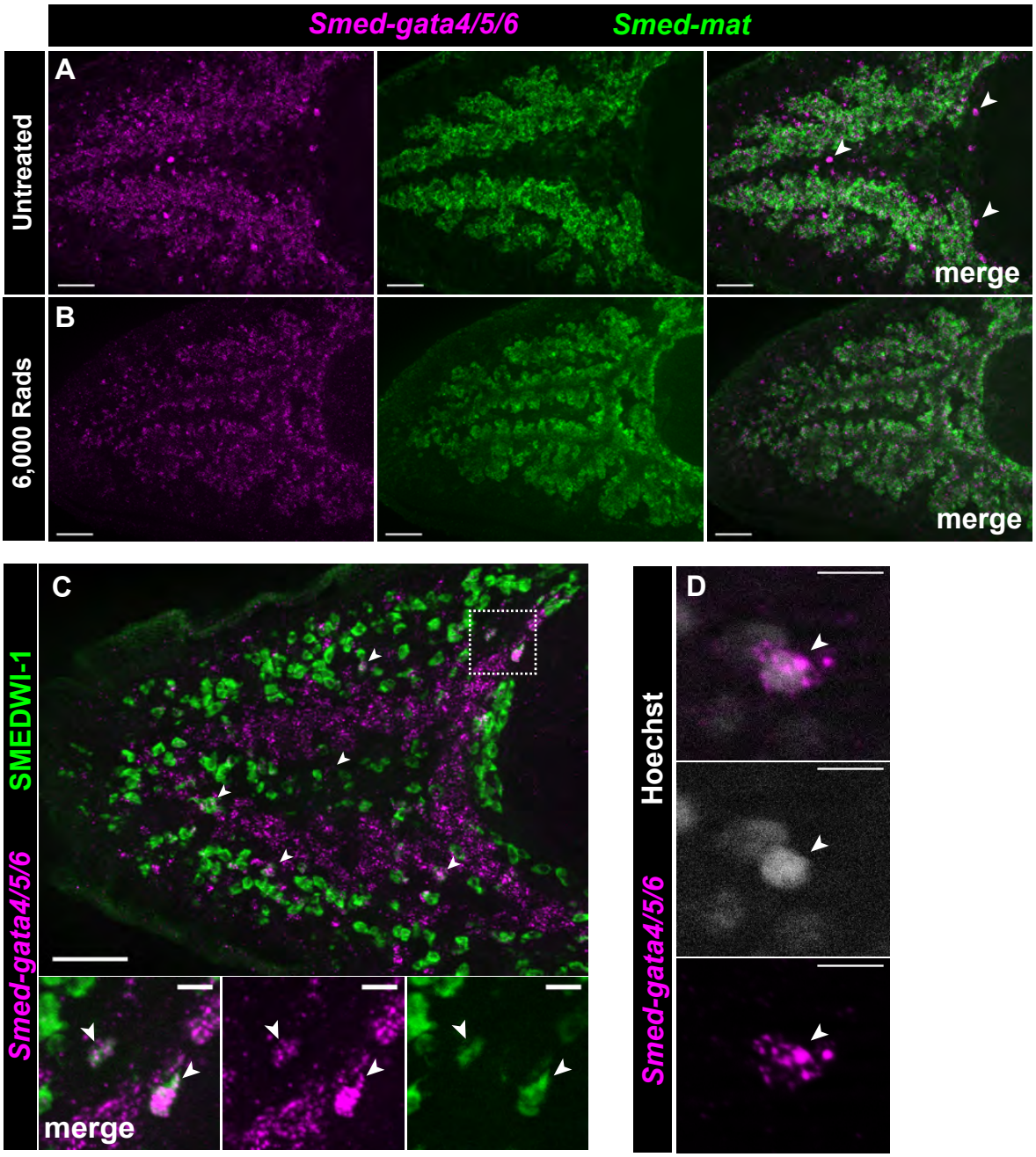

Smed-gata4/5/6
Smed-mat
Untreated
A
merge
6,000 Rads
B
merge
SMEDWI-1
Smed-gata4/5/6
C
merge
Hoechst
Smed-gata4/5/6
D

Figure S8. A subset of SMEDWI-1$^+$ cells express *Smed-gata4/5/6*

Double-labeling analysis of posterior intestinal branches in untreated or lethally-irradiated planarians. **(A-B)** Double FISH on untreated (A) or 5 day, lethally irradiated (B) planarians indicate that *Smed-gata4/5/6* is expressed in fully differentiated *Smed-mat*$^+$ cells of the planarian intestine. *Smed-gata4/5/6* is also expressed in isolated, irradiation-sensitive cells associated with the intestine (arrowheads). **(C)** Many isolated *Smed-gata-4/5/6*$^+$ cells were co-labeled by SMEDWI-1 (IF). **(D)** A representative FISH image shows subcellular localization of *Smed-gata4/5/6* mRNA . Scale bars, 50μm (A-C), 5μm (zoomed images, D).

Figure S9

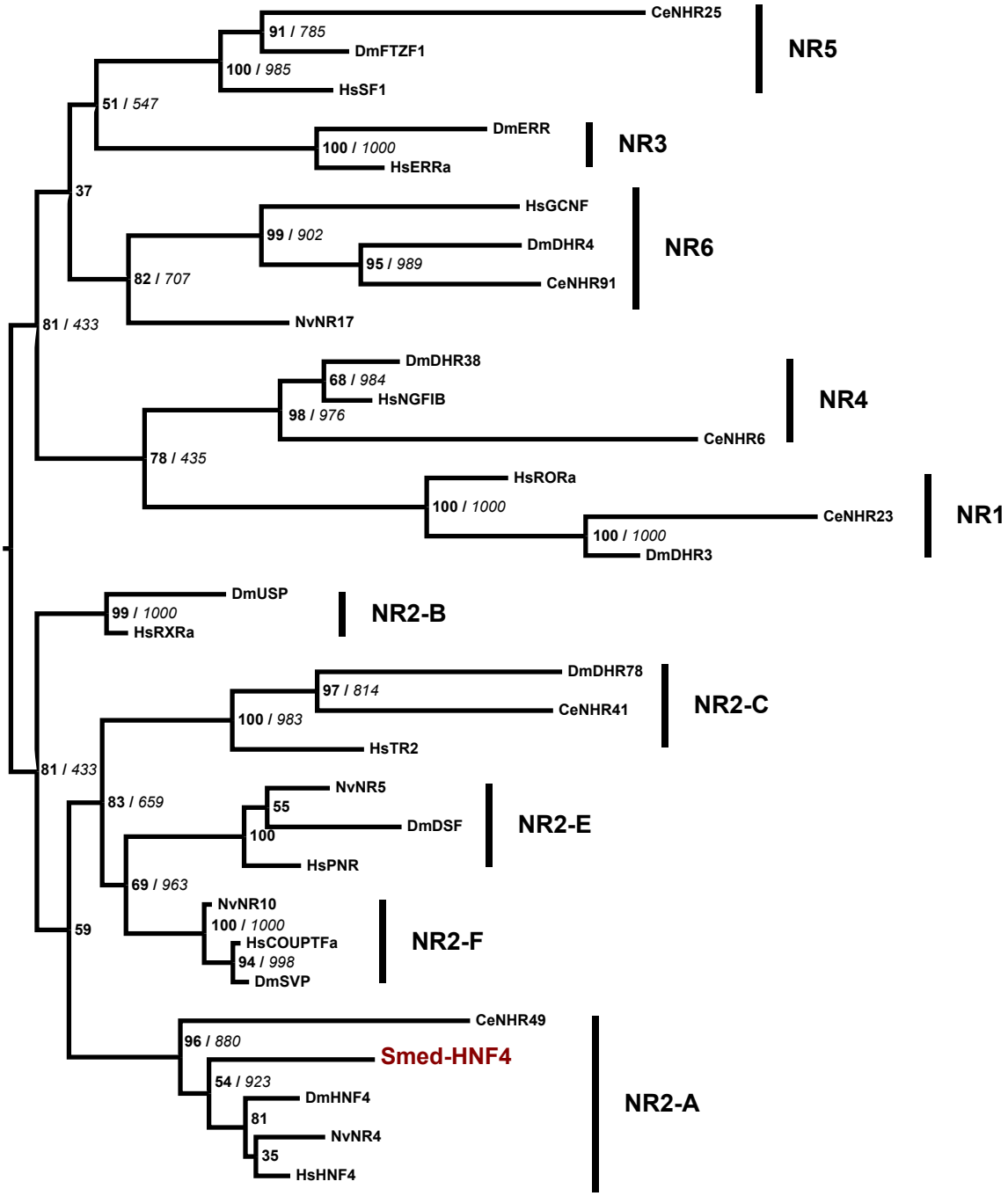

CeNHR25
91 / 785
DmFTZF1
100 / 985
HsSF1
NR5
51 / 547
DmERR
100 / 1000
HsERRa
NR3
37
HsGCNF
99 / 902
DmDHR4
95 / 989
CeNHR91
NR6
82 / 707
NvNR17
81 / 433
DmDHR38
68 / 984
HsNGFIB
98 / 976
CeNHR6
NR4
78 / 435
HsRORa
100 / 1000
CeNHR23
100 / 1000
DmDHR3
NR1
DmUSP
99 / 1000
HsRXRa
NR2-B
DmDHR78
97 / 814
CeNHR41
NR2-C
100 / 983
HsTR2
81 / 433
NvNR5
55
DmDSF
NR2-E
100
HsPNR
83 / 659
69 / 963
NvNR10
100 / 1000
HsCOUPTFa
94 / 998
DmSVP
NR2-F
59
CeNHR49
96 / 880
Smed-HNF4
54 / 923
DmHNF4
81
NvNR4
35
HsHNF4
NR2-A

Figure S9. Phylogenetic analysis of the *Smed-hnf4* gene

Maximum likelihood and neighbor-joining analysis provide strong support for the *Schmidtea mediterranea* gene *Smed-hnf4* falling within the NR2A/HNF4 family of nuclear receptors. Genes used in this analysis are well-established representatives of the six families of nuclear receptor genes NR1-6. Sequences for *Nematostella* genes used in this tree can be found in ref (*12*). Hs, *Homo sapiens;* Dm, *Drosophila melanogaster*; Nv, *Nematostella vectensis;* Ce, *Caenorhabditis elegans*; Smed, *Schmidtea mediterranea.*

Figure S10

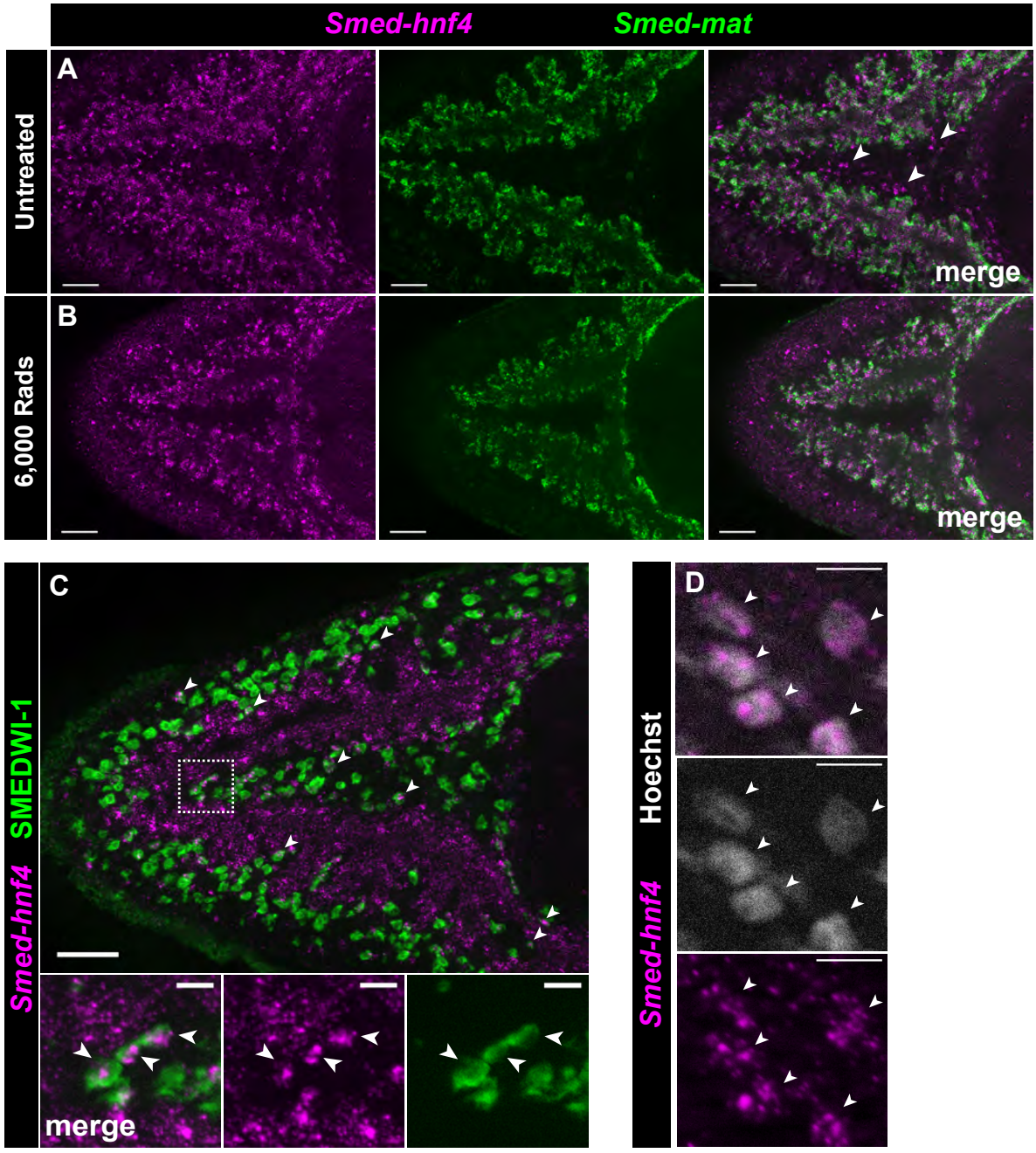

Smed-hnf4
Smed-mat
A
Untreated
merge
B
6,000 Rads
merge
C
Smed-hnf4
SMEDWI-1
merge
D
Hoechst
Smed-hnf4

Figure S10. A subset of SMEDWI-1$^+$ cells express *Smed-hnf4*

Double-labeling analysis of posterior intestinal branches in untreated or lethally irradiated planarians. **(A-B)** Double FISH on untreated (A) or 5 day, lethally irradiated (B) planarians indicate that similarly to *Smed-gata4/5/6*, *Smed-hnf4* is expressed in fully differentiated *Smed-mat*$^+$ cells of the planarian intestine. *Smed-hnf4* is also expressed in isolated, irradiation-sensitive cells associated with the intestine (arrowheads). **(C)** Many isolated *Smed-hnf4*$^+$ cells were co-labeled by SMEDWI-1 (IF). **(D)** A representative FISH image shows subcellular localization of *Smed-hnf4* mRNA. Scale bars, 50μm (A-C), 5μm (zoomed images, D).

Figure S11

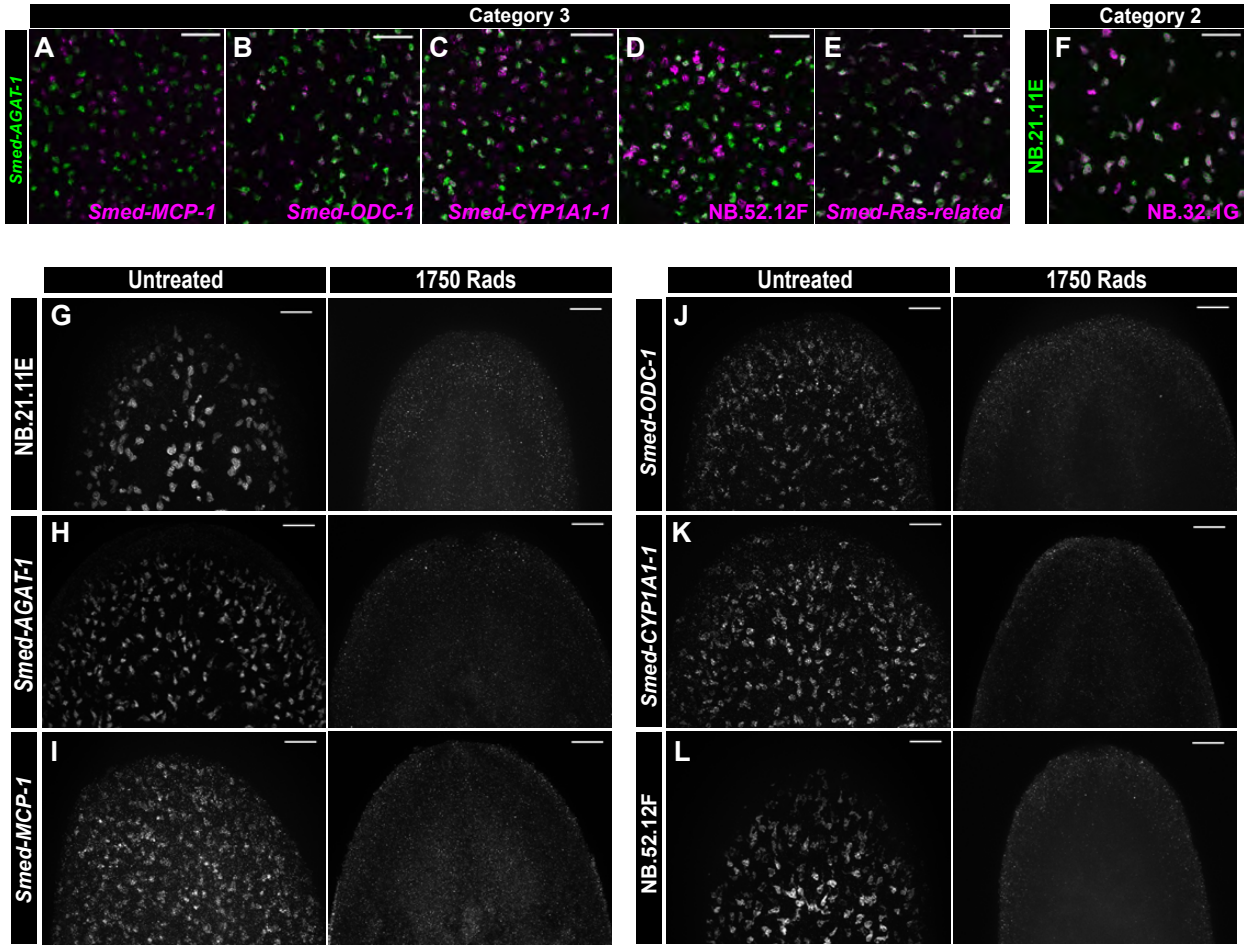

Category 3
Category 2
Smed-AGAT-1
A
B
C
D
E
F
Smed-MCP-1
Smed-ODC-1
Smed-CYP1A1-1
NB.52.12F
Smed-Ras-related
NB.21.11E
NB.32.1G
Untreated
1750 Rads
Untreated
1750 Rads
NB.21.11E
G
Smed-AGAT-1
H
Smed-MCP-1
I
Smed-ODC-1
J
Smed-CYP1A1-1
K
NB.52.12F
L

Figure S11. Characterization of post-mitotic cell populations

(A-F) Double FISH analysis of post-mitotic cell types in unirradiated, intact planarians (see also, Eisenhoffer *et. al.*, 2008). Shown are ventral, prepharyngeal regions of the animal. A previously reported panel of marker genes labels multiple populations of cells. “Category 3” gene *Smed-AGAT-1* only partially overlapped in expression with other category 3 genes *Smed-MCP-1* **(A)**, *Smed-ODC-1* **(B)**, *Smed-CYP1A1-1* **(C)**, and NB.52.12F **(D)**. However, cells expressing *Smed-Ras-related*, another category 3 gene, showed extensive overlap with the *Smed-AGAT-1*$^{+}$ population **(E)**. Similarly, “category 2” markers NB.21.11E and NB.32.1G were expressed by the same population of cells **(F)**. A revised panel of six markers NB.21.11E, *Smed-AGAT-1*, *Smed-MCP-1*, *Smed-ODC-1*, *Smed-CYP1A1-1,* and NB.52.12F therefore encompasses a heterogeneous set of cell populations with minimal redundancy. **(G-L)** FISH showing presence of NB.21.11E, *Smed-AGAT-1*, *Smed-MCP-1*, *Smed-ODC-1*, *Smed-CYP1A1-1*, and NB.52.12F-expressing cells. Shown are ventral, anterior regions from untreated or day 19-irradiated worms lacking *smedwi-1*$^{+}$ colonies. All six cell types were depleted after 1,750 rads.

Figure S12

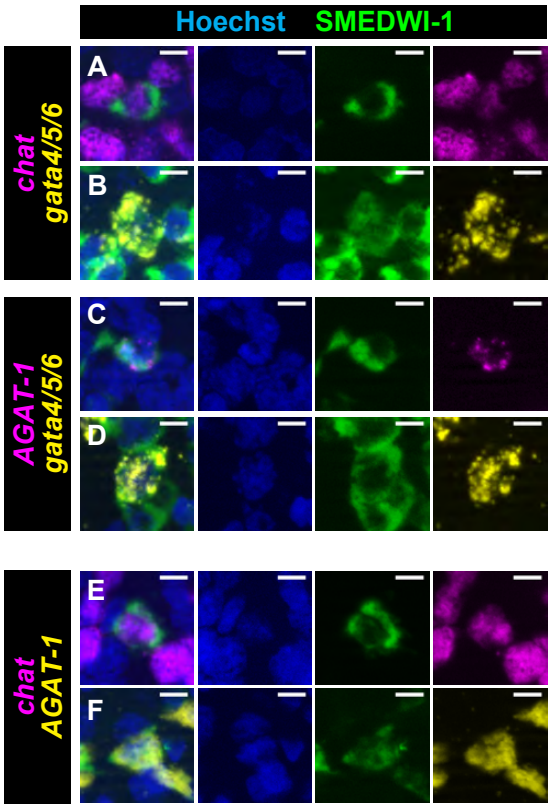

Hoechst
SMEDWI-1
chat
gata4/5/6
A
B
AGAT-1
gata4/5/6
C
D
chat
AGAT-1
E
F

Figure S12. Clonogenic neoblasts display broad differentiation capacity

Triple labeling of individual colonies 22 days after irradiation. Shown are additional cells from the same colonies depicted in Fig. 2. Each row of panels shows individual channel images for a single cell. **(A-B)** Additional examples of colony cells positive for *chat* (A) and *gata4/5/6* (B) expression from the same colony shown in Fig. 2A. **(B-C)** Additional examples of colony cells positive for *AGAT-1* (C) and *gata4/5/6* (D) expression from the same colony shown in Fig. 2B. **(D-E)** Additional examples of colony cells positive for *chat* (D) and *AGAT-1* (E) expression from the same colony shown in Fig. 2C. Scale bars, 5μm.

Figure S13

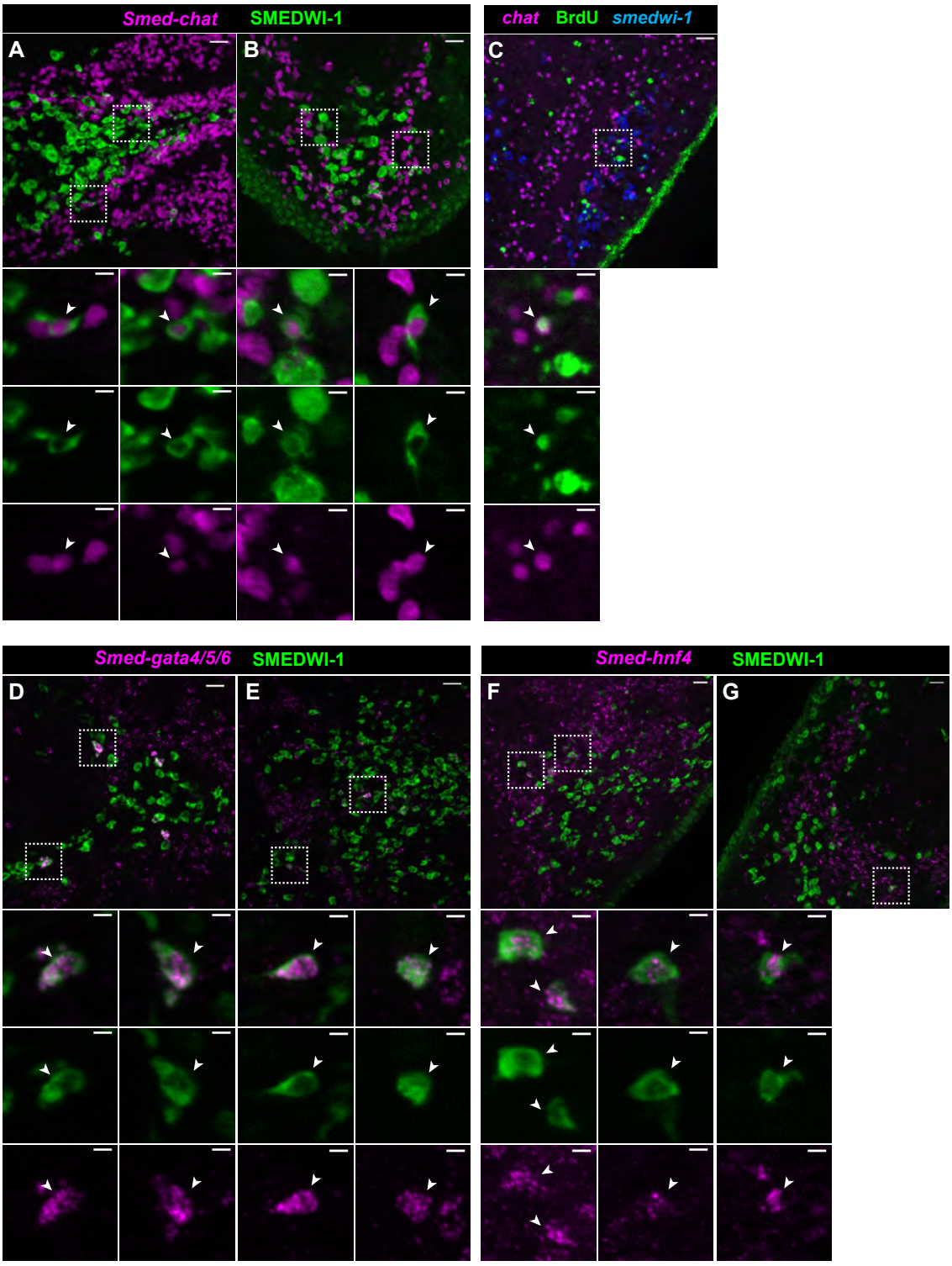

Smed-chat SMEDWI-1
A
B
chat BrdU smedwi-1
C
Smed-gata4/5/6 SMEDWI-1
D
E
Smed-hnf4 SMEDWI-1
F
G

Figure S13. A high percentage of cNeoblast colonies produce differentiated cell types

Double-labeling analysis of individual colonies 15-20 days after irradiation. Shown are representative optical sections from irradiated animals. Boxes indicate zoomed regions within the same colony that contain double-positive cells. Locations of all colonies in these analyses are shown in a scatterplot in Figure 2E. **(A-B)** Two representative colonies labeled by IF (SMEDWI-1) and FISH (*Smed-chat*) from worms 15-19 days after 1,750-1,800 rads. 29/30 such colonies contained differentiating neurons (SMEDWI$^+$; *Smed-chat*$^+$ double-positive cells). **(C)** Representative colony labeled by IF (BrdU) and double FISH (*Smed-chat; smedwi-1*) from 1,750 rad-treated animals. Worms with growing colonies were injected with a pulse of BrdU 14 days after irradiation and examined 4 or 5 days later to detect differentiating cells. Every such colony examined (n=8/8) contained *Smed-chat*$^+$; BrdU$^+$ double-positive cells. **(D-E)** Two representative colonies labeled by IF (SMEDWI-1) and FISH (*Smed-gata4/5/6*) from worms 19 days after 1,750 rads. 14/15 such colonies contained differentiating intestinal cells SMEDWI-1$^+$; *Smed-gata4/5/6*+ cells. **(F-G)** Two representative colonies labeled by IF (SMEDWI-1) and FISH (*Smed-hnf4*) from worms 19 days after 1,750 rad exposure. 10/11 such colonies contained SMEDWI-1$^+$; *Smed-hnf4*$^+$ cells. Scale bars, 20μm (5μm, zoomed images).

Figure S14

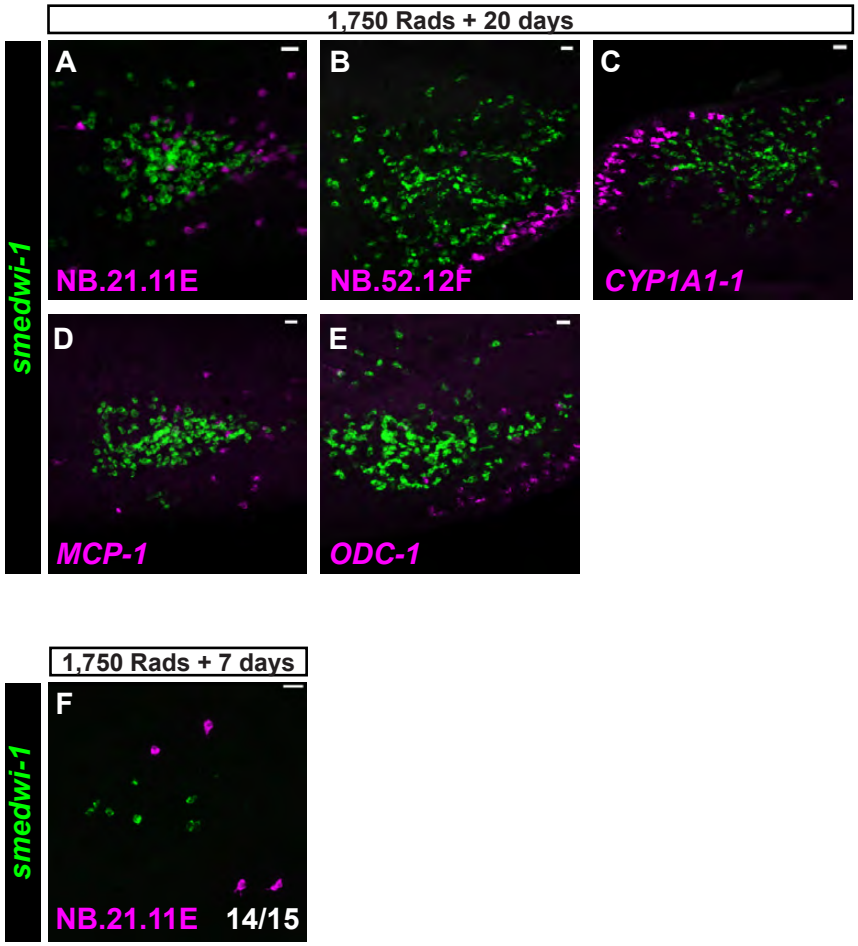

1,750 Rads + 20 days
smedwi-1
A
NB.21.11E
B
NB.52.12F
C
CYP1A1-1
D
MCP-1
E
ODC-1
1,750 Rads + 7 days
smedwi-1
F
NB.21.11E
14/15

Figure S14. Nearly all colonies locally produce post-mitotic cell types

(**A-E)** *smedwi-1*$^{+}$ colonies 20 days after 1,750 rads contained post-mitotic cells expressing NB.21.11E (A, 16/16 colonies), NB.52.12F (B, 13/15 colonies), *CYP1A1-1* (C, 15/17), *MCP-1* (D, 20/20 colonies), and *ODC-1* (E, 20/20 colonies). **(F)** Representative colony labeled by double FISH (*smedwi-1* and NB.21.11E) from worms 7 days after exposure to 1,750 rads. Nearly all individual colonies analyzed (14/15) examined at this early timepoint displayed differentiating cells. Scale bars, 20μm.

Figure S15

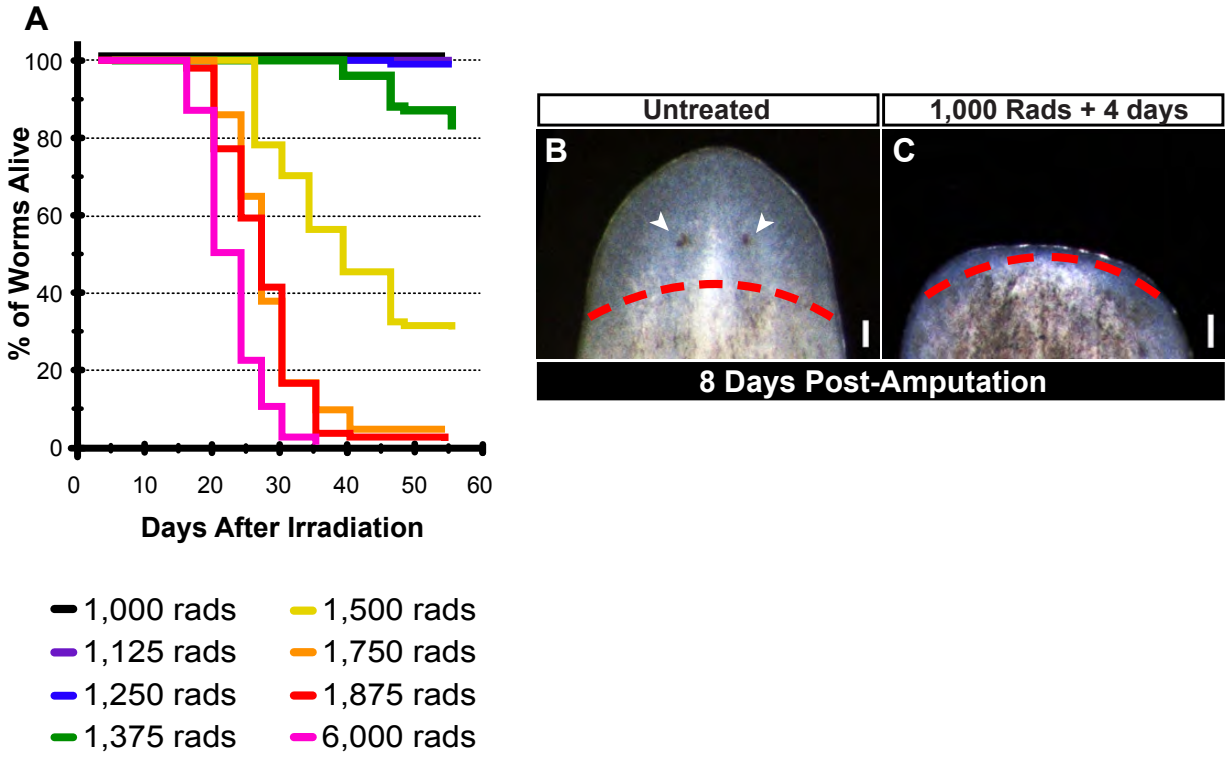

A
100
80
60
40
20
0
% of Worms Alive
0
10
20
30
40
50
60
Days After Irradiation
1,000 rads
1,500 rads
1,125 rads
1,750 rads
1,250 rads
1,875 rads
1,375 rads
6,000 rads
Untreated
1,000 Rads + 4 days
B
C
8 Days Post-Amputation

Figure S15. Effects of irradiation on planarian survival and regeneration

(A) Survival curves of irradiated worms irradiated at various doses of irradiation. Animals are from the same experiment shown in Figure 3. Viability decreased sharply above 1,500 rads ($n \geq 98$ worms/sample). **(B-C)** Head regeneration was initially impeded by even low doses of irradiation. Shown are head regions from worms 8 days post-amputation. **(B)** Control worm. **(C)** Worm amputated 4 days after exposure to 1,000 rads (49/49 animals). Similar results were obtained after 1,250 rads (50/50 worms), or 1,500 rads (50/50 worms). Scale bars, 20μm.

Figure S16

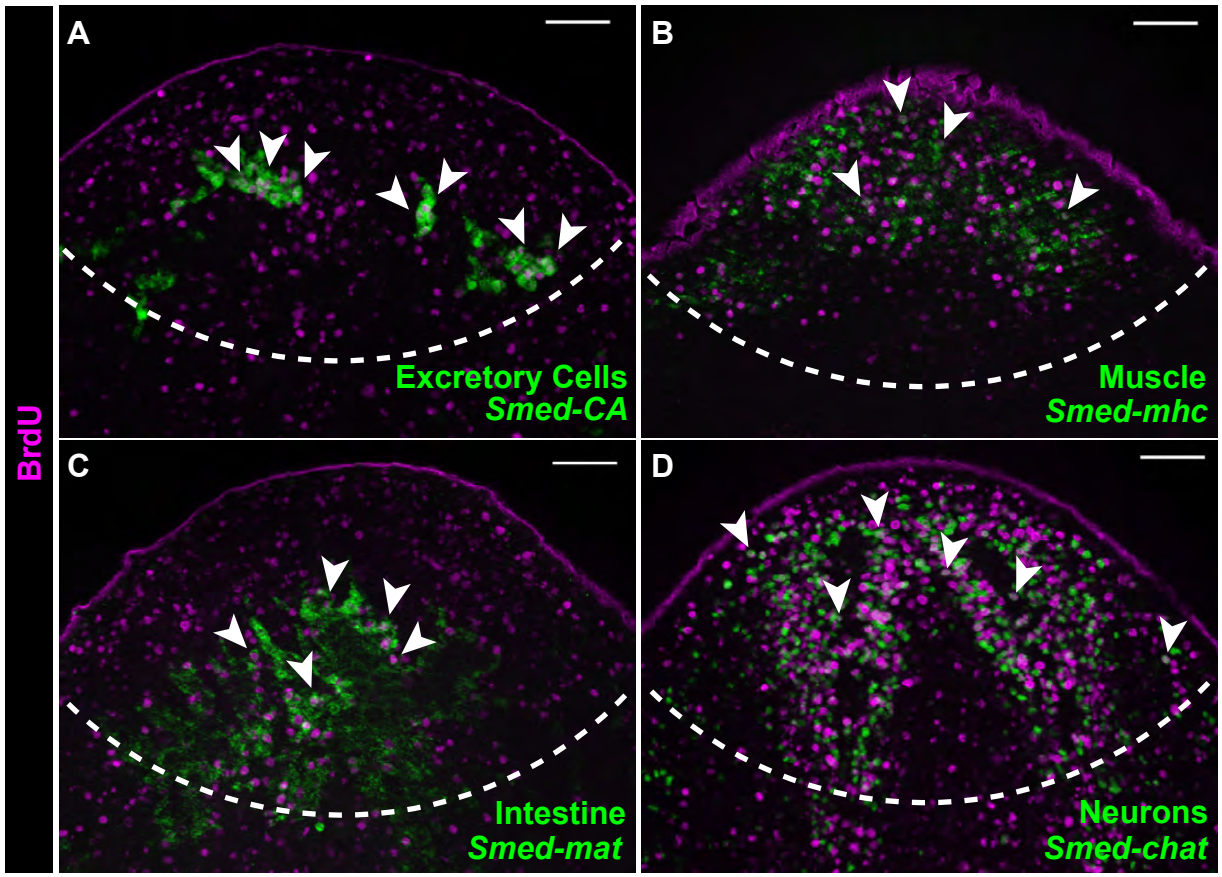

BrdU
A
Excretory Cells
Smed-CA
B
Muscle
Smed-mhc
C
Intestine
Smed-mat
D
Neurons
Smed-chat

Figure S16. Diverse types of differentiating cells are produced during head regeneration

Planarians were injected with BrdU 18 hours prior to decapitation. IF (BrdU) together with FISH shows 5-day-regenerating heads containing newly formed ($BrdU^+$) differentiated cells. These cells include **(A)** excretory, **(B)** muscle, **(C)** intestinal, and **(D)** neuronal lineages as determined by expression of *Smed-carbonic-anhydrase* (*Smed-CA*), *Smed-myosin-heavy-chain-1* (*Smed-mhc-1*), *Smed-methionine-adenosyltransferase* (*Smed-mat*), and *Smed-choline-acetyl-transferase* (*Smed-chat*), respectively. Scale bars, 100μm. Anterior, up. Dotted line, approximate amputation plane.

Figure S17

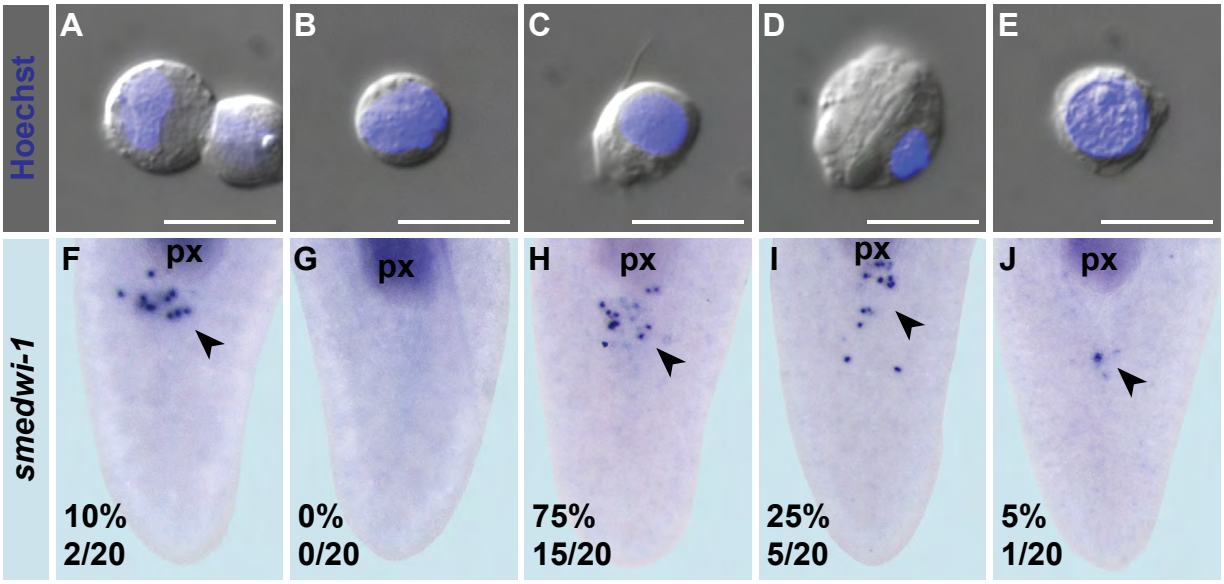

Hoechst
A
B
C
D
E
smedwi-1
F
px
10%
2/20
G
px
0%
0/20
H
px
75%
15/20
I
px
25%
5/20
J
px
5%
1/20

Figure S17. Morphological characteristics and transplant frequencies of different X1(FS) cells

(A-E) Representative images of X1(FS) cells. Nuclei were labeled with Hoechst 33342. **(F-J)** Representative images of animals 9 days after irradiation, 6 days after transplant. Anterior, up. Ventral surface shown. Transplantation of cells 10-14μm in diameter with low cytoplasmic granularity (A) resulted in few *smedwi-1*$^+$ clusters (n=2/20) (F). Transplantation of cells 8-10μm in diameter with low cytoplasmic granularity (B) resulted in zero *smedwi-1*$^+$ clusters (n=0/20) (G) Transplantation of cells 10-12μm in diameter with low cytoplasmic granularity and processes (C) resulted in many formed *smedwi-1*$^+$ clusters (n=15/20) (H). Transplantation of cells 10-14μm in diameter with high cytoplasmic granularity (D) resulted in few formed *smedwi-1*$^+$ clusters (n=5/20) (I). Transplantation of cells 8-10μm in diameter with high cytoplasmic granularity (E) resulted in few formed *smedwi-1*$^+$ clusters (n=1/20) (J). Scale bars, 10μm (A-E).

Figure S18

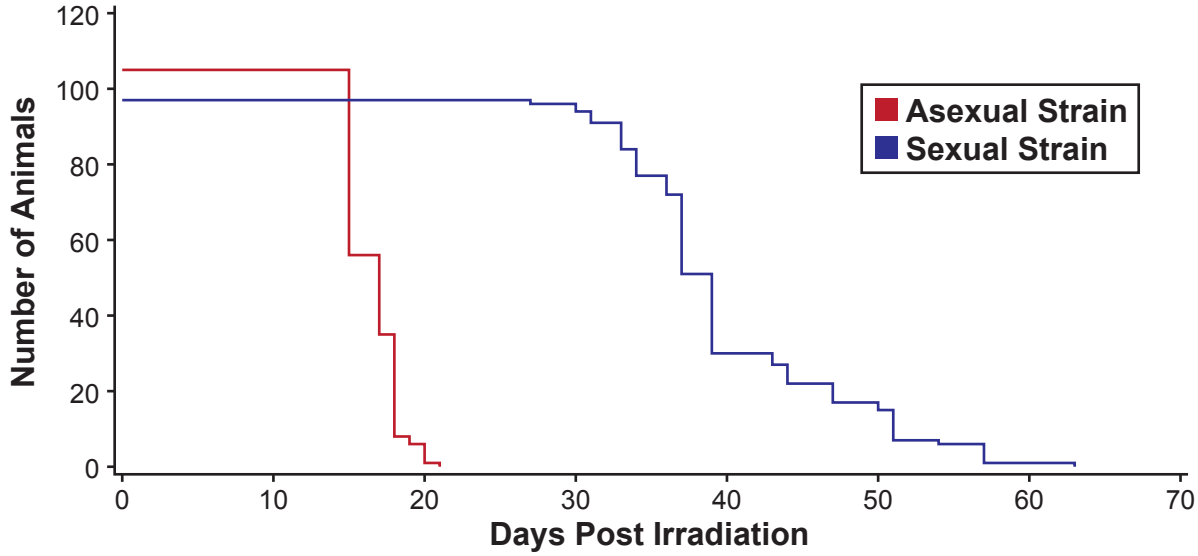

120
100
80
60
40
20
0
Number of Animals
0
10
20
30
40
50
60
70
Days Post Irradiation
Asexual Strain
Sexual Strain

Figure S18. Survival of asexual and sexual strain animals following lethal irradiation

Exact survival times vary between experiments. In this particular experiment, asexual CIW4 animals exposed to a 6,000 rad dose of radiation had a median survival period of 17 days and a longest survival period of 21 days (n=105). Sexual S2F1L3F2 strain animals exposed to identical conditions within the same experiment had a median survival period of 39 days and a longest survival period of 63 days (n=97).

Figure S19

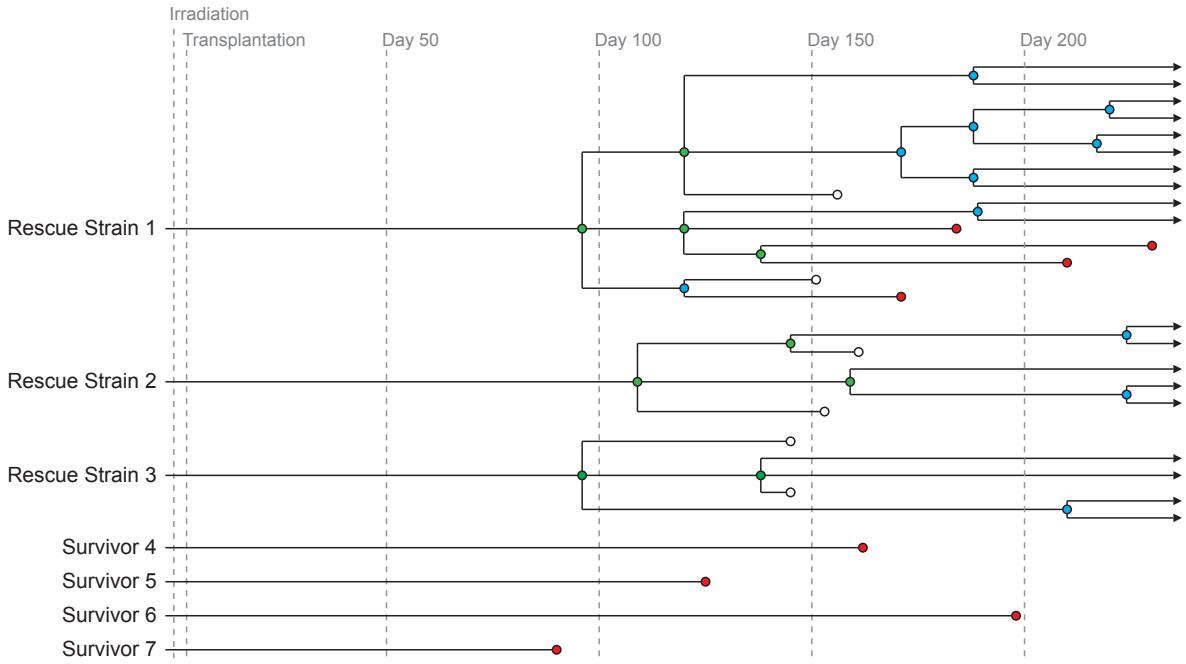

Irradiation
Transplantation
Day 50
Day 100
Day 150
Day 200
Rescue Strain 1
Rescue Strain 2
Rescue Strain 3
Survivor 4
Survivor 5
Survivor 6
Survivor 7

Figure S19. Timeline of rescued transplant hosts

Trees detailing survival of rescued animals, with branches indicating expansion of a single animal into multiple individuals by amputation or fissioning. Red end points indicate natural death. White end points indicate sacrifice of the individual for experimental purposes. Green intersections indicate amputations to expand the population. Blue intersections indicate fissioning events. Black arrowheads indicate which individuals are still alive at the time of writing.

Table S1. A small number of *smedwi-1*$^+$ colonies can rescue entire animals from irradiation and restore regenerative ability

	# (%) worms with *smedwi-1*$^+$ colonies (d7)						# (%) worms recovered (d54-55)			Fisher's Exact Test (2-tailed)		
Dose (Rads)	6 or more colonies	5 or more colonies	4 or more colonies	3 or more colonies	2 or more colonies	N	Survived	Survived & Regenerated	N	6 or more colonies	5 or more colonies	4 or more colonies
1000	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)	25	100 (100)	100 (100)	100			
1125	46 (98)	46 (98)	47 (100)	47 (100)	47 (100)	47	98 (100)	98 (100)	98			
1250	**41 (79)**	**44 (85)**	**47 (90)**	52 (100)	52 (100)	52	**98 (99)**	**97 (98)**	99	**0.0002**	**0.0032**	**0.0478**
1375	**32 (63)**	34 (67)	39 (76)	43 (84)	47 (92)	51	**82 (82)**	**80 (80)**	100	**0.0302**		
1500	**2 (4)**	**4 (7)**	11 (19)	16 (28)	24 (42)	57	**30 (30)**	**28 (28)**	100	**<0.0001**	**0.0017**	
1750	1 (3)	2 (5)	3 (8)	5 (13)	9 (24)	38	4 (4)	3 (3)	99			
1875	0	0	0	1 (3)	2 (5)	40	1 (1)	1 (1)	100			
6000	0	0	0	0	0	26	0	0	100			

Planarians were exposed to a range of irradiation doses. A portion of these animals were fixed 7 days later and colonies visualized by *smedwi-1* ISH. The number and percentage (in parentheses) of animals displaying various numbers of colonies are shown. N indicates the total number of animals analyzed. Remaining animals were followed for several weeks and decapitated 39-40 days after irradiation. Number and percentage of total worms that survived or both survived and regenerated are shown. Nearly all worms that survived also displayed normal head regeneration. Given the frequencies of *smedwi-1*$^+$ colonies observed from *in situ* data, a set of predictions can be generated based on a hypothetical minimum number of colonies required for restoring regeneration. Fisher's Exact test (2-tailed, $\alpha = 0.05$) facilitates a direct comparison between the number of colonies present and number of worms regenerated. P-values for tests in which significantly more animals regenerated than predicted are shown in bold. Together these data indicate that as few as three (P=0.0478), four (P=0.0017), or five colonies (P<0.0001) colonies are sufficient to rescue entire animals and restore regenerative ability.

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