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Supplemental Information

Genetic Regulators of a Pluripotent Adult Stem Cell System in Planarians Identified by RNAi and Clonal Analysis

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Inventory of Supplemental Material:

Figure S1 (Related to Figure 1)

Figure S2 (Related to Figure 2)

Figure S3 (Related to Figure 3)

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Figure S5 (Related to Figure 5)

Figure S6 (Related to Figure 6)

Table S1 (Related to Figure 1; *provided as a separate Excel spreadsheet*)

Table S2 (Related to Figure 1)

Table S3 (Related to Figure 2)

Table S4 (Related to Figure 3)

Table S5 (Related to Figure 4)

Supplemental Experimental Procedures

References

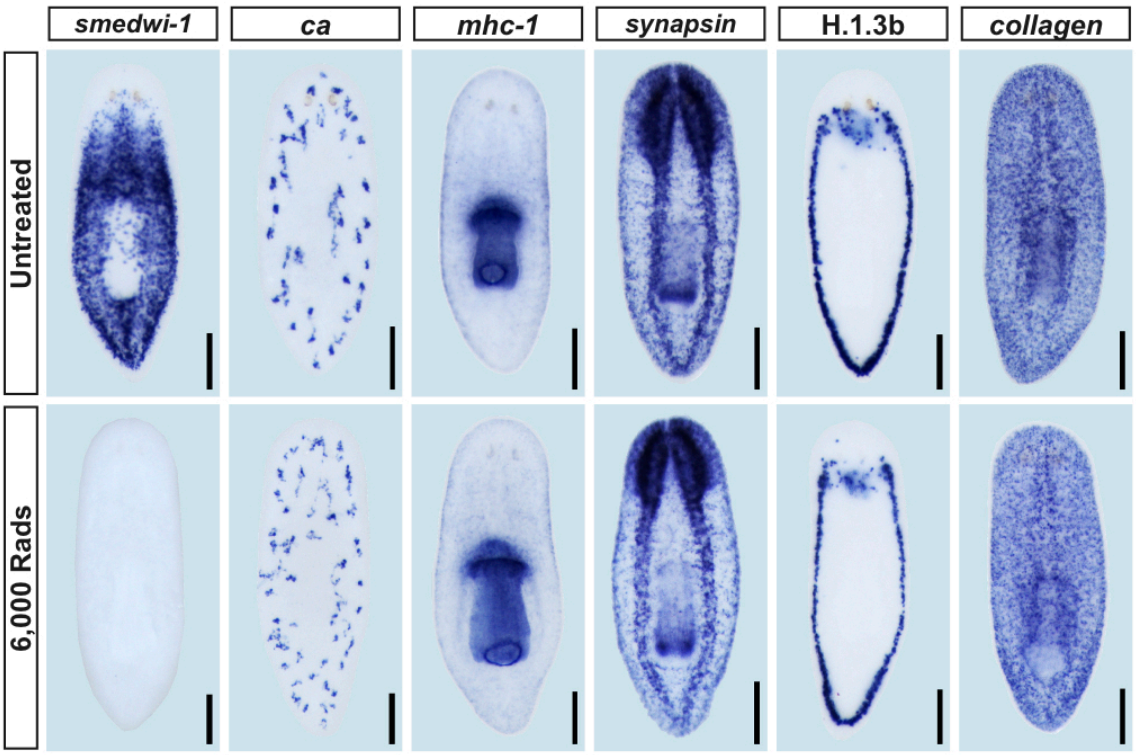


Figure S1. *smedwi-1*$^{+}$ proliferating cells are specifically depleted by γ-irradiation (Related to Figure 1)

Whole-mount *in situ* hybridizations of untreated adult planarians and planarians fixed 5 days after exposure to 6,000 rads γ-irradiation. Effects of irradiation on proliferating cells (*smedwi-1*), nephridic cells (*carbonic anhydrase / ca*), the muscular pharynx (*mhc-1*), the central nervous system (*synapsin*), and two distinct populations of subepidermal cells (H.1.3b and *collagen*) are shown. Animals are anterior up. Scale bars, 200 μm.

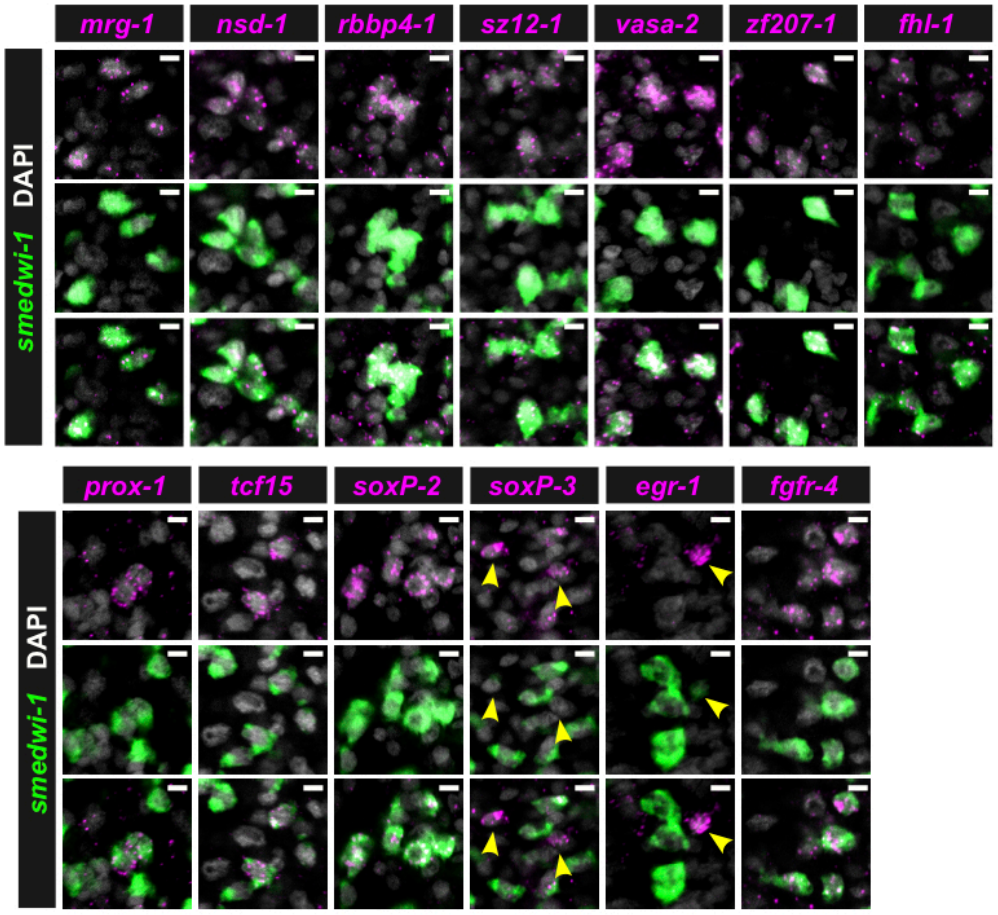


Figure S2. Irradiation-sensitive transcripts are expressed in *smedwi-1*$^{+}$ proliferating cells (Related to Figure 2)

Shown are additional genes identified by microarray, analyzed by double FISH with the gene *smedwi-1*. Most cells detected by FISH co-expressed *smedwi-1*; cells with little/no *smedwi-1* gene expression are labeled by arrowheads. Some transcripts (e.g., *mrg-1*, *rbbp4-1*, *vasa-2*, *zf207-1*, *fhl-1*, *fgfr-4*) are expressed at low levels with background signal (scattered magenta dots) also visible. Scale bars, 10 μm.

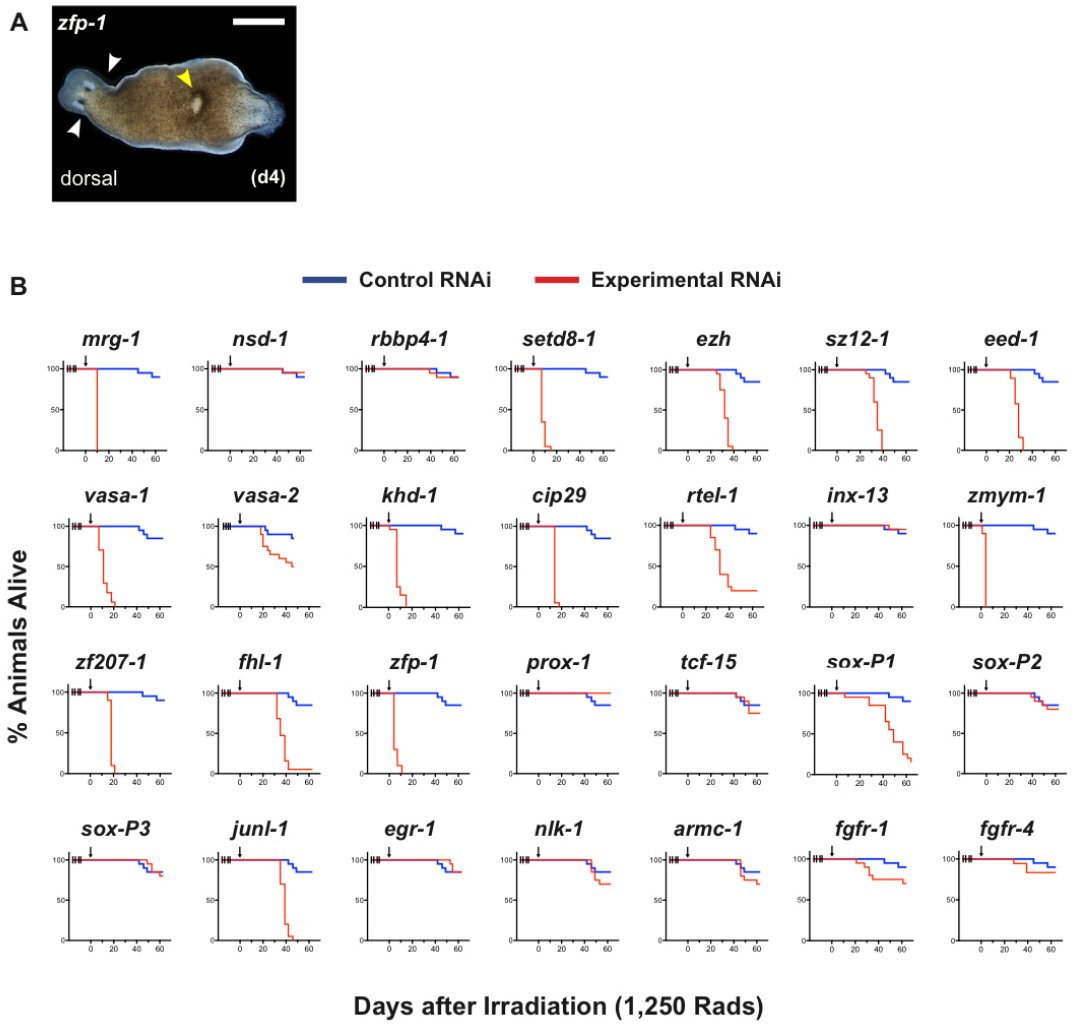
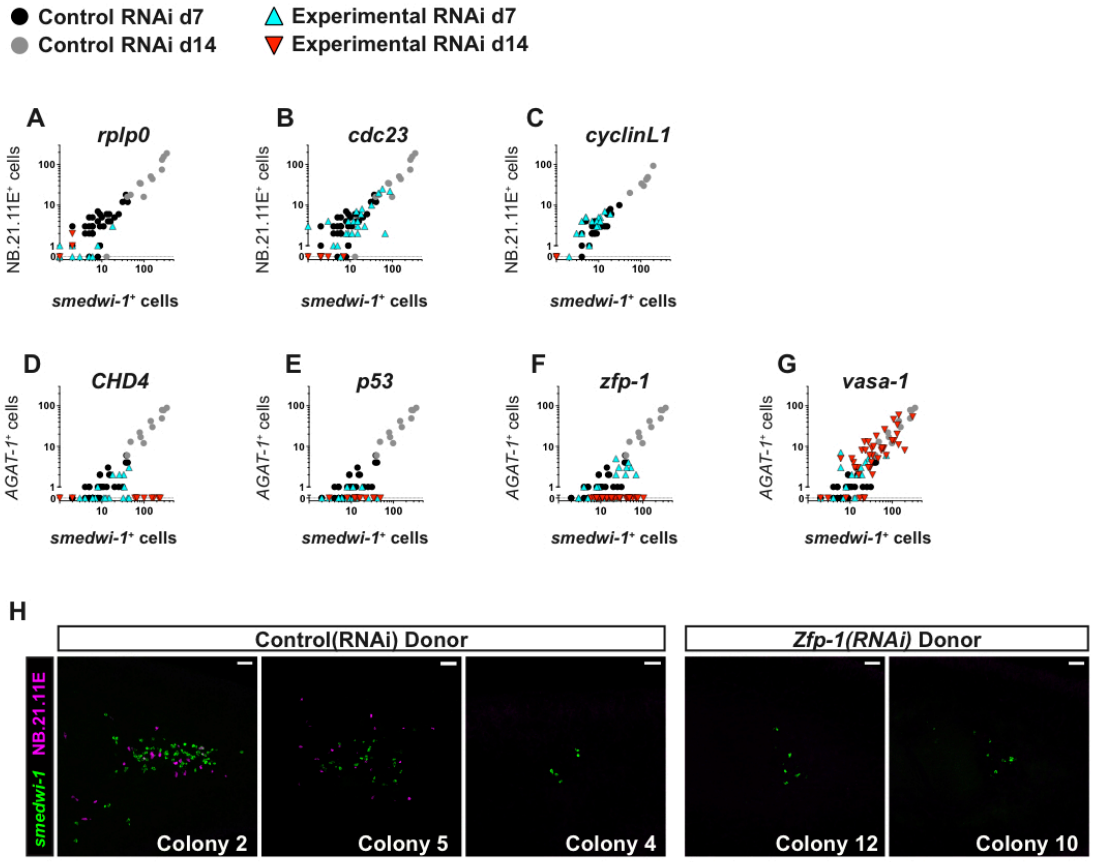


Figure S3. Identification of RNAi phenotypes after sublethal irradiation (Related to Figure 3)

(A) Representative dorsal view of a *zfp-1(RNAi)* animal 4 days after 1,250 rad exposure, anterior left. Animal is from the same experiment shown in Figure 3. In addition to ventral curling (Fig. 3E) and head regression (white arrowheads), *zfp-1(RNAi)* animals also developed dorsal epidermal lesions (yellow arrowhead). Scale bar, 500 µm. (B) Survival curves for animals exposed to 1,250 rads of irradiation after RNAi. Animals are from the same experiment shown in Figure 3. Vertical lines indicate approximate times of RNAi feedings; arrows denote the time of irradiation (n = 17-21 animals per sample). See also Supplemental Table 4.

Figure S4. Analysis of colony RNAi phenotypes (Related to Figure 4)



Donor Cell Type	Colony #	Transplant Type	Time Before Fixation	# *smedwi-1*$^+$ cells	# NB.21.11E$^+$ cells
Control(RNAi)	1	bulk	10 Days	73	46
	2	single cell X1(FS)	9 Days	81	27
	3	single cell X1(FS)	9 Days	10	1
	4	single cell X1(FS)	8 Days	10	0
	5	single cell X1(FS)	8 Days	27	31
Zfp-1(RNAi)	6	bulk	10 Days	5	0
	7	bulk	10 Days	6	0
	8	single cell X1(FS)	9 Days	2	0
	9	single cell X1(FS)	9 Days	11	0
	10	single cell X1(FS)	9 Days	15	0
	11	single cell X1(FS)	9 Days	5	0
	12	single cell X1(FS)	9 Days	15	0
	13	single cell X1(FS)	8 Days	3	0
	14	single cell X1(FS)	8 Days	3	0

Figure S4. Analysis of colony RNAi phenotypes (Related to Figure 4)

(A-G) Additional plots of individual colony cell counts following RNAi. (H) Analysis of the *zfp-1*(RNAi) colony phenotype by transplantation of RNAi-exposed cNeoblasts into lethally irradiated (6,000 Rads) non-RNAi host animals. Donor animals were administered two feedings of control or *zfp-1* dsRNA over a seven-day period prior to transplantation. Host animals were irradiated 3 days prior to transplantation and fixed 8-10 days after transplantation. Representative confocal projections (anterior, left) of individual colonies produced by transplantation and labeled by double FISH are shown. Scale bars, 50 μm. Shown is a table of *smedwi-1*$^{+}$ and NB.21.11E^{+} cell counts for all colonies observed following transplantations. A total of 75 control(RNAi) and 72 *zfp-1*(RNAi) transplants (using either bulk macerated cell preps or individual cells) were performed in order to obtain the 14 colonies listed. 60% (3/5) of control(RNAi) colonies displayed large numbers of NB.21.11E^{+} cells. By contrast, 0% (0/9) of *zfp-1*(RNAi) colonies displayed even a single NB.21.11E^{+} cell. *zfp-1*(RNAi) colonies, furthermore, were much smaller than control(RNAi) colonies. These results suggest that both failed colony expansion and failed differentiation observed in *zfp-1*(RNAi) animals are due to requirement for *zfp-1* within cNeoblasts and/or their immediate descendants.

Figure S5. Phylogenetic analysis of planarian *Sox* genes

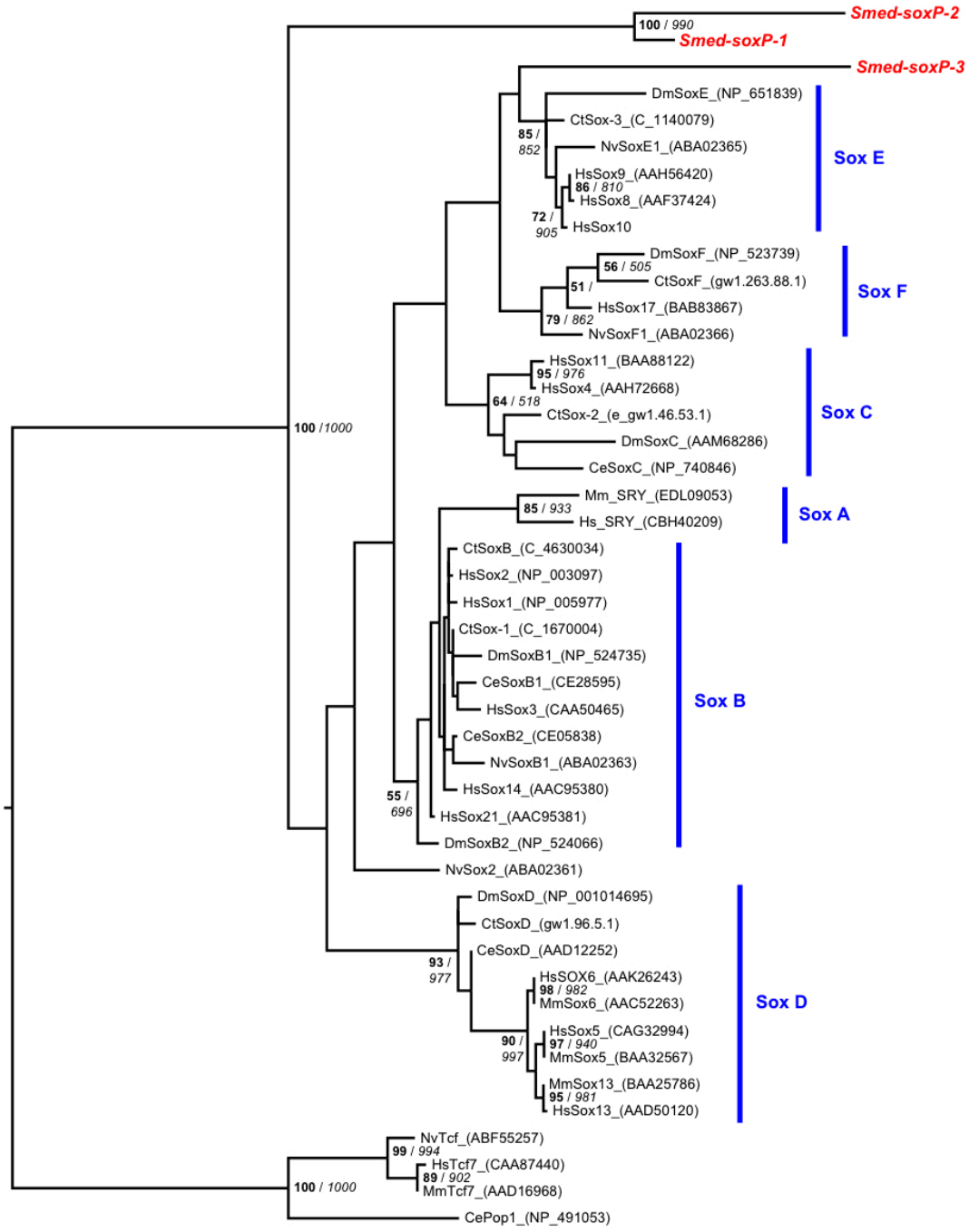


Figure S5. Phylogenetic analysis of planarian *Sox* genes

Maximum likelihood and neighbor-joining analyses provide strong support for the *Schmidtea mediterranea* genes *Smed-soxP-1, Smed-soxP-2*, and *Smed-soxP-3* falling within the SRY-box (SOX) family of transcription factors. Sequences used in this analysis are well-established representatives of six families of Sox transcription factors SoxA-F. T-cell factor (Tcf) transcription factor sequences are used as an outgroup. 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. This analysis did not generate strong support for *Smed-soxP-1, Smed-soxP-2*, or *Smed-soxP-3* belonging to any specific Sox families. Accession numbers for sequences used are listed in the tree. Mm, *Mus musculus*; Hs, *Homo sapiens*; Ce *Caenorhabditis elegans*; Dm, *Drosophila melanogaster*; Nv, *Nematostella vectensis*; Ct, *Capitella teleta*; Smed, *Schmidtea mediterranea*.

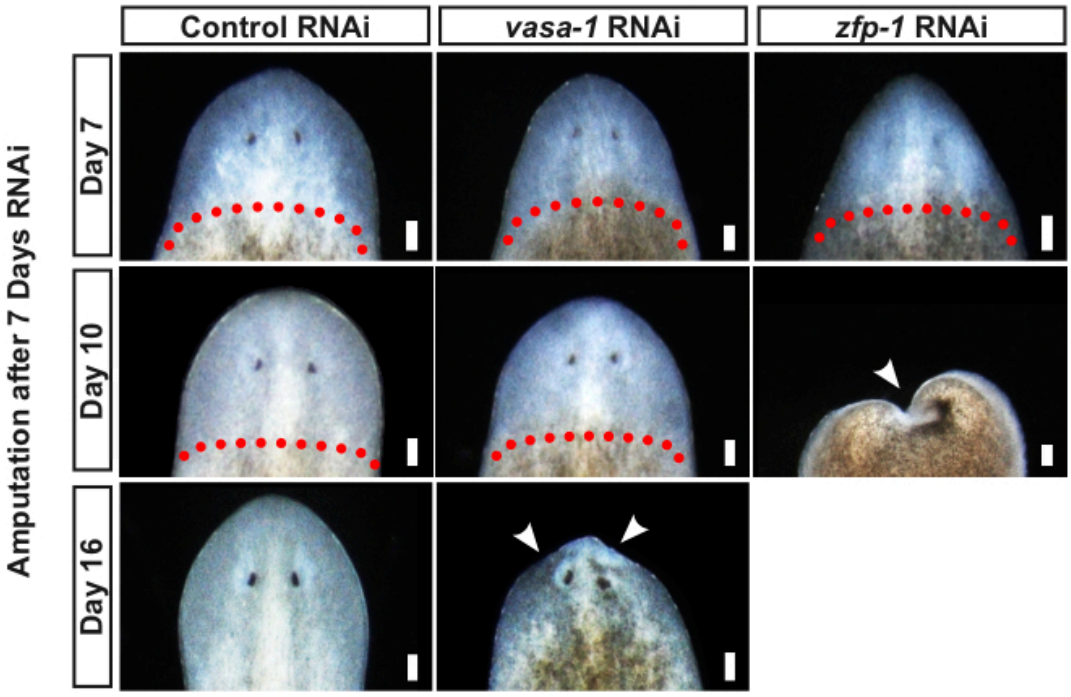


Figure S6. Assessment of regeneration ability following *Smed-zfp-1* and *Smed-vasa-1* RNAi (Related to Figure 6)

(A) Intact animals were fed RNAi food 4 times over 7 days prior to amputation. Shown are representative animal head regions from RNAi animals 7, 10, and 16 days following decaptiation. Anterior, up (n = 20-30 amputated animals per sample). Red dots indicate approximate amputation plane. Successful formation of regenerative blastemas was followed by regression of head tissues (arrowheads). Scale bars, 100 μm.

Table S1. Expression profiles of genes differentially expressed 24 hours after 6,000 rads γ-irradiation (Related to Figure 1)

This table is provided as a separate Excel spreadsheet

Statistical analysis of microarray data was performed with the limma package of Bioconductor (See Methods). Differentially expressed genes were designated based on comparisons between untreated and 24-hour irradiated samples; tabulated are 578 genes which displayed significant changes (fdr-adjusted $p < 0.05$) and a two-fold or more change in expression. Shown are log2-transformed values of mean intensity ratios (irradiated/untreated) and adjusted p-values for all irradiation timepoints (6, 12, 24, and 48 hrs). 60-mer probes sequences (also listed) are based on annotated gene models. Shown are most similar *H. sapiens, M.musculus, D. melanogaster,* and *C. elegans* proteins, determined by BLASTx.

Table S2. Gene set enrichment analysis (GSEA) of irradiation-sensitive transcripts

GO Category	GO Term	# of Genes	Enrichment Score (ES)	Normalized Enrichment Score (NES)	Nominal P-value	False Discovery Rate (FDR)
BP	DNA_REPLICATION	71	-0.78647405	-1.9618517	0	0
BP	DNA_DEPENDENT_DNA_REPLICATION	41	-0.7913672	-1.9073597	0	0
BP	CHROMOSOME_ORGANIZATION_AND_BIOGENESIS	85	-0.7124289	-1.8013581	0	0.003185955
BP	DNA_METABOLIC_PROCESS	163	-0.70437914	-1.8040102	0	0.003923937
BP	MEIOTIC_CELL_CYCLE	20	-0.7973665	-1.7849616	0	0.004126123
BP	M_PHASE	68	-0.71268564	-1.780008	0	0.003602103
BP	ESTABLISHMENT_AND_OR_MAINTENANCE_OF_CHROMATIN_ARCHITECTURE	56	-0.7173133	-1.769904	0	0.004071353
BP	DNA_REPAIR	87	-0.6905502	-1.7437253	0	0.006157725
BP	DNA_RECOMBINATION	28	-0.7382471	-1.7314062	0	0.008221576
BP	CELL_CYCLE_PROCESS	119	-0.6747928	-1.7122712	0	0.010667152
BP	CELL_CYCLE_PHASE	104	-0.67165273	-1.7099036	0	0.010057764
BP	RESPONSE_TO_DNA_DAMAGE_STIMULUS	109	-0.6726093	-1.7082855	0	0.0096307
BP	CHROMOSOME_SEGREGATION	21	-0.7447649	-1.707657	0.002066116	0.008966003
BP	MRNA_PROCESSING_GO_0006397	64	-0.68561107	-1.7063606	0	0.008396816
BP	REGULATION_OF_DNA_METABOLIC_PROCESS	30	-0.7158175	-1.6953417	0	0.009876917
BP	REGULATION_OF_CYCLIN_DEPENDENT_PROTEIN_KINASE_ACTIVITY	20	-0.7375845	-1.6770225	0.001037345	0.013271049
BP	RESPONSE_TO_ENDOGENOUS_STIMULUS	130	-0.6578717	-1.674086	0	0.013070003
BP	CHROMATIN_MODIFICATION	43	-0.6880702	-1.6725019	0	0.012782617
BP	RNA_SPLICING__VIA_TRANSESTERIFICATION_REACTIONS	31	-0.70018923	-1.6618803	0.001011122	0.014602413
BP	RNA_SPLICING	74	-0.66738105	-1.6607758	0	0.014119196
BP	MRNA_METABOLIC_PROCESS	72	-0.66198313	-1.6589657	0	0.013867962
BP	RNA_PROCESSING	139	-0.6394601	-1.6418678	0	0.018167317
BP	CELL_CYCLE_GO_0007049	192	-0.6288135	-1.6317589	0	0.02067871
BP	MITOSIS	53	-0.6588593	-1.6186252	0	0.024749776
BP	DOUBLE_STRAND_BREAK_REPAIR	17	-0.72717416	-1.6144097	0.00750268	0.025260521
BP	M_PHASE_OF_MITOTIC_CELL_CYCLE	54	-0.6580027	-1.6089724	0	0.02648995
BP	CHROMATIN_ASSEMBLY_OR_DISASSEMBLY	21	-0.70598996	-1.5996069	0.003112033	0.028907608
BP	MITOTIC_CELL_CYCLE	98	-0.62772375	-1.5894548	0	0.032034095
BP	DNA_INTEGRITY_CHECKPOINT	18	-0.71956867	-1.5862489	0.006269592	0.032731045
BP	CELL_CYCLE_CHECKPOINT_GO_0000075	30	-0.66589314	-1.5844908	0	0.032594044
BP	HISTONE_MODIFICATION	22	-0.69059443	-1.5821396	0.005197505	0.032687634
BP	CHROMATIN_REMODELING	17	-0.7156775	-1.5772376	0.010626992	0.03394659
BP	MICROTUBULE_CYTOSKELETON_ORGANIZATION_AND_BIOGENESIS	23	-0.6905639	-1.5750258	0.003080082	0.03390448
BP	COVALENT_CHROMATIN_MODIFICATION	23	-0.6858036	-1.5667869	0.004127967	0.037696093
BP	ANTI_APOPTOSIS	47	-0.6382287	-1.5638623	0	0.03839632
BP	RIBONUCLEOPROTEIN_COMPLEX_BIOGENESIS_AND_ASSEMBLY	68	-0.6247947	-1.5571553	0	0.04103016
BP	PROTEIN_DNA_COMPLEX_ASSEMBLY	26	-0.6513428	-1.5402777	0.011282051	0.04954915
MF	STRUCTURAL_CONSTITUENT_OF_RIBOSOME	69	-0.71315175	-1.7777117	0	0.008807067
MF	CHROMATIN_BINDING	21	-0.75098026	-1.6877774	0	0.039085653
MF	NUCLEOTIDYLTRANSFERASE_ACTIVITY	35	-0.69321775	-1.6499338	0	0.044717517
MF	ENDONUCLEASE_ACTIVITY	17	-0.7450352	-1.6628473	0.001057082	0.045939174
CC	ACTIN_CYTOSKELETON	75	-0.5865613	-1.9644306	0	0.001573409
CC	CELL_SURFACE	39	-0.5873265	-1.7604924	0	0.015122602
CC	EXTRACELLULAR_MATRIX	48	-0.55256414	-1.7090467	0.002547771	0.021118334
CC	PROTEINACEOUS_EXTRACELLULAR_MATRIX	47	-0.56286085	-1.7621076	0	0.022683902
CC	VESICLE	78	-0.5044195	-1.6723069	0	0.025404679
CC	EXTRACELLULAR_MATRIX_PART	32	-0.59454244	-1.7120501	0.001333333	0.025796393
CC	ENDOPLASMIC_RETICULUM	184	-0.4547668	-1.6763616	0	0.028210392
CC	COLLAGEN	18	-0.6398526	-1.6351613	0.004126547	0.030123707
CC	CYTOPLASMIC_VESICLE	75	-0.49301866	-1.6384537	0	0.03198353
CC	MEMBRANE_BOUND_VESICLE	74	-0.49771395	-1.6422218	0.00120773	0.03484871
CC	CYTOSKELETON	227	-0.4358009	-1.6122313	0	0.03571381
CC	MICROSOME	23	-0.56997657	-1.565528	0.016304348	0.03737842
CC	ENDOPLASMIC_RETICULUM_PART	66	-0.4815508	-1.5696026	0.003690037	0.0377254
CC	CELL_CORTEX	26	-0.5681521	-1.5940422	0.014844804	0.03854217
CC	VESICULAR_FRACTION	23	-0.56997657	-1.5697169	0.01615074	0.039840948
CC	CYTOPLASMIC_MEMBRANE_BOUND_VESICLE	72	-0.48943025	-1.5942947	0.001222494	0.04161096
CC	ENDOPLASMIC_RETICULUM_MEMBRANE	58	-0.47691047	-1.5254717	0.011180124	0.041728713
CC	MEMBRANE_FRACTION	182	-0.4294742	-1.5705521	0	0.041757077
CC	NUCLEAR_ENVELOPE_ENDOPLASMIC_RETICULUM_NETWORK	67	-0.46944284	-1.5259937	0.008547009	0.04326511
CC	CELL_FRACTION	243	-0.41614917	-1.5772883	0	0.04370404
CC	EXTRACELLULAR_REGION_PART	108	-0.43462032	-1.5107384	0.004587156	0.044182397
CC	CELL_CORTEX_PART	16	-0.6070221	-1.5271928	0.030428769	0.044469092
CC	VESICLE_MEMBRANE	20	-0.592809	-1.5705531	0.01920439	0.044540882
CC	VACUOLE	37	-0.5254954	-1.5392063	0.014012739	0.0451661
CC	ENDOSOME	45	-0.49883056	-1.5440586	0.012738854	0.04527182
CC	CELL_PROJECTION	62	-0.46599868	-1.5113217	0.007633588	0.045395
CC	EXTRACELLULAR_REGION	135	-0.42866713	-1.5340794	0.001135074	0.04547036
CC	LYSOSOME	34	-0.522725	-1.5135127	0.025710419	0.04596842
CC	LYTIC_VACUOLE	34	-0.522725	-1.5279459	0.019430052	0.046144865
CC	CONTRACTILE_FIBER_PART	15	-0.6101051	-1.4963323	0.036465637	0.04990748

Table S2. Gene set enrichment analysis (GSEA) of irradiation-sensitive transcripts (Related to Figure 1)

Gene set enrichment analysis was performed with javaGSEA using default settings (see Methods). Listed are Biological Process (BP), Molecular Function (MF), and Cellular Component (CC) Gene Ontology gene sets significantly enriched (fdr-adjusted $p < 0.05$) in a gene list pre-ranked by 24-hour microarray log2 ratios (irradiated/untreated) and annotated by most similar (BLASTx) human symbol. Tabulated for each gene list are Gene Ontology (GO) term names, gene set size, enrichment scores, normalized enrichment scores, nominal p-value, and fdr-corrected p value. See Subramanian et al. (2005) for further details on the GSEA method.

Table S3. Homology of genes expressed in proliferative cells of Schmidtea mediterranea (Related to Figure 2)

Gene Name	Probe Base Name	Top Human BLASTx Hit (Gene Symbol) [E-value]	Top Mouse BLASTx Hit [E-value]	Top Fly BLASTx Hit [E-value]	Top C.elegans BLASTx Hit [E-value]	PFAM Domains [E-value]
Smed-mrg-1	SMED_31964_V2	mortality factor 4 like 1 (MORF4L1) [1e-18]	predicted gene, EG627352 [2e-19]	MRG15 [1e-28]	locus:mrg-1 [0.0001]	MRG [6.6e-30]; Tudor-knot (chromodomain) [9.9e-09]
Smed-nsd-1	SMED_20741_V2	Wolf-Hirschhorn syndrome candidate 1 (WHSC1) [6e-24]	Wolf-Hirschhorn syndrome candidate 1 (human) [5e-24]	CG1716 [6e-08]	locus:met-1 [1e-06]	SET [4.8e-06]
Smed-rbbp4-1	SMED_02582_V2	retinoblastoma binding protein 4 (RBBP4) [2e-71]	retinoblastoma binding protein 4 [2e-71]	Chromatin assembly factor 1 subunit [5e-71]	locus:lin-53 [1e-72]	WD40 [0.017; 6.1e-05; 1.3e-05; 1.6e-07; 8.9e-05]
Smed-eed-1	SMED_00806_V2	embryonic ectoderm development (EED) [9e-82]	embryonic ectoderm development [8e-82]	escl [4e-80]	locus:mes-6 [5e-17]	WD40 [0.0013; 0.00029]
Smed-ezh	SMED_04593_V2	enhancer of zeste homolog 1 (Drosophila) (EZH1) [3e-57]	enhancer of zeste homolog 1 (Drosophila) [1e-58]	Enhancer of zeste [7e-58]	locus:mes-2 [1e-24]	SET [1.7e-21]
Smed-sz12-1	SMED_28607_V2	suppressor of zeste 12 homolog (Drosophila) (SUZ12) [8e-25]	suppressor of zeste 12 homolog (Drosophila) [8e-25]	Su(z)12 [1e-16]		VEFS-Box (of Polycomb protein) [2.7e-17]
Smed-setd8-1	SMED_06179_V2	SET domain containing (lysine methyltransferase) 8 (SETD8) [4e-39]	SET domain containing (lysine methyltransferase) 8 [8e-39]	pr-set7 [8e-35]	locus:set-1 [1e-34]	SET [2.1e-18]
Smed-vasa-1	SMED_00256_V2	DEAD (Asp-Glu-Ala-Asp) box polypeptide 4 (DDX4) [3e-111]	DEAD (Asp-Glu-Ala-Asp) box polypeptide 4 [4e-110]	vasa [5e-102]	locus:laf-1 [5e-97]	DEAD/DEAH box [9.6e-44]; Helicase (conserved C-terminal domain) [3.2e-26]
Smed-vasa-2	SMED_01829_V2	DEAD (Asp-Glu-Ala-Asp) box polypeptide 4 (DDX4) [2e-100]	DEAD (Asp-Glu-Ala-Asp) box polypeptide 4 [1e-99]	vasa [3e-96]	locus:laf-1 [3e-86]	DEAD/DEAH box helicase [1.7e-44]; Helicase (conserved C-terminal domain) [8.8e-26]
Smed-khd-1	SMED_01038_V2	KH domain containing, RNA binding, signal transduction associated 2 (KHDRBS2) [4e-27]	KH domain containing, RNA binding, signal transduction associated 2 [3e-27]	quaking related 58e-1 [8e-27]	hypothetical protein E02D9.1c [2e-16]	KH_1 [0.00055]
Smed-cip29	SMED_10768_V2	cytokine induced protein 29 kDa (CIP29) [1e-15]	predicted gene, EG625193 [2e-15]	CG8149 [5e-10]	status:Confirmed [1e-07]	SAP [3.4e-12]
Smed-rtel1	SMED_13828_V2	regulator of telomere elongation helicase 1 (RTEL1) [5e-38]	regulator of telomere elongation helicase 1 [2e-39]	CG4078 [1e-28]	CHL1 protein [5e-26]	DEAD_2 (RAD3-like DNA-binding helicase) [5.1e-46]
Smed-inx-13	SMED_28576_V2	pannexin 1 (PANX1) [0.025]	pannexin 1 [0.01]	innexin 2 [0.00002]	locus:eat-5 [7e-22]	Innexin [2.9e-38]
Smed-zmym-1	SMED_02942_V2	zinc finger, MYM-type 2 (ZMYM2) [8e-35]	zinc finger, MYM-type 3 [4e-35]	without children [1e-33]	locus:pro-1 [9e-07]	DUF3504 (unknown function) [3e-14]
Smed-znf207-1	SMED_05644_V2	zinc finger protein 207 (ZNF207) [1e-52]	zinc finger protein 207 [8e-53]	CG17912 [6e-54]	hypothetical protein B0035.1b [9e-45]	zf_C2H2_jaz [0.011]; zf-FCS [0.047]
Smed-fhl-1	SMED_12559_V2	four and a half LIM domains 3 (FHL3) [1e-56]	four and a half LIM domains 5 [4e-60]	Limpet [1e-39]	locus:lim-9 [1e-106]	LIM [2.6e-08; 1.4e-08; 1.1e-09; 1.8e-10; 1.4e-07]
Smed-prox-1	SMED_26251_V2	prospero homeobox 1 (PROX1) [2e-28]	prospero homeobox 2 [5e-36]	prospero [4e-23]	locus:ceh-26 [3e-30]	Prox1 Homeobox [7.8e-13]
Smed-tcf15	SMED_02941_V2	scleraxis homolog A (mouse) (SCXA) [7e-14]	transcription factor 15 [1e-13]	cousin of atonal [5e-06]	locus:hlh-13 [9e-06]	HLH [3.6e-16]
Smed-soxP-1	SMED_11833_V2	SRY (sex determining region Y)-box 13 (SOX13) [2e-13]	SRY-box containing gene 13 [2e-13]	Sox box protein 14 [5e-13]	locus:egl-13 [7e-13]	HMG_box [1.4e-12]
Smed-soxP-2	SMED_25793_V2	SRY (sex determining region Y)-box 12 (SOX12) [1e-09]	SRY-box containing gene 12 [1e-09]	Sox100B [5e-10]	locus:egl-13 [4e-09]	HMG_box [2e-10]
Smed-soxP-3	SMED_05061_V2	SRY (sex determining region Y)-box 9 (SOX9) [3e-18]	SRY-box containing gene 9 [3e-18]	Sox box protein 15 [9e-19]	locus:sem-2 [2e-18]	HMG_box [4.8e-19]
Smed-zfp-1	SMED_10510_V2	zinc finger protein 701 (ZNF701) [1e-13]	zinc finger protein 341 [3e-14]	CG14710 [2e-12]	locus:pag-3 [3e-11]	zf_C2H2 [4.3e-06; 0.00021]
Smed-junl-1	SMED_21705_V2	transcription factor AP-1 (JUN) [0.0004]	transcription factor AP-1 [0.0001]	CG15040 [6e-06]	hypothetical protein T25F10.6 [2e-05]	Jun [0.00053]
Smed-egr-1	SMED_00244_V2	early growth response 2 (Krox-20 homolog, Drosophila) (EGR2) [1e-25]	early growth response 2 [1e-25]	stripe [2e-25]	locus:egrh-1 [1e-24]	zf_C2H2 [0.00094; 0.0019]
Smed-nlk-1	SMED_08795_V2	nemo-like kinase (NLK) [3e-65]	nemo like kinase [2e-65]	nemo [1e-64]	locus:lit-1 [1e-63]	Pkinase [2.4e-29]
Smed-armc1	SMED_06686_V2	armadillo repeat containing 1 (ARMC1) [1e-13]	armadillo repeat containing 1 [4e-13]			no domains found
Smed-fgfr-1	SMED_38140_V2	fibroblast growth factor receptor 1 (FGFR1) [8e-34]	fibroblast growth factor receptor 1 [7e-34]	breathless [3e-32]	locus:egl-15 [7e-30]	Pkinase_Tyr [6.2e-23][1e-05]
Smed-fgfr-4	SMED_38138_V2	fibroblast growth factor receptor 4 (FGFR4) [2e-38]	fibroblast growth factor receptor 4 [2e-37]	breathless [7e-38]	locus:egl-15 [4e-30]	I-set (Immunoglobulin I-set) [1e-05]; Pkinase_Tyr [1.5e-25]

Table S3. Homology of genes expressed in proliferative cells of *Schmidtea mediterranea* (Related to Figure 2)

Annotations for microarray candidate genes validated by *in situ* hybridization (Fig 2). Gene names, descriptions, and E-values are tabulated for top BLASTx matches of candidate genes in *H. sapiens*, *M. musculus*, *D. melanogaster*, and *C. elegans.* Protein domains identified by PFAM are also listed.

Experiment	RNAi Condition	% of Total Animals: Visual Defects by Day 21	% of Total Animals: Dead by Day 42	N
I	Control (*unc-22*)	0%	5%	20
	bruli	100%	100%	20
	armc1	0%	0%	20
	cip29	100%	100%	20
	vasa-1	100%	100%	17
	egr-1	0%	0%	20
	fhl-1	53%	95%	19
	junl-1	95%	95%	20
	nlk-1	0%	0%	20
	prox-1	0%	0%	20
	tcf15	0%	5%	20
	soxP-3	10%	0%	20
	zfp-1	100%	100%	20
	ezh	100%	100%	20
	sz12-1	95%	100%	20
	eed-1	100%	100%	19
II	Control (*unc-22*)	0%	0%	20
	Control (*unc-22*; 6,000 Rads)	100%	100%	19
	Control (No RNAi)	0%	0%	21
	khd-1	100%	100%	20
	rbbp4-1	11%	5%	19
	zf207-1	100%	100%	20
	zmym-1	100%	100%	20
	inx-13	0%	0%	20
	rtel1	70%	80%	20
	nsd-1	0%	0%	22
	mrg-1	100%	100%	20
	setd8-1	100%	100%	20
	fgfr-1	95%	25%	20
	fgfr-2	44%	17%	18
	soxP-1	60%	35%	20
	soxP-2	20%	5%	20
	rplp0	100%	100%	20
	bruli	100%	100%	20
III	Control (*unc-22*)	60%	10%	20
	Control (*unc-22*; 6,000 Rads)	100%	100%	20
	vasa-2	90%	45%	20

Table S4. Identification of RNAi phenotypes after Sublethal Irradiation (1,250 Rads) (Related to Figure 3)

Quantitative analysis of RNAi phenotypes identified after sublethal irradiation (See also Figs. 3 and S3). Listed are results for genes without major RNAi phenotypes, in addition to those depicted in Fig. 3. Percentages of animals showing visible defects in tissue homeostasis (e.g., head regression, ventral curling, lesions, or lysis) by day 21 post-irradiation are listed. Also listed are percentages of animals that died by day 42 post-irradiation. All other animals appeared normal, and showed no signs of tissue failure. Total numbers of animals analyzed per condition are also noted. Results for each RNAi condition should be compared to those of internal control samples. Control animals in Experiment III, for example, developed early signs of head regression and curling but subsequently recovered and nearly all survived past day 42 post-irradiation.

		Numbers of Cells per Colony												Analysis of Cell Type Ratios	
	Associated	# *smedwi-1*$^+$ cells (d7)			# *smedwi-1*$^+$ cells (d14)			# NB.21.11E$^+$ cells (d7)			# NB.21.11E$^+$ cells (d14)			*smedwi-1* / NB.21.11E (d14)	
RNAi condition	Control	Mean	St.Dev	t TEST	Mean	St.Dev	t TEST	Mean	St.Dev	t TEST	Mean	St.Dev	t TEST	Ratio of Means	ANCOVA
smedwi-2	I	10.8	9.5	0.6112	n/a	n/a	n/a	3.2	2.8	0.1232	n/a	n/a	n/a	n/a	n/a
smedwi-3	I	13.1	12.7	0.8054	3.3	3.4	**0.0008**	4.5	3.2	0.8299	0.9	1.5	**0.0047**	3.8	0.927
bruli	I	9.6	6.0	0.2976	17.8	16.8	**0.0016**	3.9	3.2	0.5091	4.1	4.0	**0.0065**	4.3	0.3702
CHD4	I	21.1	14.9	**0.0457**	108.3	84.9	0.3039	3.8	2.5	0.3242	8.9	9.0	**0.0107**	12.2	**<0.0001**
p53	I	11.4	9.7	0.7650	19.9	13.8	**0.0018**	0.0	0.0	**<0.0001**	0.0	0.0	**0.0044**	undef	n/a
rplp0	I	4.4	5.1	**0.0025**	1.7	0.6	**0.0007**	0.5	0.9	**<0.0001**	1.0	1.0	**0.0048**	1.7	0.9699
cdc23	I	21.0	22.5	0.0958	2.8	2.1	**0.0007**	5.9	7.0	0.4719	0.0	0.0	**0.0044**	undef	n/a
rpa1	II	11.7	5.4	0.7942	n/a	n/a	n/a	3.8	1.9	0.7719	n/a	n/a	n/a	n/a	n/a
cyclinL1	II	8.2	4.2	0.0889	1.0	0.0	**0.0002**	3.8	1.7	0.8512	0.0	0.0	**0.0022**	undef	n/a
cip29	I	13.1	9.5	0.7789	13.9	16.3	**0.0013**	5.5	6.1	0.6181	2.1	2.7	**0.0054**	6.6	0.2905
soxP-1	I	15.7	14.4	0.4999	57.3	28.4	**0.0139**	4.2	4.1	0.7477	19.5	10.7	**0.0310**	2.9	0.142
fhl-1	I	18.6	11.9	**0.0426**	34.1	35.8	**0.0039**	4.4	3.7	0.7495	8.4	7.3	**0.0101**	4.0	**0.02527**
zfp-1	I	21.0	18.0	0.0514	26.3	22.9	**0.0025**	1.7	3.2	**0.0042**	0.02	0.2	**0.0044**	1077.0	**<0.0001**
vasa-1	I	16.5	17.6	0.4171	55.8	60.2	**0.0132**	4.9	4.9	0.8725	10.8	16.1	**0.0130**	5.1	**<0.0001**
setd8-1	III	8.8	5.2	0.7764	n/a	n/a	n/a	3.0	2.3	0.6725	n/a	n/a	n/a	n/a	n/a
zmym-1	III	8.8	5.4	0.7828	3.2	1.5	**<0.0001**	3.6	2.2	0.2018	0.8	0.8	**<0.0001**	3.9	0.917
khd-1	III	9.3	4.5	0.9678	19.3	12.4	**<0.0001**	2.5	1.8	0.8447	3.6	3.4	**<0.0001**	5.4	0.1935
junl-1	IV	5.3	1.6	**0.0003**	31.7	19.8	**0.0009**	1.8	0.8	**0.0016**	12.6	9.6	**0.0006**	2.5	0.4051
sz12-1	IV	8.0	5.4	0.0381	45.7	40.7	**0.0054**	3.6	1.5	0.1565	15.8	13.9	**0.0019**	2.9	0.8295
eed-1	IV	9.1	5.5	0.0873	43.0	35.1	**0.0041**	4.5	3.8	0.7529	20.9	20.1	**0.0193**	2.1	0.1181
ezh	V	7.4	1.7	0.0501	31.6	16.8	**<0.0001**	5.2	1.3	0.9176	11.4	6.5	**<0.0001**	2.8	0.2609
control	I	12.3	10.7		152.8	112.4		4.7	4.0		63.9	61.9		2.4	
control	II	11.2	6.7		128.4	43.7		3.7	2.5		45.1	23.4		2.8	
control	III	9.4	7.8		108.8	61.0		2.6	2.2		36.5	23.8		3.0	
control	IV	12.5	8.7		111.8	66.6		4.9	4.3		43.3	24.3		2.6	
control	V	11.2	8.5		105.3	66.1		5.1	4.9		54.5	38.9		1.9	

		Numbers of Cells per Colony						Analysis of Cell Type Ratios	
	Associated	# *AGAT-1*$^+$ cells (d7)			# *AGAT-1*$^+$ cells (d14)			*smedwi-1* / *AGAT-1* (d14)	
RNAi condition	Control	Mean	St.Dev	t TEST	Mean	St.Dev	t TEST	Ratio of Means	ANCOVA
CHD4	I	0.7	1.0	0.2291	0.0	0.0	**0.0018**	n/a	**<0.0001**
p53	I	0.2	0.4	**0.0010**	0.0	0.0	**0.0018**	n/a	0.0794
zfp-1	I	1.2	1.5	0.8064	0.0	0.0	**0.0018**	n/a	**<0.0001**
vasa-1	I	2.5	2.3	0.0547	11.0	13.8	**0.0165**	5.1	**0.0077**
control	I	1.1	1.4		36.4	30.9		4.2	

Table S5. Statistical analysis of colony RNAi phenotypes (Related to Figure 4)
For all quantitative clonal analyses, each experimental condition was compared to an internal RNAi control in which animals were cultured, irradiated, fixed, stained and analyzed in parallel. Tabulated are mean and standard deviations for *smedwi-1*$^+$, NB.21.11E$^+$, and *AGAT-1*$^+$ cell counts for all timepoints and RNAi conditions. Listed are p values from a Student's t-test (2-tailed) comparing cell counts at each timepoint to respective internal controls. Relative ratios of mean population sizes (per colony) were also assessed (see right). To determine whether cell type ratios significantly deviated from those of control colonies, linear regression together with analysis of covariance (ANCOVA, an f-test statistic) was performed using Graphpad Prism. Resulting p values are listed. Conditions in which no colony cells were present are denoted by "n/a". Tests resulting in significant differences from controls ($p < 0.05$) are highlighted in bold.

Supplemental Experimental Procedures:

Accession Numbers

Raw microarray data are deposited in GEO, accession number GSE34969. Full-length sequences of genes cloned for this study are deposited in Genbank, accession numbers JQ425133- JQ425160. Additional candidate genes with associated EST clone names and Genbank accession numbers are *Smed-rbbp4-1* (clone H.87.8a; accession number AY066201.1),*Smed-setd8-1* (accession numbers PL06004A2H08, PL06005A2B11, and PL06007A1C07), *Smed-khd-1* (H.62.2h; AY068551.1), *Smed-zmym-1* (SAAH-aaa29c01, EG409125.1), *Smed-znf207-1* (H.118.1c, AY067556.1), and *Smed-nlk-1* (H.118.1c, AY067556.1). Cell cycle genes with deposited RNAi clone names and accession numbers are *Smed-cyclinL1* (NBE.2.09B, AY967575.1), *Smed-rpA1* (NBE.6.12e, AY967663.1), *Smed-rplp0* (NBE.7.7g, AY967679.1), and *Smed-cdc23* (NBE.4.10b, AY967619.1). Accession numbers for additional genes used in this study are DQ186985.1 (*smedwi-1*), EG413862.1 (*mat*), EC616347 (*carbonic anhydrase / ca*), EC386316 (*mhc-1*), AY067773 (*synapsin*), AY067799 (H.1.3b), DN308230 (*collagen*).

Microarray Analysis

Total RNA was harvested with Trizol (Invitrogen) from untreated animals and animals 6, 12, 24, and 48 hours after 6,000 rads γ-irradiation. Three biological replicates were used. Cy3 and Cy5-labeled cRNA was prepared using a QuickAmp labeling kit (Agilent) starting with 1µg total RNA. Custom planarian 60-mer 4x44,000 oligonucleotide expression arrays (Agilent) were hybridized according manufacturer instructions and scanned using an Agilent DNA microarray scanner. Array images were quantified and statistical significance of differential expression was calculated using Agilent's Feature Extraction Image Analysis software with the default two-color gene expression protocol. Agilent two-color arrays were within-array normalized by loess, followed by between-array quantile normalization of average intensities across channels (Aquantile). Differential expression analysis was performed with a moderated t-test, as implemented in the limma package of Bioconductor, with p-value correction by false discovery rate. Genes were considered differentially expressed if they met a corrected p-value threshold of 0.05 and displayed greater than two-fold change in expression (log2 ratio > 1). Volcano plots were generated using R.

Gene Cloning

Molecular clones of candidate gene sequences were obtained from existing EST libraries (Robb et al., 2007; Sánchez Alvarado et al., 2002) and by direct cloning from cDNA. cDNA libraries were generated with Superscript III reverse transcriptase (Invitrogen) from total RNA extracted (Trizol, Invitrogen) from mixed-stage regenerating animals. Gene-specific primers were designed from gene predictions and EST databases (Robb et al., 2007) and contained Gateway adapter sequences for downstream applications (see below). Amplicons generated by PCR were cloned into the pGEM vector (pGEM T-easy, Promega). In some cases, a second (nested) round of amplification was performed to obtain PCR products for cloning. Templates for RNA probe synthesis were generated by PCR using primers recognizing Gateway adapter sequences with a T7 promoter sequence appended to the reverse primer. For RNAi experiments, Gateway recombination (Invitrogen) was used to clone genes into the pPR244 vector, as described (Reddien et al., 2005a). For many genes, putative full-length transcripts were also assembled de novo from Illumina reads with the Trinity software package (Grabher et al., 2011) using default settings. Resulting contigs were mapped to the current version of the genome by BLAT and were compared to the Bowtie mapped reads to verify the assembly. Contigs for some genes were assembled from existing published EST sequences, or by performing 5' and 3' Race (FirstChoice RLM-RACE, Ambion).

Gene Set Enrichment Analysis

GSEA was performed as described (Subramanian et al., 2005) using javaGSEA. A gene list annotated by top human BLASTx gene symbol and pre-ranked by log2 ratios (24 hour irradiated / untreated) was analyzed by Gene Ontology (GO) gene sets (GO:molecular function, GO:biological process, and GO:cellular component) using default settings. Gene sets were considered significantly enriched if they met an fdr-corrected p-value threshold of 0.05.

Phylogenetic Analysis

Peptide sequences for *Smed-soxP-1*, *Smed-soxP-2, Smed-soxP-3* were aligned with well-known members of Sox family transcription factors using ClustalW with default settings (Thompson et. al., 1994). Alignments were trimmed using GBlocks (Castresana,

2000). 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 with WAG model of amino acid substitution, four substitution rate categories, and the proportion of invariable sites estimated from the dataset (Guindon and Gascuel, 2003). 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.

Fluorescence-Activated Cell Sorting

Tissue fragments were macerated in calcium-free, magnesium-free medium plus BSA (CMFB) as described (Reddien et al., 2005b) containing 1 mg/ml of collagenase for 45 minutes at RT. Tissues were passed through syringes with 25 gauge 5/8 inch needles and through a 40 μm cell-strainer cap (BD Biosciences). Cells were centrifuged at 1,250 rpm, 5 min, and resuspended in CMFB containing Hoechst 33342 (Invitrogen, 10 mg/ml) for 45 minutes at RT. Calcein (Invitrogen, 0.5 mg/ml) was incubated with the cells for 15 minutes at RT. 5 mg/ml propidium iodide was added to cells prior to flow cytometry. Sorts used a MoFlo3 FACS sorter. The X1 population (Hayashi et al., 2006) was assessed and quantified in triplicate using FlowJo software as described (Scimone et al., 2010).

Cell Transplantation

Isolation and transplantation of bulk macerated cells and individual X1(FS) cells from RNAi donor animals into lethally irradiated hosts were performed as described (Wagner et al., 2011).

Primers used for Gene Cloning

Smed-mrg-1
For 5'-TGCCTCTGAAATCTGATATAAAG
Nest 5'-CTATCACGGACCCTTGCTTT
Rev 3'-ACATGGAACCGTAAATGCTG

Smed-nsd-1
For 5'-ACATGCACGAAATGGTTTCA
Rev 3'-TCCAATGCAAAAAGTGACAAA

Smed-eed-1
For 5'-TCAATGATCGCATCCGTAAA
Nest 5'-GTCTGATCCTATTTTATTCGTCTCC
Rev 3'-GATCAAAGCGAGCAATCAGG

Smed-ezh
For 5'-GATGACGTTCGGCAAATCTT
Nest 5'-TGAACAGATTGCAATGGTTAGT
Rev 3'-TCGAATCAGTGCCGTTATTG

Smed-sz12-1
For 5'-AAGTCACATAGCGTAGAATTTCAAGA
Nest 5'-GCGGCACAAGACAAATCCTA
Rev 3'-AGCCATTTCATGCATTCGAG

Smed-setd8-1
For 5'-TTTCTCCCAAAGAAAGTTCTAAAAA
Nest 5'-TATCAAATGAAATTCAAGGCAAAA
Rev 3'-CAACAACAATAAAATACACAAAATCG

Smed-vasa-1
For 5'-TGATGAAGAATGGGGAGCAT
Nest 5'-CTCAAAATGGCTTTGGCAGT
Rev 3'-TCGAGCCATTCAGAAGTCG

Smed-vasa-2
For 5'CGGAGATTGAATAATGTAGTTAGCAA
Rev 3'-CGATAAAATCCATAAAAGATGCAC

Smed-cip29
For 5'-GTGGTATAATGGAGGACTTGACG
Rev 3'-ATCTAGCAGCGCGAGCTTT

Smed-rtel1
For 5'-TTTTCCATTCGAACCTTATGC
Nest 5'-TGCCAAATAATATACATGGAAAAA
Rev 3'-TCCATGTCCAATTTCAGAGTTTC

Smed-inx13
For 5'-TGATAGCTTCTGAATTGCTTTCTT
Nest 5'-AATGGATTCTCTGTCGCTCAA
Rev 3'-GGTCGGTTTGAGGTTTTCAG

Smed-fhl-1
For 5'-ATAAAATGGCCTTGAAACAAGA
Nest 5'-AATTGTACAGGTTTTAAAATTCATGG
Rev 3'-AACTGCTGCAATTGGGACAC

Smed-prox-1
For 5'-TAAAGTCAGCCGGAATAGCA
Nest 5'-TCCAAAAATGAATTCACCACA
Rev 3'-ACTCTGGCAACATCTGATCG

Smed-tcf15
For 5'-GCAAAAGAACGCGAAAGGT
Rev 3'-TATACAAAAGGCAACGAAATGC

Smed-soxP-1
For 5'-AAGACAAATGCAACACAATCAAA
Nest 5'TACTTTGAAATTATGGATGGTCCATTT
Rev 3'-TGTTGAAATAATGAATTAAGATTTGG

Smed-soxP-2
For 5'-GCTTCAAATTCAGAAATAAGCAAA
Nest 5'-GGAATAACATTCCAGCTACCATT
Rev 3'-GCAACCATGAAAATCGCTTC

Smed-soxP-3
For 5'-TTGTTGAGCATGTTTCTAAATACTC
Nest 5'-TGAATTATTGTGAAAACACCGAAA
Rev 3'-AAATCAAACTACAAAAACAATTCATGT

Smed-zfp-1
For 5'-GAATTTCATGGAACAAAATAATTCA
Nest 5'-CATCAACTACTCCATTCTCATTGG
Rev 3'-ACGTCCATGGAGTCAGTTGG

Smed-junl-1
For 5'-AATCGGAATTCGGTATTTTGG
Nest 5'-AAATGCTCTCAGACCCGATT
Rev 3'-TTGAAAAACAAGCGAATTTGG

Smed-egr-1
For 5'-CGAGACTGCTAATGATGATCCA
Nest 5'-CATCGTTTGAGGTTCATTCG
Rev 3'-TGGCAATATTTGCAGTCATGT

Smed-armc1
For 5'-AATGTCTCTTAATCCGCTGTCTG
Nest 5'-CGGTTGTTAGGCCAGGATT
Rev 3'-TTTTCAAGGTCCTTTTGTGAAA

Smed-fgfr-1
For 5'-TGGAATGTTCGATTTTCCATC
Nest 5'-CTGTACGATGGGCTTGGTTT
Rev 3'-TCAACCAACTGGAAAGTGTGA

Smed-fgfr-4
For 5'-ACATGCATCCAGAAATGAAGAA
Nest 5'-GATGTGCATGGTGAAGGTTG
Rev 3'-CACTGAAAATCGGCCTCATT

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