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Figures and figure supplements

Selective amputation of the pharynx identifies a FoxA-dependent regeneration program in planaria

Carolyn E Adler, et al.

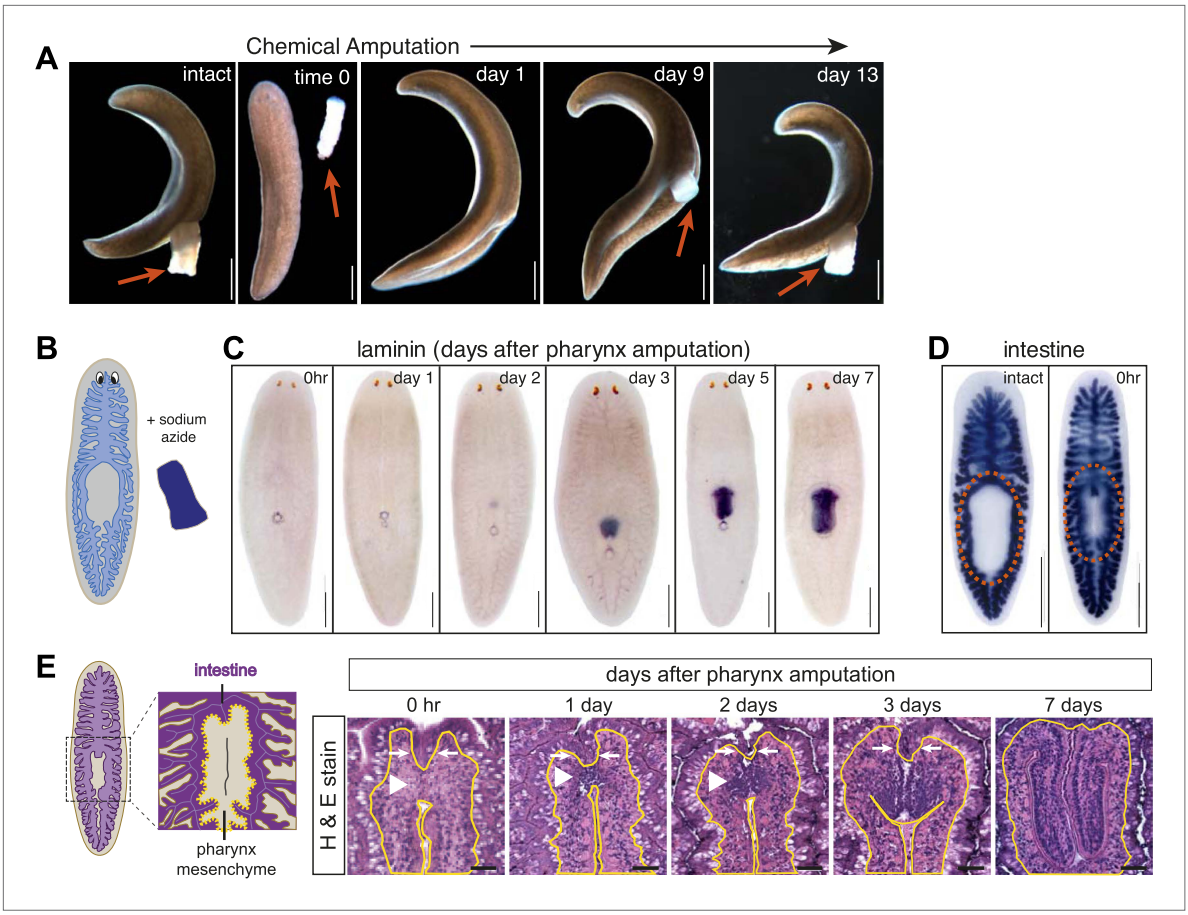

Figure 1. Sodium azide selectively removes the pharynx. (**A**) Live animals before and after sodium azide treatment, showing pharynges (arrows). (**B**) Schematic of chemical amputation. (**C**) Pharynx (labeled with *Smed-laminin*) reappears 2–3 days after pharynx removal. (**D**) Intestine (labeled with *Smed-porcupine*) before and immediately after chemical amputation. (**E**) Representative hematoxylin/eosin sections of the regenerating pharynx (white arrowheads). Yellow lines outline mesenchyme and white arrows highlight intestine. Scale bars, **A**–**D**: 500 μm, **E**: 50 μm.

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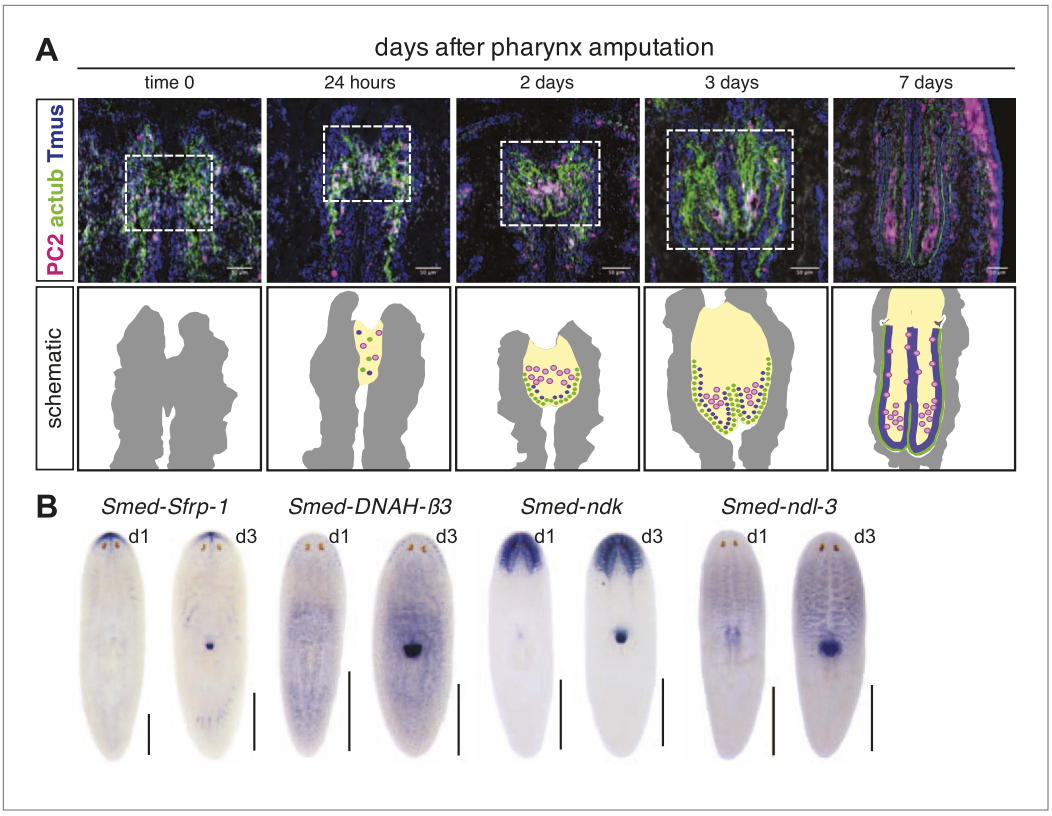

Figure 1—figure supplement 1. Histological analysis of regenerating pharynx.

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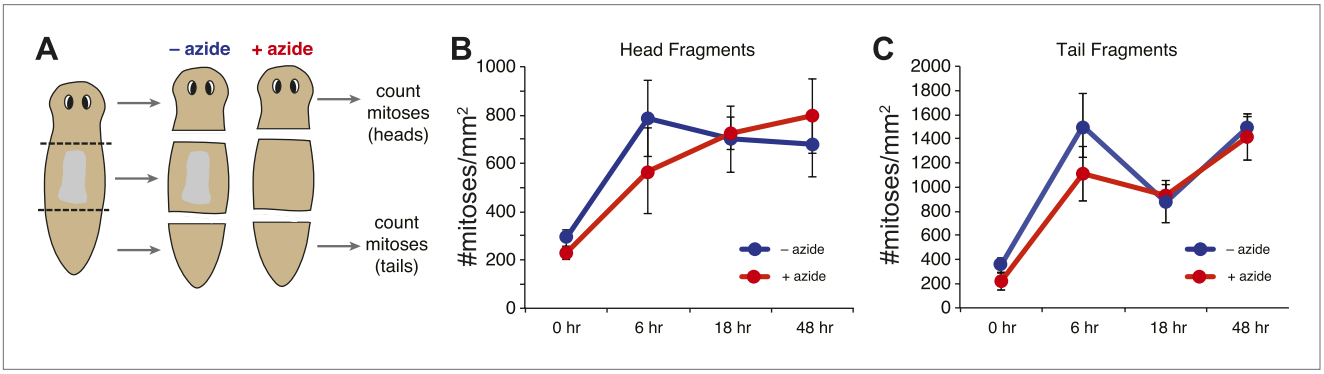

Figure 1—figure supplement 2. Effects of sodium azide exposure.

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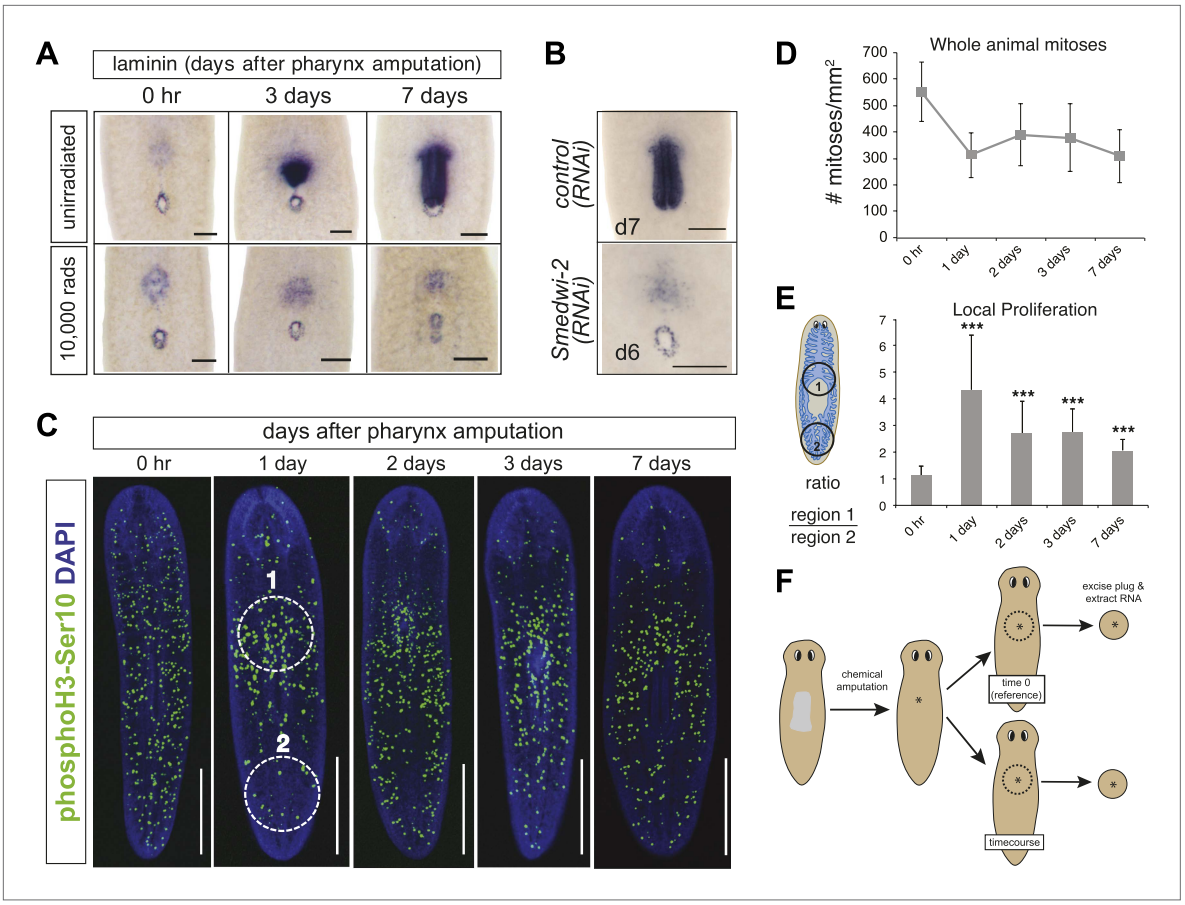

Figure 2. Local proliferation of stem cells drives regeneration. (**A**) Irradiated animals fail to regenerate the pharynx (100%; n >50), as indicated by *Smed-laminin* ISH. (**B**) *Smedwi-2(RNAi)* inhibits pharynx regeneration (100%, n >30). (**C**) Representative confocal images of animals during pharynx regeneration, stained with anti-phosphoH3-Ser10. Circles are representative of those used for quantification in (**E**). (**D**) Quantification of phosphoH3-Ser10 staining in whole animals. Error bars = SD. (**E**) Local proliferation measured in two equal-sized circles, (1) centered over the pharynx and (2) centered in the tail as marked in (**C**). Error bars = SD; *** equals p<.0001; significance determined with Student's *t* test. (**F**) Schematic of strategy for expression profiling. Scale bars, **A** and **B**: 200 μm, **C**: 500 μm.

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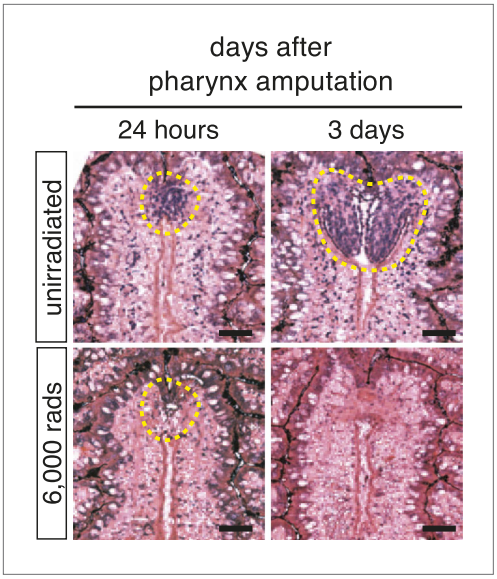

Figure 2—figure supplement 1. Irradiation prevents accumulation of cells at the blastema.

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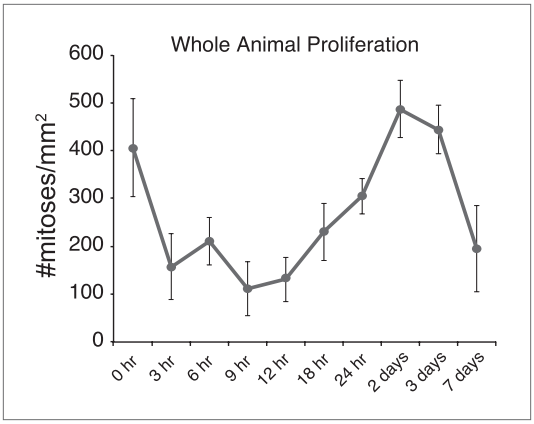

Figure 2—figure supplement 2. Body-wide mitotic activity after chemical amputation.

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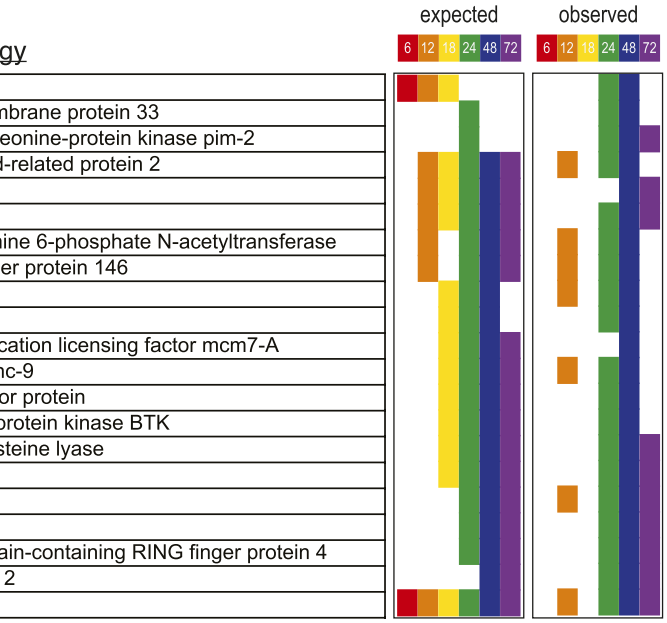
A

Clone ID	Probe ID	Homology
2A05	SMED20002798	novel
2E02	SMED10023438	Transmembrane protein 33
5B09	SMED10015047	Serine/threonine-protein kinase pim-2
5B07	SMED10000192	Rhomboid-related protein 2
5B06	SMED20009699	novel
5E08	SMED10004653	novel
2A03	SMED10015196	Glucosamine 6-phosphate N-acetyltransferase
5B10	SMED10018687	RING finger protein 146
2C03	SMED10039154	novel
5B02	SMED10001332	singed
2A11	SMED10005551	DNA replication licensing factor mcm7-A
2D12	SMED10023201	Innexin unc-9
3D09	SMED10004216	Brain tumor protein
5B11	SMED10039115	Tyrosine-protein kinase BTK
2F11	SMED10018951	Selenocysteine lyase
4B12	SMED10017580	novel
3C02	SMED10036377	novel
1G06	SMED20012969	Musculin
5F04	SMED10022969	PDZ domain-containing RING finger protein 4
1C07	SMED10019822	RuvB-like 2
5C04	SMED10046108	novel



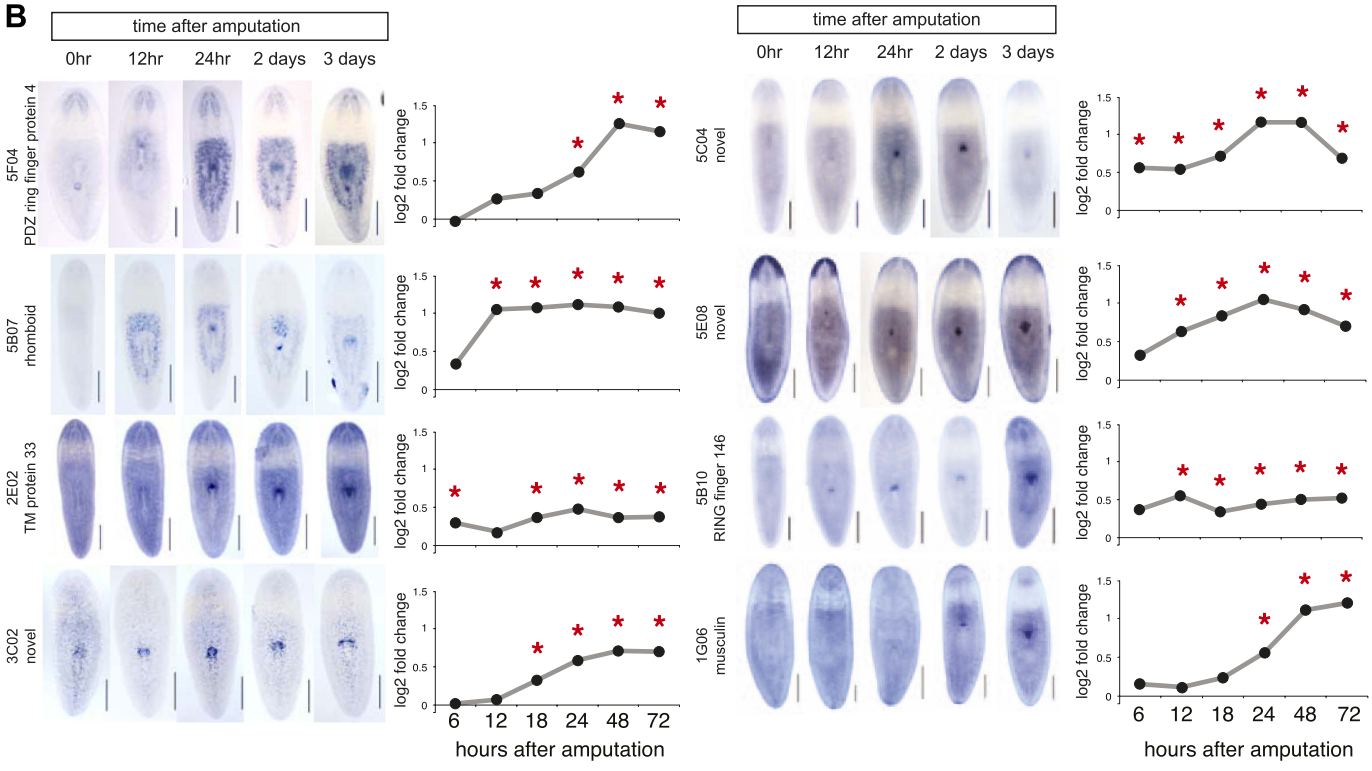


Figure 2—figure supplement 3. Validation of microarray by in situ timecourses.
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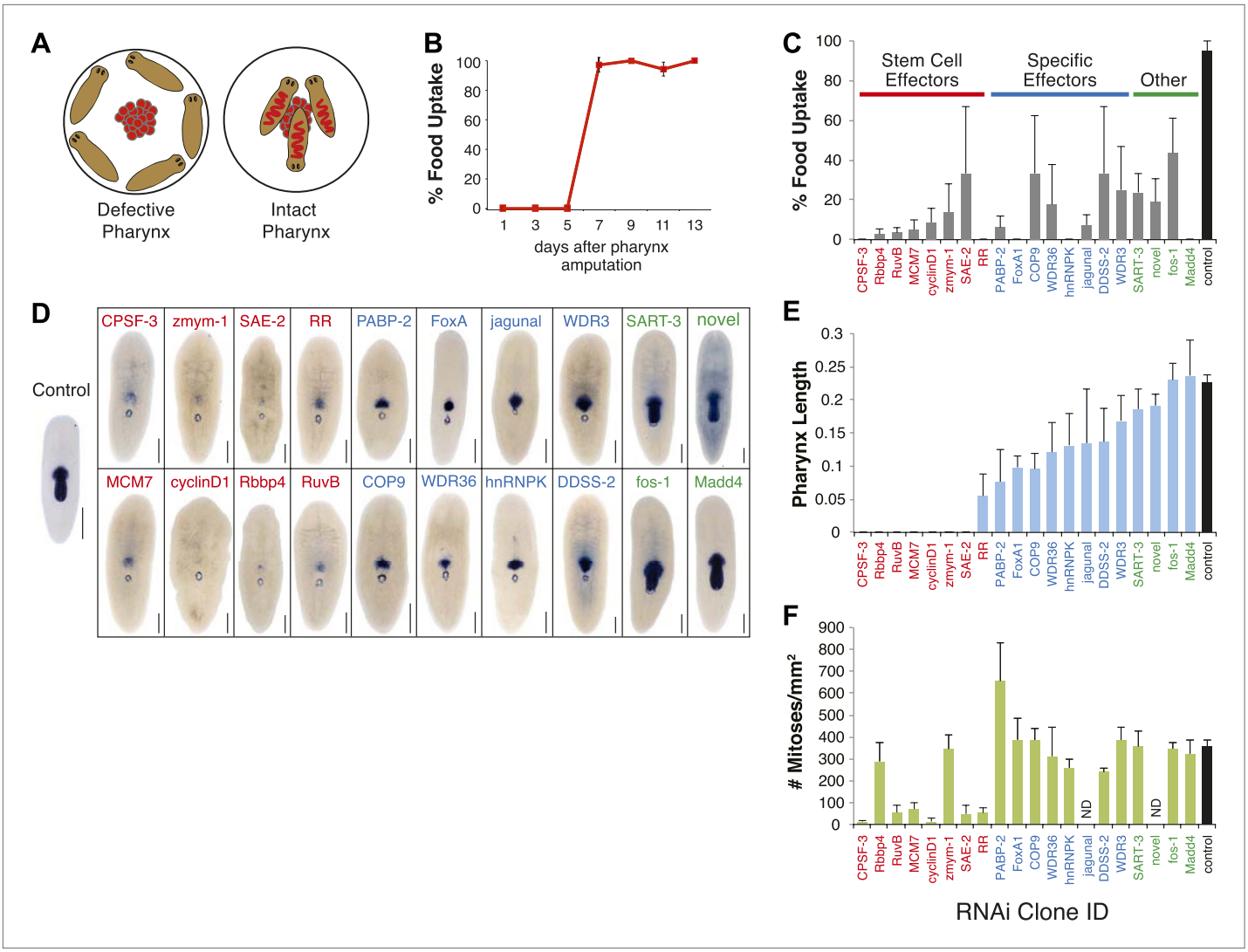

Figure 3. RNAi screen for genes affecting pharynx regeneration. (**A**) Schematic of feeding assay. (**B**) Animals recover ability to ingest food 7 days after chemical amputation. For each timepoint, n = 10 animals, repeated in triplicate. Error bars = SD. (**C**) Quantification of feeding behavior of RNAi-treated animals 10 days after amputation. Shown are averages of three independent experiments; error bars = SEM, n ≥30 animals. *Smed-laminin* in situ hybridization shows extent of pharynx regeneration defects in RNAi-treated animals. Scale bars = 250 μm. (**E**) Quantification of pharynx length in RNAi animals 11 days after amputation. For each bar, n = 6–10 animals; error bars = SD. (**F**) Mitotic activity of whole animals 3 days after pharynx amputation measured by phosphoH3-Ser10 staining. Error bars represent SD, and n = 8 animals for each condition.

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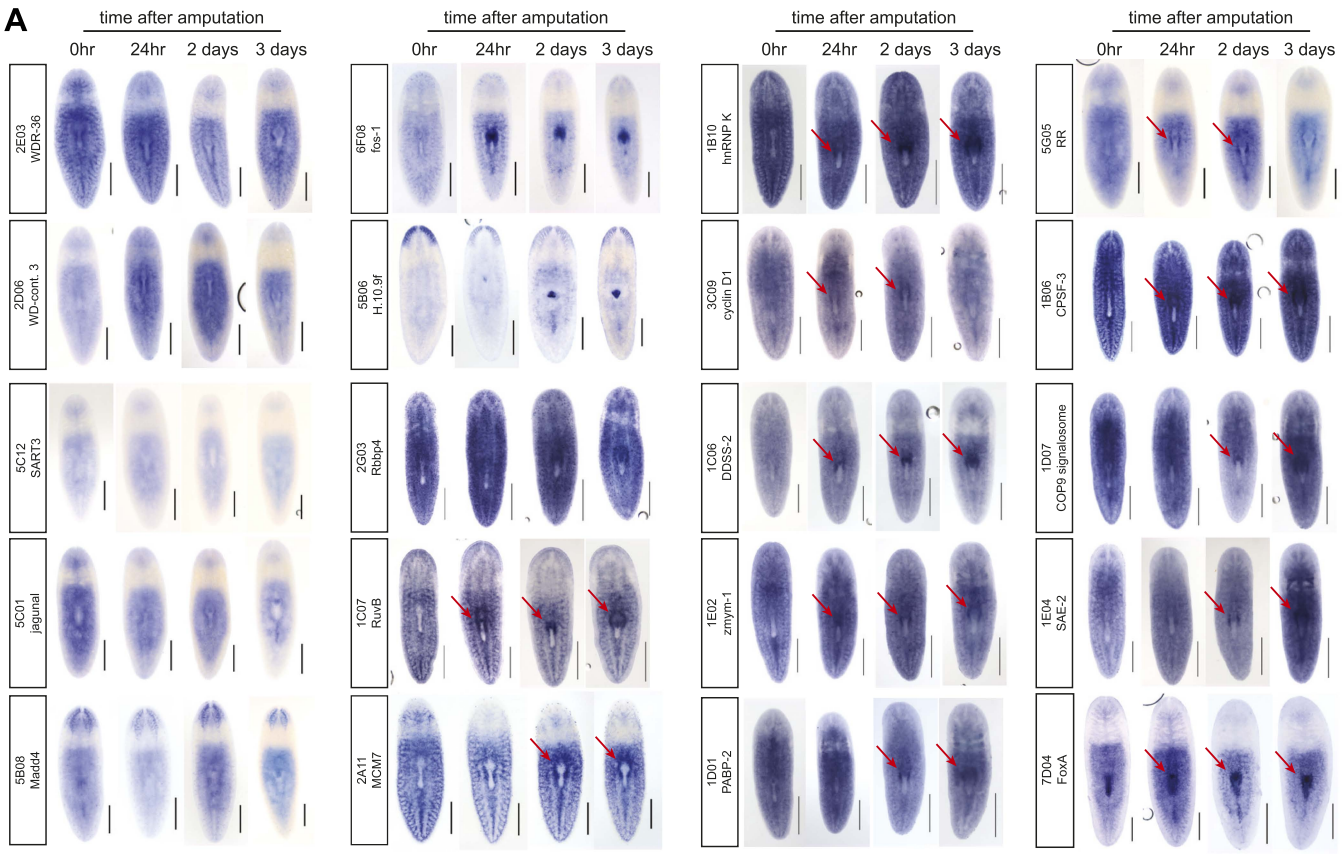

B

		Fold Change (log2)					Adjusted p-value						
Probe ID	Description	u6	u12	u18	u24	u48	p.adj.u6	p.adj.u12	p.adj.u18	p.adj.u24	p.adj.u48	Spatial Distribution	Temporal Change
SMED10001012	fos-1	0.357	0.438	0.269	0.524	0.383	1.29E-01	3.63E-02	2.00E-01	4.03E-03	1.52E-02	midbody mesenchyme, new pharyr	12, 24, 48
SMED10002251	jagunal	0.269	0.521	0.311	0.312	0.277	1.02E-01	4.03E-04	2.52E-02	1.82E-02	1.45E-02	diffuse	nc
SMED10005551	MCM7	0.125	0.114	0.684	0.662	0.465	6.52E-01	6.29E-01	5.97E-06	6.24E-06	2.57E-04	neoblasts	24, 48
SMED10012366	Squamous cell carcinoma antigen recognized by T-ce	0.56	-0.119	-0.0081	-0.039	-0.3	2.39E-02	7.69E-01	9.83E-01	9.24E-01	1.07E-01	diffuse	nc
SMED10015791	Mothers against decapentaplegic homolog 4/medea	0.648	0.458	0.514	0.43	0.742	7.74E-03	6.84E-02	2.22E-02	5.15E-02	1.59E-04	CNS, PrNeph, diffuse	nc
SMED10022308	Ribonucleoside-diphosphate reductase subunit M2	-0.101	0.115	0.253	0.6	0.547	8.01E-01	6.94E-01	1.77E-01	3.00E-04	2.67E-04	mesenchyme, diffuse	24, 48
SMED20002207	WD repeat-containing protein 3	0.0831	0.055	0.544	0.389	0.394	7.22E-01	7.96E-01	4.27E-06	3.48E-04	9.65E-05	diffuse	nonspecific
SMED20002254	WD repeat-containing protein 36	0.143	0.068	0.615	0.558	0.404	4.01E-01	7.30E-01	4.71E-07	1.69E-06	6.76E-05	mesenchyme	nc
SMED20009699	clone H.10.9f(T3)	0.286	0.675	0.69	0.989	0.842	2.72E-01	8.90E-04	2.52E-04	6.69E-07	3.51E-06	CNS, distal pharynx	12, 24, 48
SMED10000127	Forkhead transcription factor A1	-0.025	0.043	-0.101	-0.091	0.337	9.63E-01	8.98E-01	6.01E-01	6.74E-01	8.51E-03	midbody mesenchyme, new pharyr	12, 24, 48
SMED10002178	Cleavage and polyadenylation specificity factor subur	-0.194	0.099	0.00795	0.0794	0.412	2.51E-01	6.21E-01	9.69E-01	6.42E-01	1.78E-04	mesenchyme	24, 48
SMED10006322	COP9 signalosome complex subunit 5	-0.098	0.111	0.117	0.144	0.503	7.09E-01	5.82E-01	4.44E-01	3.27E-01	2.24E-05	mesenchyme, diffuse	48
SMED10009302	SUMO-activating enzyme subunit 2	-0.182	0.05	-0.12	0.0549	0.491	1.27E-01	7.76E-01	2.70E-01	6.90E-01	4.00E-07	mesenchyme, diffuse	48
SMED10018587	Polyadenylate-binding protein 2	-0.029	0.196	0.249	0.205	0.415	9.62E-01	4.33E-01	1.94E-01	2.94E-01	4.48E-03	mesenchyme, diffuse	48
SMED10019822	RuvB-like 2	-0.124	0.147	0.0417	0.146	0.655	6.71E-01	5.17E-01	8.51E-01	4.14E-01	4.72E-06	neoblasts	24, 48
SMED20001888	Heterogeneous nuclear ribonucleoprotein K	-0.026	0.181	0.00602	0.0801	0.367	9.37E-01	1.45E-01	9.72E-01	5.46E-01	7.89E-05	mesenchyme	48
SMED20002134	Decaprenyl-diphosphate synthase subunit 2	-0.041	0.066	0.301	0.42	0.483	8.93E-01	7.38E-01	6.61E-03	1.13E-04	5.36E-06	mesenchyme, diffuse	24, 48
SMED20003618	G1/S-specific cyclin-D1	0.169	0.179	0.355	0.282	0.402	4.29E-01	3.35E-01	9.68E-03	3.69E-02	6.73E-04	diffuse	48
SMED10021079	Zinc finger MYM-type protein 4	0.151	0.038	-0.0041	-0.041	0.417	5.99E-01	9.16E-01	9.89E-01	8.82E-01	2.01E-03	mesenchyme, diffuse	24, 48
SMED10015488	Histone-binding protein RBBP4	0.136	0.434	0.337	0.419	0.644	7.34E-01	3.70E-02	9.13E-02	2.29E-02	1.31E-04	unknown cells	48

Figure 3—figure supplement 1. Candidate gene summary.

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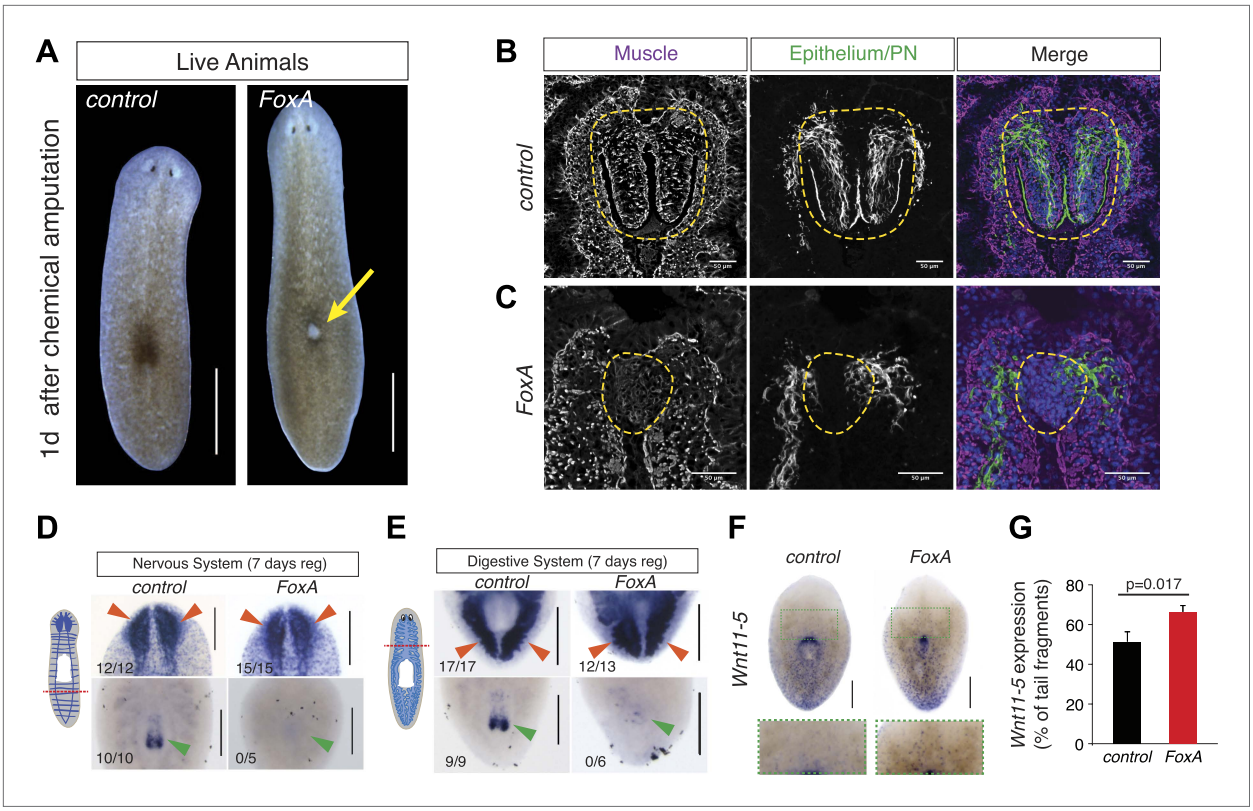

Figure 4. *FoxA* is required for pharynx regeneration. (**A**) *FoxA(RNAi)* animals develop dorsal lesions (arrow). (**B** and **C**) Confocal images of cryosections stained with antibodies recognizing muscle (α-Tmus), epithelial cells and protonephridia (α-acetylated tubulin), and nuclei (DAPI). Control (**B**) and *FoxA(RNAi)* animals (**C**) are shown 3 days after pharynx removal. Dashed green lines highlight the regenerating pharynx. (**D**) Tail fragments amputated at dashed red line regenerate brain tissue (*Smed-PC2*, red arrowheads) but not a pharynx (*Smed-PKD2*, green arrowheads). (**E**) Head fragments regenerate posterior intestinal branches (*Smed-porcupine*, red arrowheads) despite the absence of a pharynx (*Smed-PKD2*, green arrowhead). (**F**) Whole-mount ISH for *Wnt11-5* in *control* and *FoxA(RNAi)* tail fragments 7 days after amputation. Green boxes highlight insets shown below. (**G**) Ratio of *Wnt11-5* expression to total length of tail fragment. Significance determined by Student's *t* test. Error bars = SEM. N = 14 fragments. Scale bars, (**A**), 500 μm, (**B** and **C**), 50 μm, (**D**–**F**), 200 μm.

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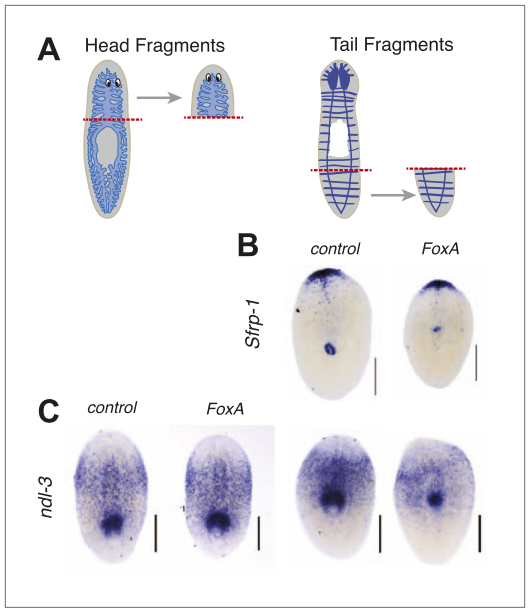

Figure 4—figure supplement 1. FoxA is not required for anterior/posterior patterning during regeneration.

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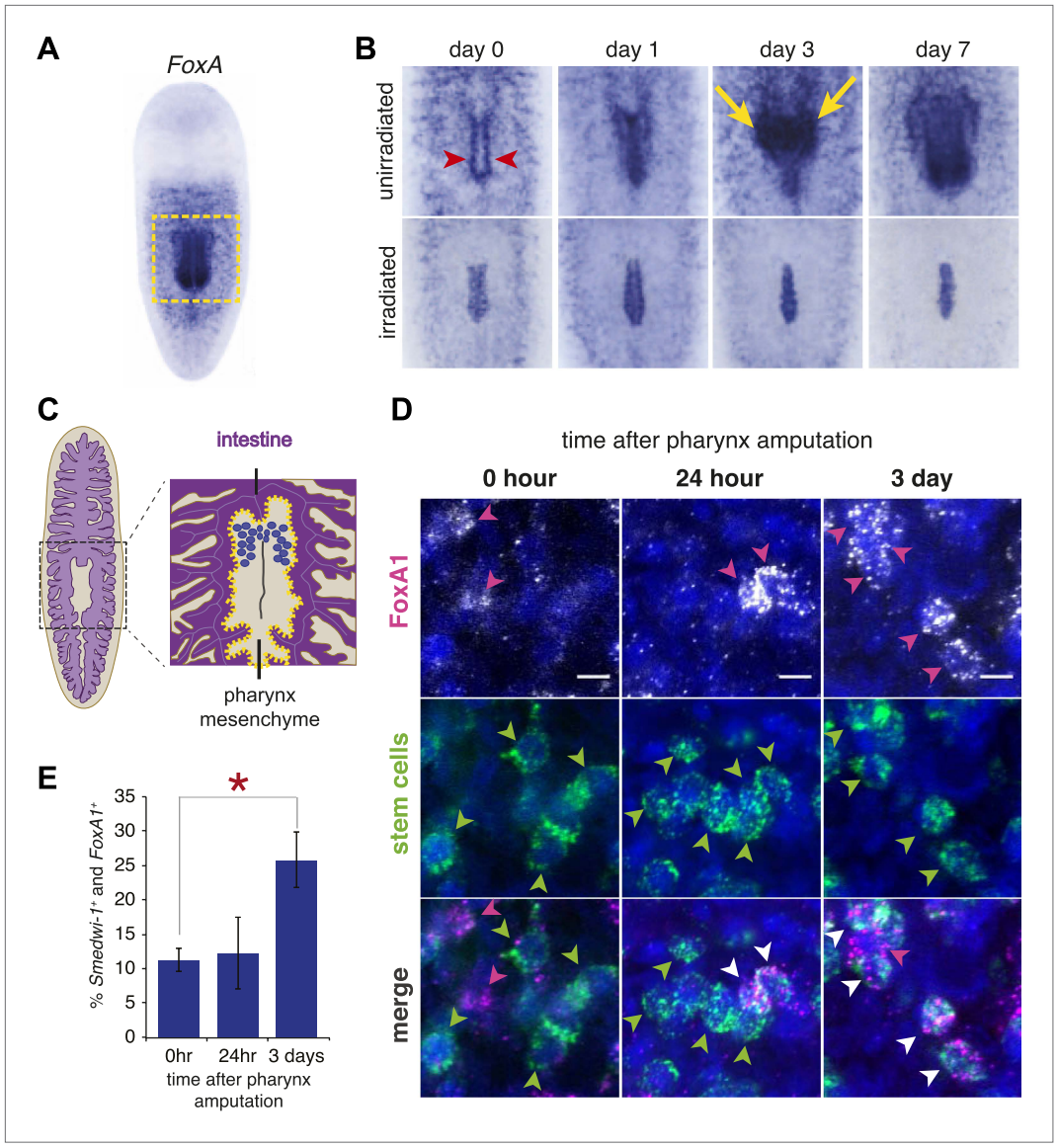

Figure 5. FoxA expression in neoblasts increases after amputation. (**A**) Whole-mount ISH for *Smed-FoxA* in intact animals. Boxed region highlights areas shown in (**B**). (**B**) *Smed-FoxA* expression in pharyngeal region during regeneration, in unirradiated animals (top) and lethally irradiated animals (bottom). Yellow arrowheads point to accumulation of *FoxA+* cells in mesenchyme surrounding pharynx and red arrows highlight pharyngeal pouch. (**C**) Schematic of mesenchymal pouch surrounding the pharynx, where *FoxA+* cells concentrate during regeneration. (**D**) Double-FISH with *smedwi-1* and *Smed-FoxA* at different times after pharynx removal. Arrowheads highlight positive cells. Scale bars = 10 μm. (**E**) Quantification of percentage of *smedwi-1+* cells that co-express *FoxA* during regeneration. For each timepoint, n = 100–150 *smedwi-1+* cells.

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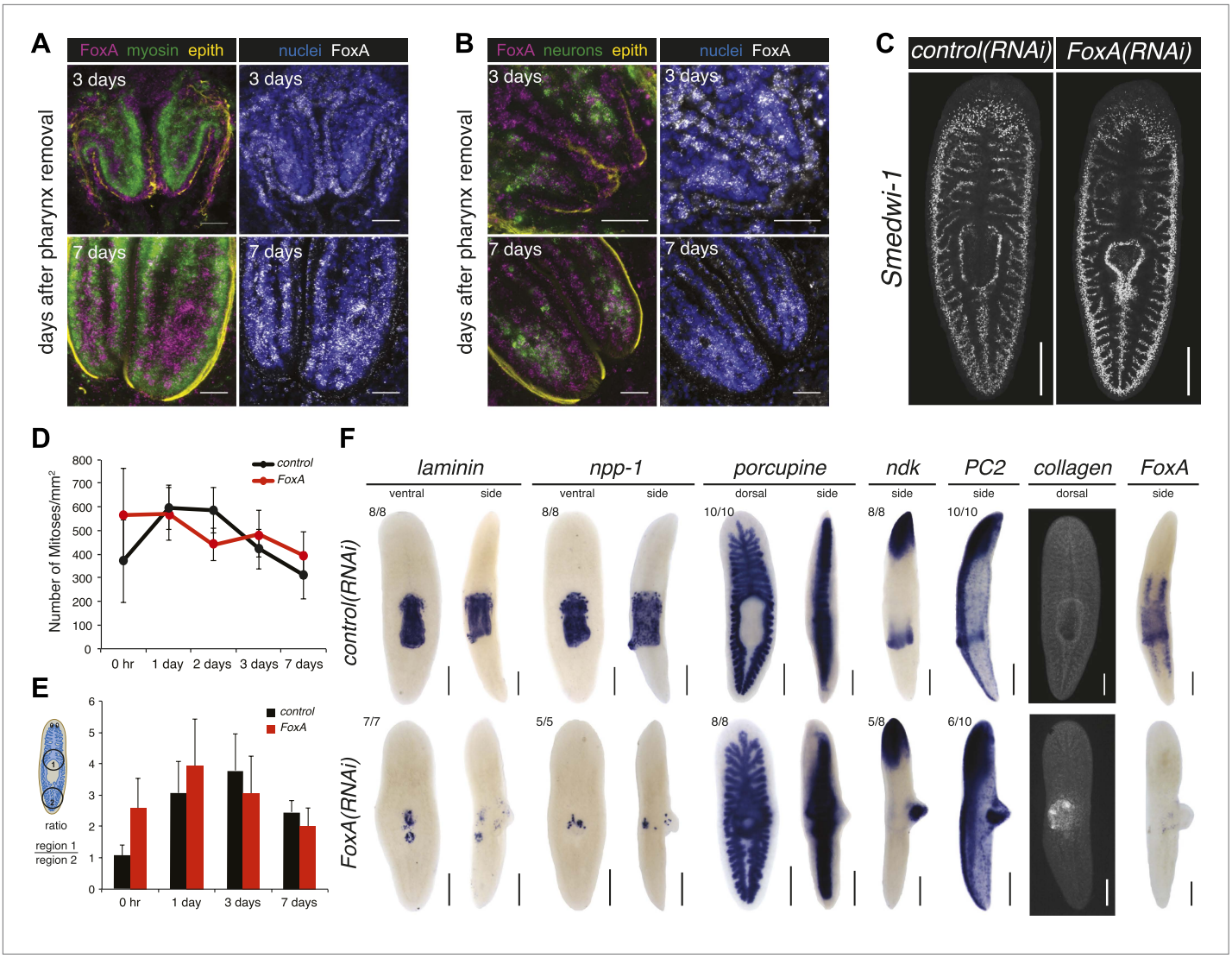

Figure 6. FoxA functions as a master regulator of the pharyngeal lineage. (**A** and **B**) Confocal images of animals stained for *FoxA*, *myosin* (muscle), *PC2* (neurons), α-acetylated tubulin (epithelial cells) and nuclei showing *FoxA* enrichment in epithelial cells 3 days after pharynx amputation, and shifting to mesenchyme 7 days after amputation. (**C**) Confocal images of *Smedwi-1* FISH (7 days after pharynx amputation) showing distribution of stem cells. (**D**) Body-wide phosphoH3-Ser10 staining in *FoxA(RNAi)* animals during pharynx regeneration. Error bars = SD. (**E**) Local phosphoH3-Ser10 staining during pharynx regeneration in *FoxA(RNAi)* animals. Error bars = SD. (**F**) Dorsal outgrowths in *FoxA(RNAi)* animals (day 20) lack pharyngeal tissue. Tissue-specific markers include: *laminin*, *npp-1* (pharynx), *porcupine* (intestine), *ndk*, *PC2* (neurons), *collagen* (muscle). In all images, anterior is up; in side views, dorsal is to the right. Scale bars (**A** and **B**) 50 μm, (**C**) 500 μm, (**F**) 250 μm.

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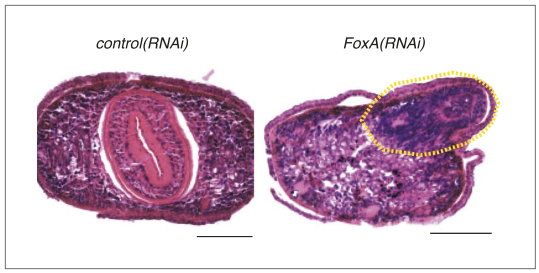

Figure 6—figure supplement 1. Dorsal outgrowths in *FoxA(RNAi)* are disorganized.

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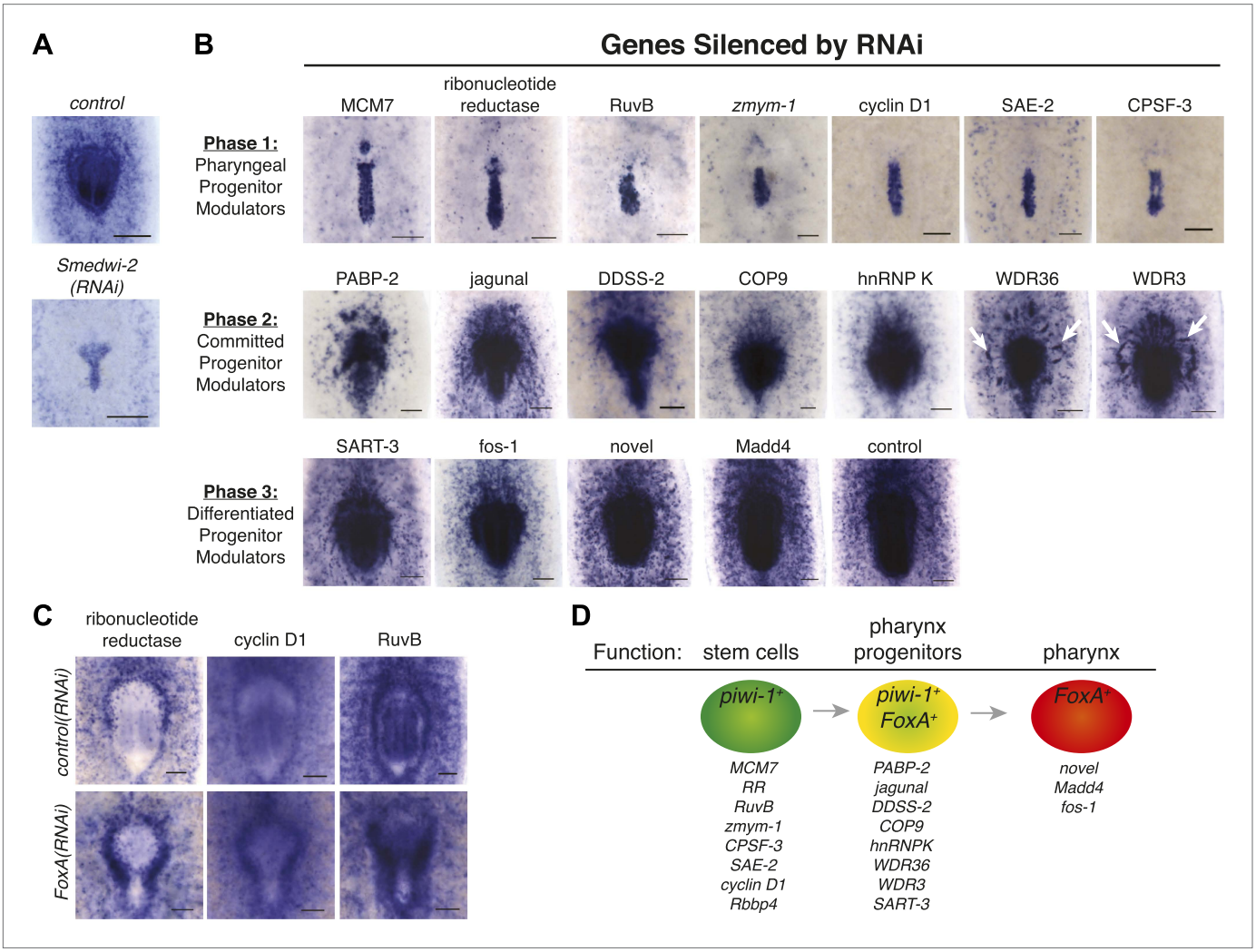

Figure 7. *FoxA* expression resolves a molecular pathway for pharynx regeneration. (**A**) *Smed-FoxA* expression 7 days after amputation in *Smedwi-2(RNAi)* animals. (**B**) *Smed-FoxA* expression 7 days after amputation following knockdown of the indicated genes. (**C**) Gene-specific in situ hybridization in *FoxA(RNAi)*. (**D**) Model for molecular control of pharynx regeneration. Scale bars = 100 μm.

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